Glyphosate &

the IPA-, K-, NH_4 - and DMA salts of glyphosate

Herbicide

Application for Renewal of Approval (AIR 2) according to Commission Regulation (EC) N° 1141/2010

ANNEX II

Summary documentation, TIER II

Document M:

Point 5: Toxicological and toxicokinetic studies

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IIA 5 Toxicological and toxicokinetic studies on the active substance

The data presented in this dossier based on studies from several Glyphosate Task Force members. Within the studies the name of the active substance (a.s.) or test substance **glyphosate** differs between glyphosate, glyphosate acid, glyphosate technical, as well as company specific codes (e.g. HR-001). All these test substance descriptions refer to glyphosate acid. Glyphosate salts will be clearly identified with their counterion (or defined sympbol; e.g. K= potassium) in the test substance name.

IIA 5.1 Absorption, distribution, excretion and metabolism in mammals

This assessment of the mammalian metabolism of glyphosate includes detailed reports of four studies which were not available for the 2001 EU glyphosate evaluation, as well as the existing data (see Table 5.1-1).

Absorption, distribution and elimination

The 2001 EU evaluation of glyphosate concluded that following oral administration, glyphosate is rapidly absorbed from the gastrointestinal tract but only to a limited extent of approximately 30 - 40 %. Data on the extent of biliary excretion in bile-cannulated rats, that was not available for the 2001 EU glyphosate evaluation, confirms the systemically available glyphosate is excreted exclusively in the urine ([redacted] and [redacted]; 1996; IIA 5.1.1/02). Elimination of ingested glyphosate via faeces and systemic glyphosate via urine is rapid and is nearly complete within 48 hours. The pulmonary route of elimination is negligible (< 0.2%, [redacted] 1995 and [redacted] 1996; IIA, 5.1.3/02). Faeces contain unabsorbed glyphosate.

Distribution into the organs and tissues after an oral dose is rapid but limited with generally low residues found in organs and tissues at termination. After a period of 3 to 7 days following oral administration, total body burden accounted for less than 1% of the applied radioactivity. There is no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 72h -168h. Elimination from bone is slower than from other tissues. However, the amount of radiolabel in bone after 168 h after a single oral dose was relatively low at 0.02 – 0.03% of the applied dose ([redacted] 1995). The highest residues were measured in bone, followed by kidney and liver. This pattern of absorption, distribution and elimination was not significantly changed either by single high doses administered or by repeated administration of low doses. Similarly, the sex of the test animals did not affect the results. The pattern of distribution of radioactivity in whole-body autoradiograms showed the greatest intensity of radioactivity to be in bone and gastrointestinal tract at up to 24 hours after dosing which was reduced to negligible amounts within 48 hours ([redacted] 1996 (IIA, 5.1.3/02)). Peak plasma levels were observed within 4 – 6 h and elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination of radiolabel in plasma has been suggested from the plasma radiolabel in a range of studies and terminal half lives have been estimated at 8 – 10h. Radiolabel in plasma was negligible after 24h and not detected at 168 h.

Metabolism

Metabolism of glyphosate is very limited. Most of the parent glyphosate is eliminated unchanged and a small amount, just under 0.5% of the applied dose is eliminated as aminomethylphosphonic acid (AMPA).While AMPA is known to be the major metabolite of glyphosate in plants, metabolism in mammals has been shown to be very limited, perhaps due to gastrointestinal microflora activity rather than mammalian metabolic pathways. The metabolite AMPA has been subjected to an extensive range of metabolism and toxicity studies which demonstrate that it is of lower toxicity than glyphosate acid (see Section IIA 5.8).

Studies in rabbits, goats and laying hen demonstrated a similar pattern of toxicokinetics and metabolism.

Percutaneous absorption of glyphosate is very limited and is discussed in Section 5.9.9.

Table 5.1-1: Overview of ADME studies

	Reference	Study Type (Ref. Point in dossier or Monograph)	Dosing regime	Scope of study
Studies from the 2001 evaluation	[redacted] (1992a)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single oral gavage dose of 30 or 1000 mg/kg bw [^{14}C]-glyphosate	Provides information on the balance of distribution of radiolabel in excreta and in tissues and organs over 168h.
	[redacted] (1992a)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single oral 30 mg/kg bw [^{14}C]-glyphosate after repeat dosing with unlabelled glyphosate	Provides information on the balance of distribution of radiolabel in excreta and in tissues and organs over 168h.
	[redacted] (1992a)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single intravenous 30 mg/kg bw [^{14}C]-glyphosate	Provides information on the distribution of radiolabel in excreta and tissues after administration via a non-parental route
	[redacted] (1992a)	ADME – rat Annex B-5.1 Glyphosate Monograph	Metabolism study	Metabolites in urine and faeces from all dose groups investigated by HPLC and TLC and quantitative investigation of parent glyphosate and potential metabolite AMPA.
	[redacted] (1992b)	ADME – rat Annex B-5.1 Glyphosate Monograph	Toxicokinetics in blood after a 30 mg/kg bw oral gavage dose of [^{14}C] –glyphosate and whole body autoradiography over 24h.	Provides information on the distribution of radiolabel in plasma with time and by whole body autoradiography.
	[redacted]. (1995)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single oral gavage dose of 0.2 or 200 mg/kg bw [^{14}C]-glyphosate. 168h collection of excreta	Provides information on the balance of distribution of radiolabel in excreta and in tissues and organs over 168h.
	[redacted] (1995)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single intravenous 0.2 mg/kg bw [^{14}C]-glyphosate	Provides information on the distribution of radiolabel in excreta and tissues after administration via a non-parental route. IV study also used to facilitate the estimation of oral absorption from the oral route study.
	[redacted] (1995)	ADME – rat Annex B-5.1 Glyphosate Monograph	Investigation of radiolabel in excreta, tissues and organs and investigation of urine metabolites after single high dose study group	Provides information of metabolite profiles in urine and faeces and forms part of the preceding studies by Leuschner
	[redacted] (1988)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single oral dose of 10 mg/kg bw or 1000 mg/kg bw [^{14}C]-glyphosate	Provides information on the balance of distribution of radiolabel in excreta and in tissues and organs over 168h.
	[redacted] (1988)	ADME – rat Annex B-5 Glyphosate Monograph	Single oral 10 mg/kg bw [^{14}C]-glyphosate after repeat dosing with 10 mg/kg bw unlabelled glyphosate	Provides information on balance of distribution of radiolabel in excreta and in tissues and organs over 168h.after repeat dosing
	[redacted] (1988)	ADME – rat Annex B-5.1 Glyphosate Monograph	Single intravenous 10 mg/kg bw [^{14}C]-glyphosate	Provides information on the distribution of radiolabel in excreta and tissues and organs after administration via a non-parental route. Intravenous dosing study also used to facilitate the estimation of oral absorption from the oral route study.
	[redacted] (1990):	ADME – rat Annex B-5.1 Glyphosate Monograph	Single gavage oral dose (radioactivity but dose not specified)	Previous Monograph considered the study supplementary. Provides some information on the distribution of radiolabel in excreta and tissues and organs and the distribution of parent and metabolites.
	[redacted] (1988)	ADME – rat Annex B-5.1 Glyphosate Monograph	Oral and intravenous administration of [^{14}C]-glyphosate	Metabolism study. Analysis of excreta, blood, tissues and organs for the for the presence and distribution of glyphosate and metabolites using HPLC and mass spectroscopy

	Reference	Study Type (Ref. Point in dossier or Monograph)	Dosing regime	Scope of study
Studies from the 2001 evaluation	[redacted] (1973a)	ADME – rat Annex B-5.1 Glyphosate Monograph	Dietary administration of glyphosate at concentrations of up to 100 ppm and assessment of radioactivity in excreta over days 2, 6, 10 and 14 of treatment and days 1, 3, 6 and 10 of recovery period.	Noted to be the only study of this kind to investigate metabolism after admixture in diet and considered to provide supplementary information. Previous Monograph considered dose levels to be too low.
	[redacted] (1973b)	ADME – rabbit Annex B-5.1 Glyphosate Monograph	Single gavage oral dose of [^{14}C]-glyphosate (5.7-8.8 mg/kg bw) labelled at one of 3 sites, collection of excreta over 120h	Includes investigation of metabolic fate of the metabolite AMPA
Studies not reviewed in the 2001 evaluation	[redacted] (1995)	Metabolism – rat IIA, 5.1.1/01 Excretion study (168 h post-dosing), GLP	Single oral gavage of 10 or 600 mg/kg bw [^{14}C]-glyphosate (feed provided *ad libitum* but feeding status before dosing is not stated)	Elimination of radiolabel in urine, faeces, pulmonary route and residual carcass, determined. Plasma concentrations and determination of pharmacokinetic parameters; Quantitative tissue distribution; Identification of major urinary and faecal metabolites
	[redacted] (1996)	ADME – rat IIA, 5.1.1/02 Excretion study Bile excretion study GLP	Single oral gavage to fasted rats of 1 or 100 mg/kg bw glyphosate (feed provided *ad libitum* but rats fasted pre-dosing and for 4 h post-dosing)	Elimination of radiolabel in urine, faeces, and residual carcass, determined. Plasma concentrations and determination of pharmacokinetic parameters; biliary excretion measurement; quantitative tissue distribution; Identification of major urinary and faecal metabolites
	[redacted] (1996)	Biotransformation – rat IIA, 5.1.3/03 Bile Excretion over 48 h. GLP	Single oral gavage to rats of 1000 mg/kg bw (non-fasted).	Examination of biliary elimination after a single high oral dose and extent of systemic absorption; Identification of urinary and faecal metabolites
	[redacted] (1996a, b)	ADME – rat IIA, 5.1.1/03, /04 GLP	Single oral gavage of 10 or 1000 mg/kg bw [^{14}C]-glyphosate (non-fasted).	Examination of urinary and faecal excretion, plasma concentrations as well as tissue retention in selected tissues and residual carcasses; these studies corroborate findings from other studies.
	[redacted] (1996c)	ADME – rat IIA, 5.1.3/01 GLP	Single oral dose of 10 mg/kg bw [^{14}C]-glyphosate by gavage after repeated dosing of the unlabelled test substance (non-fasted).	Examination of urinary and faecal excretion as well as residual radioactivity in blood, selected tissues and residual carcasses; this study corroborates findings from other studies obtained at the same dose level but without pre-administration of unlabelled test substance.
	[redacted] (1996d)	ADME – rat IIA, 5.1.3/02 GLP	Single oral dose of 10 mg/kg bw of [^{14}C]-glyphosate (non-fasted).	Performance of whole body autoradiogram; examination of urinary, faecal and pulmonary excretion

Table 5.1-2: Comparison of the distribution of radiolabelled glyphosate acid in excreta and tissues in critical studies in the rat

Reference	Dosing regime	Excretion (mean % of applied dose)								Overall recovery (mean % of applied dose)	
		Urine		Faeces		Residual Carcass		Bile			
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
[redacted] (1995)$	Single oral gavage (no info about fasted/non-fasted)										
	10 mg/kg bw	22.5	19.4	74.6	84.3	0.33	0.27	--	--	97.5[a]	104 [a]
	600 mg/kg bw	30.3	29.5	74.7	74.2	0.31	0.39	--	--	105	104
[redacted] (1996)$	Single oral gavage to fasted rats and fed 4h after dosing										
	1 mg/kg bw	24.9	34.9	72.6	62.4	0.75	0.98	--	--	98.3	98.8
	100 mg/kg bw	55.3	55.0	41.2	42.4	0.84	0.98	--	--	96.3	98.5
	1 mg/kg bw	27.5	24.2	55.3	61.0	4.99	3.82	0.03	0.08	94.6	96.0
[redacted] (1996)$	Single oral gavage (no info about fasted/non-fasted) 1000 mg/kg bw	20.2	16.3	39.1	30.5	--	--	0.06	0.06		
[redacted] (1996a)$	Single oral gavage (no info about fasted/non-fasted) 10 mg/kg bw	13.3	11.1	88.5	88.7	--	--	--	--	102	99.6
[redacted] (1996b)$	Single oral gavage to non-fasted rats 1000 mg/kg bw	16.9	17.8	89.5	84.6	--	--	--	--	106	102.
[redacted] (1996c)$	Single oral dose (gavage) after repeated dosing; (non-fasted) rats										
	10 mg/kg bw	10.6	10.7	86.8	90.7	--	--	--	--	97.5	102
[redacted] (1996d)$	Single oral dose (gavage), (no info about fasted/non-fasted) 10 mg/kg bw (24 h post-dosing)	22.3	11.9	55.5	83.8	--	--	--	--	77.8 [a]	95.7 [a]
	10 mg/kg bw (48 h post-dosing)	34	12.5	60.5	91.2	--	--	--	--	94.5 [a]	103.7 [a]

Reference	Dosing regime	Excretion (mean % of applied dose)								Overall recovery (mean % of applied dose)	
		Urine		Faeces		Residual Carcass		Bile			
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
[redacted] 1992a	30 mg/kg bw ^{14}C-glyphosate after repeated dosing with 30 mg/kg bw unlabelled glyphosate (Feeding status not reported)	34.3	34.4	49.6	46.7	0.96	0.83				
[redacted] 1988	10 mg/kg bw single oral dose	28.6	22.5	62.4	69.4	0.48	0.36				
	1000 mg/kg bw single oral dose	17.8	14.3	68.9	69.4	<0.4	<0.4				
	10 mg/kg bw repeated oral dose	30.9	23.1	61.0	70.9	<0.7	<0.7				
[redacted] / (1995)	0.2 mg/kg bw single oral dose	12.3	9.6	82.9	83.3	--	--				
	200 mg/kg bw single oral dose	17.1	13.2	81.8	84.4	--	--				

$ Not submitted in the 2001 EU glyphosate evaluation
[a] excretion by the pulmonary route was investigated but not considered in the overall recoveries given within this table, since the measured values in the studies were below 0.2%
Note: The results of the previously evaluated studies of [redacted] (1995), [redacted] (1990), [redacted] (1988), and [redacted] (1973 a,b) (see Table 5.1-1) are not considered in Table 5.1-2 due to reporting deficiencies.

IIA 5.1.1 Toxicokinetic studies - Single dose, oral route, in rats

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.1.1/01	[redacted]	1995	HR-001: Metabolism in the rat [redacted] Data owner: Arysta Lifescience SAS Report No.: SNY 332/951256 Date:1995-08-16 GLP: yes not published
Guideline:			Japanese MAFF, 59 NohSan, Notification No. 4200 (1985) OECD 417

	US-EPA FIFRA 85-1
Deviations:	None
Dates of experimental work:	1995-02-08 - 1995-06-15

Executive Summary

[^{14}C]-labelled N-(phosphonomethyl)glycine (glyphosate, >98% pure) was administered by gavage, as aqueous solution (with addition of $NaHCO_3$ to achieve dissolution) at 10 or 600 mg/kg/day as a single dose to rats to determine tissue distribution, as well as biliary and urinary excretion of ^{14}C and plasma ^{14}C concentrations. The proportion and ratio of metabolites has also been investigated.

The results showed that at least about a quarter (19-30%) of the administered dose is absorbed from the gut. Absorption was similar for both sexes and at both dose levels, with peak plasma ^{14}C concentrations observed around 3 to 6 h after dosing. The decline in plasma ^{14}C concentration was monophasic with a half-life of about 8 hours at 10 mg/kg/day and 5.9 h at 600 mg/kg bw/day dosing, indicating a rapid clearance. The areas under the plasma concentrations *versus* time curves (AUC_t) after the high dose level indicated a minimal 120 fold increase compared to the low dose level. There was neither a noticeable difference in excretion in males and females nor at different dose levels. Over 90% of the radioactivity was excreted with the urine and faeces within 48 h. About 74 - 84% of the administered ^{14}C are detected in faeces and 19 - 30% is excreted via urine. No significant radioactivity was detected in exhaled air (< 0.2% of dose). The distribution of radioactivity in tissues was similar for males and females after both the dose levels with maximum tissue concentrations generally decreasing after 3 to 6 h. Concentrations of radioactivity retained in the tissue 7 days post application were generally very low. Highest concentrations were detected in the gastrointestinal tract and its content, stomach and its content, kidneys and bones. Major urinary (18 - 27%) and faecal (65 - 78%) component was the parent compound. A minor component was also observed (0.1 - 1.6%) and identified as aminomethyl phosphonic acid by TLC and HPLC co-chromatography.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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
2. Radiolabelled test material:	[^{14}C] - HR001
Identification:	[^{14}C] - N-(phosphonomethyl)glycine (CAS No. 1071-83-6)
Position of radiolabel:	N-(phosphono[^{14}C]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

3. Reference substances:

Identification:	Aminomethylphosphonic acid (AMPA) (CAS No. 1066-51-9)
Description:	solid
Lot/Batch #:	09203L2
Purity:	99%
Stability of test compound:	Not reported

4. 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

5. Vehicle and/ or positive control: Water, solubility was increased by addition of sodium hydrogen carbonate

6. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	Charles River UK Ltd, Magate, Kent, UK
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), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	During acclimatisation: Individual housing in suspended, wire bottom, stainless steel cages. 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

B: 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 CO_2. 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 ^{14}C 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: Sperisorp SAX HPLC column (Hichrom, UK) and guard column, eluent A: water, eluent B: 0.75 M KH_2PO_4, pH 3.35) and HPLC system 2 (isocratic method; column: glyphosate analytical column (BioRad, USA), eluent: 0.005 M KH_2PO_4 + 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.

II. RESULTS AND DISCUSSION

A. 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 5.1-3). 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 5.1-3: Excretion balance (in mean % of applied dose) at 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**

B. CONCENTRATION OF RADIOACTIVITY IN THE PLASMA

After a single oral dose of 10 mg/kg bw ^{14}C 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 5.1-3).

After a single oral dose of 600 mg/kg bw ^{14}C 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 5.1-4). 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_t) 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 5.1-4: Kinetic parameters in plasma after single oral dose 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.

C. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

Radioactivity concentrations in tissues were very low at all times (see Table 5.1-5 and Table 5.1-6). 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 5.1-5: 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
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 5.1-6: 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	13.19	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

D. 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. The nature of the other component (<2%) was not investigated further.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.1.1/02	[redacted]	1996	[^{14}C]-Glyphosate: Absorption, distribution, metabolism and excretion following oral administration to the rat [redacted] Data owner: Nufarm Report No.: 1413/2-1011 Date: 1996-10-23 GLP: yes not published

Guideline:	Japanese MAFF, 59 NohSan, Notification No. 4200 (1995)
Deviations:	None
Dates of experimental work:	1995-12-14 - 1996-07-17

Executive Summary

[^{14}C]-labelled glyphosate was administered by gavage, as a single dose of 1 or 100 mg/kg/day to fasted rats to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C]-concentrations. The biliary excretion was measured directly via bile duct cannulation. The proportion and ratio of metabolites were also investigated.

Absorption from the gut was slow and limited. Mean maximum plasma concentrations of 0.016 µg equiv./mL (male) and 0.0362 µg equiv./mL (female) were observed between 1.5 and 12 h post-dose at a nominal dose level of 1 mg/kg bw. Following oral administration at a nominal dose level of 100 mg/kg bw maximum plasma levels were 8.909 µg equiv./mL (male) and 7.634 µg equiv./mL (female) at about 4 h following administration. A comparison of the AUC_{0-24} values shows an approximate 100-fold increase with a corresponding increase in dose, indicating that absorption is independent of the dose level. The distribution of ^{14}C-glyphosate was rapid and widespread. Maximum tissue concentrations were observed between 4 and 12 h post-dose (1 mg/kg bw) and about 6 h post-dose (100 mg/kg bw). Examination of tissue levels indicated that [^{14}C]-Glyphosate was not retained in the tissues with the exception of less than 1% in bone at 72 h post-dose.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC analysis. Only one major peak was detected in urine and faeces (>90% of total activity) which was subsequently identified as glyphosate by LC/MS/MS in representative samples. A minor component was observed in the radiochromatogramm which had a similar retention time to AMPA, however, due to the very low levels this could not be definitively identified. Within 168 h the mean total recovery was 98.31% (male) and 98.81% (female) at a nominal dose level of 1 mg/kg bw, with 72.62% (male) and 62.40% (female) being recovered in faeces and 18.44% (male) and 27.15% being detected in urine. At the high dose level (100 mg/kg bw) elimination in urine (39.42% male; 43.07% female) was quantitatively more significant than for the low dose group. Faecal elimination accounted for 41.23% (male and 42.38% (female). Negligible amounts of radioactivity were detected in the bile (<0.08 µg equiv/g) at the dose level of 1 mg/kg bw, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The increase in renal elimination following administration at 100 mg/kg bw suggest an increase in absorption. However a high interanimal variation is also observed which prevents a firm conclusion being drawn. Furthermore, the pharmacokinetic data indicates that absorption is linear which contradicts the suggestion of increased absorption. The highly ionisable nature of the test substance could be a contributory factor to the variability in the degree of absorption.

In conclusion, there was no apparent sex difference in the absorption, metabolism, distribution and excretion of ^{14}C-glyphosate following oral administration at both dose levels. glyphosate had no potential for accumulation in the body.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Radiolabelled test material:

Identification:	[^{14}C] – Glyphosate
Position of radiolabel:	N-(phosphono[^{14}C]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

3. 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

4. Vehicle and/ or positive control: Deionised water

5. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD BR)
Source:	Charles River UK Ltd, Margate, Kent, UK
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*

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

B: 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 CO_2. 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 centrifuges 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 CO_2.

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 analyzed 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 duplicats.

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: Sperisorp SAX 250 x 4.6 mm id, eluent A: water, eluent B: 0.75 M KH_2PO_4, 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.

II. RESULTS AND DISCUSSION

A. 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 5.1-7).

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 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 5.1-7: Excretion balance (in mean % of applied dose) at 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
Carcass	0.75	0.98	0.84	0.98
Total	**98.31**	**98.81**	**96.31**	**98.50**

B. 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 5.1-8), providing strong evidence that low doses of systemic glyphosate are eliminated almost exclusively in the urine.

Table 5.1-8: 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**

C. 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 5.1-9). 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 AUC_{0-24} (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 5.1-9). Mean AUC_{0-24} 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 5.1-9: 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

D. 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 5.1-10). 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 (see Table 5.1-12).. After administration of 100 mg/kg bw all tissues were exposed to radiolabelled material by 4 h post-dose (see Table 5.1-11). 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 (see Table 5.1-13).

Table 5.1-10: 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

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
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 5.1-11: 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 5.1-12: 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

Tissue	Males				Females			
	4 h	12 h	24 h	72 h	4 h	12 h	24 h	72 h
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 5.1-13: 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	-	-	-	-

E. 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 [^{14}C]-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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.1.1/03	[redacted]	1996a	Glyphosate acid: Excretion and tissue retention of a single oral dose (10 mg/kg) in the rat [redacted] Data owner: Syngenta Report No.: CTL/4940 Date: 1996-04-26 GLP: yes not published

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

Deviations: None

Dates of experimental work: 1995-10-16 - 1996-02-04

Executive Summary

A single dose of 10 mg/kg bw of [^{14}C]-labelled glyphosate acid was administered by oral gavage to rats (5 per sex) to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most administered dose was eliminated, principally in the faeces, within 24 h. Males excreted means of 13.3% and 88.5% of the dose in urine and faeces respectively over 72h. Females excreted means of 11.1% and 88.7% of the dose in urine and faeces respectively over the same period. At the end of the observation period (72 h) a low percentage of radioactivity was present in all tissues examined, with highest concentrations found in the bone and intestinal tract plus contens.

In conclusion, after oral application glyphosate acid was excreted rapidly and extensively, predominantly in faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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

3. Vehicle and/ or positive control: Deionised water

4. Test animals:

Species:	Rat
Strain:	Alpk:AP_fSD
Source:	Biological Services Section, Zeneca Pharmaceuticals, Alderly Park
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), *ad libitum*
Water:	Tap water, *ad libitum*
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

B: 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 [^{14}C] 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 CO_2. 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 than 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 [^{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 [^{14}C]-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 [^{135}Ba] as the external source.

II. RESULTS AND DISCUSSION

A. EXCRETION AND RETENTION OF RADIOACTIVITY

After a sinlge 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 5.1-14). There were no differences in the cumulative excretion patterns between the sexes.

Table 5.1-14: 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**

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

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 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 5.1-15). 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 5.1-15: 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.1.1/04	[redacted]	1996b	Glyphosate acid: Excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat [redacted] Data owner: Syngenta Report No.: CTL/4942 Date: 1996-06-19 GLP: yes not published

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

Deviations: None

Dates of experimental work: 1995-10-16 - 1996-05-10

Executive Summary

[^{14}C] -labelled glyphosate acid was administered by gavage, as a single dose of 1000 mg/kg/day to non-fasted rats (5 per sex) to determine tissue distribution, excretion of [^{14}C] and plasma [^{14}C] concentrations 72 h post administration.

Excretion of radioactivity was rapid for rats of both sexes and most administered dose was eliminated, principally in faeces, within 24 h. Males excreted means of 16.7% and 89.6% of the dose in urine and faeces respectively over 72h. Females excreted means of 17.5% and 84.5% of the dose in urine and faeces respectively over the same period. The rates of excretion were thus similar for both sexes. Upon termination of the experiment (72 h) the mean total percentage of administered radioactivity present in all tissues examined and the residual carcass was 0.5% for males and 0.6% for females.

In conclusion, there was no apparent sex difference in distribution and excretion of [^{14}C]-glyphosate acid following oral administration at 1000 mg/kg bw. glyphosate acid was excreted rapidly and extensively, principally in the faeces.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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:	1.580GBq/mMol

Stability of test compound:	Stable throughout the experiment
3. Vehicle and/ or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP_fSD
Source:	Biological Services Section, Zeneca Pharmaceuticals, Alderly Park
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

B: 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 CO_2. 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 than

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.

II. RESULTS AND DISCUSSION

A. 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 (see Table 5.1-16).

Table 5.1-16: 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**

B. DISTRIBUTION OF RADIOACTIVITY IN TISSUE

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 5.1-17).

Table 5.1-17: 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

III. CONCLUSION

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.

IIA 5.1.2 Toxicokinetic studies – Second single dose, oral route, in rats

EU-subpoint does not exist.

IIA 5.1.3 Toxicokinetic studies – Repeated dose, oral route, in rats

Annex point	Author(s)	Year	Study title
IIA, 5.1.3/01	[redacted]	1996c	Glyphosate acid: Excretion and Tissue Retention of a Single Oral Dose (10 mg/kg) in the Rat Following Repeat Dosing [redacted] Data owner: Syngenta Report No.: CTL/P/4944 Date: 1996-05-22 GLP: yes not published
Guideline:			OECD 417 (1984)

Deviations:	None
Dates of experimental work:	1995-10-16 - 1996-03-26

Executive Summary
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 rats/sex were given a single oral dose (10 mg/kg) of [^{14}C]-phosphonomethyl glyphosate acid. After monitoring the excretion of radioactivity in urine and faeces for 72 hours after dosing, the rats were killed and the residual radioactivity was measured in blood, selected tissues and in the residual carcasses.
The results showed that excretion of radioactivity was rapid for rats of both sexes and most of the administered dose was eliminated, principally in faeces, within 24 hours. Over 72 hours, males and females excreted means of 86.6% and 90.7% of the dose in faeces, respectively.
Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (see IIA 5.1.1/03) showed no significant differences on either the routes or rates of elimination. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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
2. 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.
3. Vehicle and/ or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	Biological Service Section, Zeneca Pharmaceuticals, Alderley Park, England (UK)
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), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	After administration of the 14th 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

B: 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 ($60F_{254}$) 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/µg equivalents glyphosate acid/g of tissue (µg 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.

II. RESULTS AND DISCUSSION

A. 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 (see also Table 5.1-18)

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 5.1-18: Excretion of radioactivity in urine and faeces in male and female rats

	Excretion of radioactivity [%]			
	Males			
Time after dosing (hours)	**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
	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

B. 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 5.1-19)

Table 5.1-19: 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
	Females			
Tissue	% 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

C. 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.

III. CONCLUSION

Comparison of the results with those obtained at the same dose level but without pre-administration of unlabelled test substance (see IIA 5.1.1/03) 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.

Annex point	Author(s)	Year	Study title
IIA, 5.1.3/02	[redacted]	1996d	Glyphosate acid: Whole body autoradiography in the rat (10 mg/kg) [redacted] Data owner: Syngenta Report No.: CTL/P/4943 Date: 1996-06-10 GLP: yes not published

Guideline: OECD 417 (1984)

Deviations: None

Dates of experimental work: 1995-10-25 - 1996-04-04

Executive Summary

Two male and two female rats were administered a single oral dose of 10 mg [^{14}C]-phosphonomethyl labelled glyphosate acid/kg. One male and one female was terminated 24 hours after dosing and the other pair were terminated 48 hours after dosing, qualitative whole body autoradiogram was performed on all animals. In addition, radioactivity was measured in urine, faeces and exhaled air.

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 whole body autoradiograms showed no marked differences in the tissue 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.

In conclusion, glyphosate acid was excreted rapidly and predominantly in the faeces. 48 hours after dosing the greatest intensity of radiolabelling was found in the bone and intestinal tract and contents.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Radiolabelled test material: [^{14}C]-phosphonomethyl-labelled glyphosate acid

Identification:	[^{14}C]-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
3. Vehicle and/ or positive control:	Deionised water
4. Test animals:	
Species:	Rat
Strain:	Alpk:AP_fSD
Source:	Biological Service Section, Zeneca Pharmaceuticals, Alderley Park, England (UK)
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

B: 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 10 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 ($60F_{254}$) 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.

II. RESULTS AND DISCUSSION

A. 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 5.1-20.

Table 5.1-20: Excretion of radioactivity in urine, faeces, cage wash and expired air by male and female rats

	Time After Dosing (hours)	Males		Females	
		Rat 1 (% of applied dose)	Rat 2 (% of applied dose)	Rat 3 (% of applied dose)	Rat 4 (% 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
Exhaled $^{14}CO_2$	**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

B. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.1.3/03	[redacted]	1996	Glyphosate acid: Biotransformation in the rat [redacted] Data owner: Syngenta Report No.: CTL/P/5058 Date: 1996-06-28 GLP: yes not published

Guideline:	OECD 417 (1984)
Deviations:	None
Dates of experimental work:	1995-11-26 - 1996 May

Executive Summary

To examine the role of biliary elimination a single oral administration of 1000 mg [^{14}C]-glyphosate acid/kg bw was administered to bile duct cannulated rats. 48 hours after dosing the two male and two female rats were sacrificed.

To investigate additionally the biotransformation of glyphosate acid, samples of former studies (see IIA 5.1.1/03, IIA 5.1.1/04, and IIA 5.1.3/01) were analysed by chromatography and NMR for metabolites.
The results showed that following a single oral dose of [^{14}C]-glyphosate acid the excretion of radioactivity in bile is negligible, thus indicating that radioactivity recovered in faeces is most likely unabsorbed material. The radioactivity present in urine and faeces from rats given [^{14}C]-glyphosate acid at low or high dose levels or after repeated dosing was characterised as being predominantly glyphosate acid. Trace amounts of aminomethyl phosphonic acid (AMPA) were detected in urine samples.
In conclusion, following an oral dose of glyphosate acid to male and female 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 acid and trace amounts of AMPA.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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.

3. Reference substance:

Identification:	Aminomethylphosphonic acid (AMPA)
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported
Stability of test compound:	Not reported

4. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD
Source:	Biological Service Section, Zeneca Pharmaceuticals, Alderley Park, England (UK)
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), *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: 1995-11-26 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 the excretion and tissue distribution studies described in sections IIA 5.1.1/03, IIA 5.1.1/04, and IIA 5.1.3/01 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 ^{1}H-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 ($60F_{254}$) 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 rat of 0.5 mL/min.

Radioactivity was detected in both methods by liquid cell.

Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H-NMR)

Proton and phosphorus NMR spectra were acquired using a Brucker 400MHz instrument. Samples of glyphosate acid and AMPA were dissolved in D_2O 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.

II. RESULTS AND DISCUSSION

A. 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 5.1-21).

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

Table 5.1-21: Excretion of radioactivity in urine, faeces and bile by male and female bile duct cannuled rats given a single oral dose of 1000 mg [^{14}C]-glyphosate acid/kg bw (mean of two rats expressed as % of applied dose)

Time after dosing (hours)	Males rats % of applied dose			Female rats % 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	**20.185**	**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

B. 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 (see IIA 5.1.1/03, IIA 5.1.1/04, and IIA 5.1.3/01) 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 5.1-22. For glyphosate acid and AMPA the values range from 63.3-95.3% and 0.07-0.66%, respectively.

Table 5.1-22 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

III. CONCLUSION

Following an oral dose of glyphosate acid to rats approximately 10-20% of the dose was absorbed (see IIA 5.1.1/03, IIA 5.1.1/04, and IIA 5.1.3/01). 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).

IIA 5.2 Acute toxicity

The 2001 EU evaluation of glyphosate concluded that glyphosate acid and its salts exhibit a low acute toxicity in laboratory animals by the oral and dermal route with LD_{50} values greater than 2000 mg/kg bw in previously conducted studies. These results were confirmed in other and new studies recently performed by [redacted] 2009a/ IIA 5.2.1/01 (LD_{50}, oral, rat ♀ > 5000 mg/kg bw/day), [redacted] 1995a/ IIA 5.2.1/02 (LD_{50}, oral, rat ♀/♂> 5000 mg/kg bw/day) and [redacted] 1995b/ IIA 5.2.1/03 (LD_{50}, oral, mice ♀/♂> 5000 mg/kg bw/day). Given all LD_{50} values exceed the highest dose tested and differences between the EU and GHS classification criteria[1], the acute oral and dermal toxicity endpoints should be amended to greater than 5000 mg/kg bw/day. General signs of oral intoxication were breathing difficulties, reduced activity, diarrhoea, ataxia and convulsions.

The acute inhalation toxicity was also low with LC_{50} values above the limit test dose of 5 mg/L air per 4 hours obtained for the acid and the isopropylammonium salt (IPA). Achieving inhalation doses up to the limit dose of 5 mg/L were often a technical challenge, based on the physic-chemical properties (vapour pressure = 1.31 $x10^{-5}$ Pa) of the test materials. Toxic symptoms after inhalation exposure included slight dyspnoea, increased or decreased respiratory rate, slight tremor and a slight decrease in body weight but were not consistently observed throughout the studies. The low toxicity previously observed for the acid are supported by the study of [redacted] (2010b, IIA, 5.2.3), which demonstrated an LC_{50} of > 5.18 mg/L for the rat.

Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. Studies conducted since the last EU review have confirmed that glyphosate acid is either non-irritating or only slightly irritating to the skin and that no classification is required (IIA 5.2.4/01 [redacted] 2007a, IIA 5.2.4/02 [redacted] 2009a).

During the 2001 EU glyphosate review, glyphosate acid was found to be strongly irritating to rabbit eyes requiring classification; previously as R41 – **'Risk of serious damage to eyes'** and now **'Irreversible effects on the eye/serious damage to eyes (Category 1)'** under GHS. The majority of recently performed studies on the eye irritating potential of glyphosate acid supported the previous findings and classification (IIA 5.2.5/01 [redacted] 2007b, IIA 5.2.5/02 [redacted] 2009b). There was markedly less eye irritation observed with the salts which are used in formulated products, presumably due to the salts having a more neutral pH than glyphosate acid.

Neither glyphosate acid nor the salts have shown sensitizing effects in guinea pigs. However, only the acid and the IPA salt have been tested under the more stringent conditions of the Magnusson-Kligman test. The 2001 EU glyphosate evaluation on skin sensitizing effects is supported by two previously conducted Magnussion-Kligman tests which both demonstrated no sensitizing effects of glyphosate acid in the guinea pig (IIA 5.2.6/01 [redacted] 2007c, IIA 5.2.6/02 [redacted] 2010b).

IIA 5.2.1 Acute oral toxicity

The low acute toxicity of glyphosate demonstrated in the 2001 EU evaluation was confirmed by additional studies not evaluated during the 2001 review. [redacted] (2009, IIA 5.2.1/01) and [redacted] (1995/ IIA 5.2.1/02) found and LD_{50}, for rat and mice of > 5000 mg/kg bw/day. Also for glyphosatesalts (as illustrated by glyphosate IPA and glyphosate ammonium salt), low toxicity was observed (see Table 5.2-2).

[1] According to EU classification criteria, substances with oral or dermal LD_{50} values above 2000 mg/kg bw/day need not to be classified. According to GHS substances with oral and dermal LD_{50} values greater than 5000 mg/kg bw/day need not to be classified. However, it has to be noted that the GHS category 5 (i.e. for substances with LD_{50} values > 2000 but ≤ 5000 mg/kg bw/day) are not compulsory in the EU.

Table 5.2-1: 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] 1995a (Sanachem)	Rat, Sprague Dawley	5/sex/2000	97.6	Cotton seed oil	> 2000 (limit test)	Slightly congested lungs, spleno-megaly, Liver: centri-lobular congestion
	Annex B-5.2.1.1.1, Glyphosate Monograph [redacted] 1994 (Herbex)	Rat, Sprague Dawley	1/sex/2000[##] 5/sex/2000	95	Arachis oil	> 2000 (limit test)	No findings
	Annex B-5.2.1.1.1, Glyphosate Monograph Tornai, 1994b (Alkaloida)	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] 1994 (SIN)	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] 1992a (Barclay)	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] 1991a (FSG)	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] 1990a (AGC)	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] 1989a (CHE)	Rat, Sprague Dawley	5/sex/5000	98.6	0.5 % CMC	> 5000 (limit test)	Piloerection, reduced activity, ataxia (♂ only)

	Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/01 [redacted] 2009a (HAG)	Rat, Sprague Dawley	5/sex/5000	96.4	water	> 5000 (limit test)	decreased activity, diarrhoea, piloerection, polyuria, salivation
	IIA 5.2.1/02 [redacted] 1995a (ALS)	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] 1995b	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] 2009a (EXC)	Rat, Wistar	3 females/2000 (step 1) 3 females/2000 (step 2)	96.66	water	> 2000	No findings
	IIA 5.2.1/05 [redacted] 2009a (HAG)	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
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/06 [redacted] 2010c (HAG)	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] 2010d (HAG)	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] 2005a (HAG)	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] 2008a (HAG)	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] 2007d (NUF)	Rat, HanRcc: WIST	2 x 3 ♀/2000	95.1	PEG 300	> 2000 (limit test)	Slightly ruffled fur

	Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.2.1/11 [redacted] 1988a (MON)	Rat, Sprague Dawley	5/sex/5000	97.76	water	> 5000	diarrhea, apparent urinary incontinence and hair loss on the abdomen
	IIA 5.2.1/12 [redacted] 1979a (MON)	Rat, Wistar	5/sex/2500 5/sex/3500 5/sex/5000 5/sex/7000 5/sex/9900	99	water	> 5000	Mortalities: 1/10 1/10, 3/10,7/10, 10/10 at 2500, 3500, 5000, 7000 and 9900 mg/kg bw; clinical signs: ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and fecal staining of the abdomen
	IIA 5.2.1/13 [redacted] 1996a (SYN)	Rat	5/sex/5000	95.6	water	> 5000	No findings
	IIA 5.2.1/14 [redacted] 2007a (SYN)	Rat	3 ♀/5000	96.1	Water	> 5000	Ruffled fur, hunched posture
	IIA 5.2.1/15 [redacted] 2011a (SYN)	Rat	3 ♀/5000	96.3	0.5 % CMC	>5000	No findings

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-5.2.1.1.2, Glyphosate Monograph [redacted] 1991b (FSG)	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] 1994 (I.Pi. Ci)	Mice, Charles River	5/sex/2000	technical	0.5 % CMC	> 2000 (limit test)	Piloerection, hunched posture, hypoactivity
	Annex B-5.2.1.1.2, Glyphosate Monograph [redacted] 1991 (CHE)	Mice, Bom:NMRI	5/sex/2000	98.6	water	> 2000 (limit test)	Piloerection, sedation

CMC = carboxymethylcellulose

Table 5.2-2: 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] 1995b (Sanachem)	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-5.2.1.2.1, Glyphosate Monograph [redacted] 1994a (MON / CHE)	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)	Rat, Wistar	5/sex/2000	IPA 62% in water	none	> 2000 (limit test)	No findings

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] 1981a (MON / CHE)	Rat, Sprague-Dawley	5/sex/5000	IPA 65	none	> 5000 (limit test)	Pale colored kidneys and hydronephrosis in few ♀
	Annex B-5.2.1.2.2, Glyphosate Monograph [redacted] 1987a (SIN)	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] 1987b (SIN)	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] 1985 (MON)	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)	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)	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.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/01	[redacted]	2009a	Glyphosate: Acute Oral Toxicity Study (UDP) In Rats. [redacted] Data owner: Helm AG Report No.: 12170-08 Date: 2009-03-11 GLP: yes not published

Guideline:	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
Dates of experimental work:	2008-11-11 - 2008-11-27

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included activity decrease, diarrhoea, piloerection, polyuria and salivation, which were no longer evident by Day 8. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be

LD_{50}, oral, female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley
Source:	Texas Animal Specialties, Humble, TX, US
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

B: 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 euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

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

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/02	[redacted]	1995a	HR-001: Acute Oral Toxicity Study In Rats. [redacted] Data owner: Arysta Life Sciences Report No.: IET 94-0134 Date: 1995-02-20 GLP: yes not published

Guideline:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:	None
Dates of experimental work:	1995-01-24 - 1995-02-07

Executive Summary

The test substance, HR-001, was evaluated for its acute oral toxicity potential in Sprague Dawley rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity and salivation 1 and 3 hours after administration. No body weight losses were recorded on Day 7 and 14 after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rats > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance HR-001 is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: 0.5% carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crj:CD), SPF
Source:	Tsukuba Breeding Center, Charles River Japan, Inc.
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), *ad libitum* except for an overnight fast before dosing and about 3 h after dosing
Water:	Tap water, *ad libitum*
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. 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.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/03	[redacted]	1995b	HR-001: Acute Oral Toxicity Study In Mice. [redacted] Data owner: Arysta Life Sciences Report No.: IET 94-0133 Date: 1995-02-20 GLP: yes not published

Guideline:	OECD 401 (1987), JMAFF 59 NohSan 4200 (1995), US EPA (1984)
Deviations:	None
Dates of experimental work:	1995-01-24 - 1995-02-07

Executive Summary
The test substance, HR-001, was evaluated for its acute oral toxicity potential in ICR mice when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs included decreased spontaneous motor activity, sedation and crouching position 1 and 3 hours after administration. There were no body weight losses at 14 days after administration when compared with the body weights on the day of administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was calculated to be

LD_{50}, oral, mice > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance HR-001 is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/or positive control: 0.5% carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Mice
Strain:	ICR (Crj:CD-1), SPF
Source:	Atsugi Breeding Center, Charles River Japan, Inc.
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. 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.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, HR-001 is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/04	[redacted]	2009a	Glyphosate Technical: Acute Oral Toxicity Study in Rats. [redacted] Data owner: Excel Report No.: C22864 Date: 2009-04-02 GLP: yes not published

Guideline:	OECD 423 (2001) Commission Regulation (EC) No 440/2008 (2008), method B.1 tris
Deviations:	None
Dates of experimental work:	2009-01-13 - 2009-02-05

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in female HanRcc: WIST (SPF) rats when administered as a gavage dose at a level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. There was no effect on body weight gain. No macroscopic findings were recorded at necropsy. The acute oral LD_{50} was calculated to be

LD_{50}, oral, female rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	Harlan Laboratories Ltd., Laboratory Animal Services, Fuellinsdorf, Switzerland
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 rior 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 Muttenz / Switzerland).
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 30 - 70% Air changes: 10 - 15/hour 12-hour light/dark cycle

B: 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 euthanized by CO_2 asphyxiation. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the course of the study.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age.

D. NECROPSY
No macroscopic findings were recorded at necropsy.

III. CONCLUSION

The oral LD_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/05	[redacted]	2009a	Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 23910 Date: 2009-06-16 GLP: yes not published

Guideline: 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.

Dates of experimental work: 2009-02-04 - 2009-03-04

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	2010-08-01

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

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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), *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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/06	[redacted]	2010c	Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 24874 Date: 2010-01-06 GLP: yes not published

Guideline: 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.

Dates of experimental work: 2010-10-15 - 2010-11-10

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	2009051501
Purity:	96.4%
Stability of test compound:	2011-05-15

2. Vehicle and/ or positive control: 0.8% aqueous hydroxypropylmethylcellulose

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/07	[redacted]	2010d	Acute Oral Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 24602 Date: 2010-02-19 GLP: yes not published

Guideline: 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.

Dates of experimental work: 2009-10-26 - 2009-11-24

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 2000 mg/kg bw. The Acute Toxic Class Method (ATC method) was employed to establish the required information for hazard assessment and hazard classification. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20090506
Purity:	97.3%
Stability of test compound:	May 2011

2. Vehicle and/ or positive control: 0.8% aqueous hydroxypropylmethylcellulose

3. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/08	[redacted]	2005a	Glyphosate Acid Technical – Acute Oral Toxicity Up and Down Procedure in Rats. [redacted] Report No.: 15274 Date: 2005-04-04 GLP: yes not published

Guideline: US EPA OPPTS 870.1100 (2002)
OECD 425 (2001).

Deviations: There were no deviations from the study plan.

Dates of experimental work: 2004-05-04 - 2004-05-20

Executive Summary

The test substance, Glyphosate Acid Technial, was evaluated for its acute oral toxicity potential in female albino rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality occurred during the study. Clinical signs 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. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50}, oral, female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Acid Technical is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley derived
Source:	Ace Animals, Inc., Boyertown, PA, US
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

B: 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 euthanized by an overdose of CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate Acid Technical is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/09	[redacted]	2008a	Acute Oral Toxicity Study in Wistar Hannover Rats for Glyphosate Technical. [redacted] Report No.: RF-3996.305.475.07 Date: 2008-09-16 GLP: yes not published

Guideline: OECD guideline 423 (2001).

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

Dates of experimental work: 2007-09-12 - 2008-06-11

Executive Summary

The test substance, Glyphosate Technical, was tested for acute oral toxicity in female albino rats using a stepwise procedure. The test item was administered orally at single dose levels of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD_{50} was determined to be

LD_{50} cut-off value, oral, female rat = 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. **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.
2. **Vehicle and/or positive control:** Deionised water
3. **Test animals:**
   - Species: Rat albino
   - Strain: Wistar Hannover
   - Source: Bioagri Laboratórios, DF, Brazil
   - 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.), *ad libitum* except for fasting overnight before dosing
Water:	Filtered drinking water, *ad libitum*
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

B: 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 CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed in females treated with 2000 mg/kg bw.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The acute oral LD_{50} 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 Harmonized System (GHS) classification criteria Glyphosate Technical is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/10	[redacted]	2007a	GLYPHOSATE TECHNICAL (NUP05068) : Acute oral toxicity study in rats [redacted] Data owner: Nufarm Report No.: BO2272 Date: 2007-03-01 GLP: yes unpublished

Guideline: Japanese guideline Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Preparation of Study Results, Acute oral toxicity studies. Guideline 2"1-1 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 2004173/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

Dates of experimental work: 2006-12-12 to 2007-01-04

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in female albino rats when administered as a 2000 mg/kg body weight gavage dose. No mortality ccurred during the study. The only clinical sign observed was slightly ruffled fur. There was no effect on body weight gain and no macroscopic findings were recorded at necropsy.

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) > 2000 mg/kg body weight

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical (NUP 05068) does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions.

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

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	RCC Ltd, Laboratory Animal Services CH-4414 FOliinsdorf I Switzerland
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 MuttenzlSwitzerland).
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 30 - 70% Air changes: 10 - 15/hour 12-hour light/dark cycle

B: 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 mLlkg 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 acclimatization 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

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

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

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 Harmonized System (GHS) classification criteria, Glyphosate Technical (NUP 05068) is not to be classified for this endpoint.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/11	[redacted]	1988a	Acute Oral Toxicity Study of Glyphosate Batch/Lot/NBR No. XLI-55 in Sprague-Dawley Rats [redacted] Data owner: Monsanto Monsanto Report No.: FD-88-29 Date: 1988-06-08 GLP: yes not published

Guideline:	US EPA 81-1
Deviations:	None
Dates of experimental work:	1988-04-05 to 1988-04-19

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Sprague-Dawley rats when administered as a gavage dose at level of 5000 mg/kg body weight. No mortalities occurred. Clinical signs included diarrhoea, apparent urinary incontinence, and hair loss on the abdomen. Body weight gain was noted for all animals. No internal abnormalities were noted during gross necropsy examination of the animals. The acute oral LD_{50} was estimated to be

LD_{50}, oral, rat > 5000 mg/kg body weight

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	Charles River Breeding Laboratories, Inc., Wilmington, MA, US
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 – 23.9ºC
	Humidity: 40 – 70%
	Air changes: Not specified
	Light cycle: 12 hour light/dark cycle

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortalities occurred.

B. CLINICAL OBSERVATIONS

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

C. BODY WEIGHT

Body weight gain was noted for all animals.

D. NECROPSY

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

III. CONCLUSION

The oral LD_{50} of the test material (glyphosate) in rats was estimated to be 5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate should not be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/12	[redacted]	1979a	Acute Oral Toxicity Study In Rats. [redacted] Data owner: Monsanto Monsanto Report No.: BND-77-428 Date: 1979-08-06 GLP: no (pre-GLP) not published
Guideline:			None (pre-guideline)
Dates of experimental work:			Not specified

Executive Summary

The test substance, glyphosate, was evaluated for its acute oral toxicity potential in male and female Wistar strain albino rats when administered as a gavage dose at levels of 2.5, 3.5, 5.0, 7.0, and 9.9 g/kg body weight (n=5 per sex per dose level). Mortalities for the dose at levels of 2.5, 3.5, 5.0, 7.0, and 9.9 g/kg were 1/10, 1/10, 3/10, 8/10, and 10/10, respectively. 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. 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. The gross necropsy conducted at termination demonstrated discoloured lungs, liver and/or kidneys for the 2.5 g/kg body weight group, discoloured lungs, liver and/or kidneys or air filled intestines for the 3.5 g/kg body weight group, no findings for the 5.0 g/kg body weight group, and discoloured lungs, liver and/or kidneys, and air filled intestines for the 7.0 g/kg body weight group. No 9.9 g/kg body weight animals survived to necropsy. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rat 5.6 g/kg body weight, with 95% confidence limits of 4.9 to 6.3 g/kg body weight

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Marland Breeding Farms, Inc., Hewitt, NJ, US
Age:	Not specified
Sex:	Male and female
Weight at dosing:	225 - 294 g
Acclimation period:	Not specified
Diet/Food:	*ad libitum* (except when fasted for approximately 18 hours prior to dosing)
Water:	*ad libitum*
Housing:	Individually
Environmental conditions:	Temperature: Not specified
	Humidity: Not specified
	Air changes: Not specified
	Light cycle: Not specified

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortalities in the study are as indicated below in Table 5.2-3.

Table 5.2-3 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

B. 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.

C. 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.

D. 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 5.2-4.

Table 5.2-4 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

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, glyphosate is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/13	[redacted]	1996a	Glyphosate Acid: Acute Oral Toxicity Study In Rats. [redacted] Data owner: Syngenta Report No.: CTL/P/4660 Date: 1996-08-23 GLP: yes not published

Guideline: OECD 425 (2001)
US EPA OPPTS 870.1100 (2002)

Deviations: None

Dates of experimental work: 1995-01-10 – 1996-08-23

Executive Summary

In an acute oral toxicity study, a group of five male and five female, fasted, young adult Alpk:AP$_f$SD (Wistar-derived) rats were given a single oral dose of 5000 mg/kg bw of glyphosate acid in deionised water and observed for 15 days.

None of the animals died. There were no signs of systemic toxicity and no treatment-related findings at examination *post mortem*. All animals gained weight during the study.

LD_{50}, oral, male and female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate acid is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Alpk:AP$_f$SD (Wistar-derived)
Source:	Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
There were no signs of systemic toxicity.

C. 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.

D. 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.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, glyphosate acid is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/14	[redacted]	2007a	Glyphosate technical material: Acute oral toxicity study in the rat (up and down procedure). [redacted] Data owner: Syngenta Report No.: B02755 Date: 2007-02-08 GLP: yes not published

Guideline:	OECD 425 (2001) US EPA OPPTS 870.1100 (2002) Japanese MAFF 12 NohSan No. 8147
Deviations:	None
Dates of experimental work:	2006-11-29 – 2007-02-08

Executive Summary

In an acute oral toxicity study (limit test), 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) in purified water at a concentration of 5000 mg/kg body weight and administered at a dosing volume of 10 mL/kg.

The animals were examined daily during the acclimatization 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 necropsied and examined macroscopically.

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.

All animals survived until the end of the study period. Slightly 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. The body weight of the animals was within the range commonly recorded for this strain and age. No macroscopic findings were recorded at the scheduled necropsy.

LD_{50}, oral, female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate technical material is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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).

2. 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).

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	RCC Ltd, Laboratory Animal Services, CH-4414 Füllinsdorf, Switzerland
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

B: 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 acclimatization 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. 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.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age.

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate technical material is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/15	[redacted]	2011a	Glyphosate technical: Acute oral toxicity study in the rat (up and down procedure) [redacted] Data owner: Syngenta Report No.: 10/218-001P Date: 2011-04-15 GLP: yes not published

Guideline:	OECD 425 (2008) OPPTS 870.1100 (2002)
Deviations:	None
Dates of experimental work:	2010-12-09 – 2011-04-15

Executive Summary
Two limit tests with different dose levels were performed. As there were no clinical signs or macroscopic findings observed at the dose level of 2000 mg/kg a limit test at a higher dose level (5000 mg/kg bw) was requested by the Sponsor, data from the animals treated at 2000 mg/kg bw were archived in the raw data without any further reporting.
In an acute oral toxicity study (limit test), 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) in 0.5% carboxymethylcellulose (CMC) at a concentration of 5000 mg/kg body weight and administered at a dosing volume of 10 mL/kg.
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 necropsied and examined macroscopically.
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.
No deaths occurred during the study. No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw. There were no treatment related changes in the body weights. The body weights of the animals were within the range commonly recorded for this strain and age. No test item-related macroscopic findings were observed.

LD_{50}, oral, female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate technical is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: 0.5% Carboxymethylcellulose (CMC)

3. Test animals:

Species:	Rat
Strain:	RjHan:WI
Source:	Laboratoire Elevage Janvier, B.P. 4105, Route des Chênes Secs, 53940 Le Genest-St-Isle, France
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

B: 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 bodyweight 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities.

B. CLINICAL OBSERVATIONS
No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

C. BODY WEIGHT
The body weight of the animals was within the range commonly recorded for this strain and age.

D. NECROPSY
No macroscopic findings were recorded at the scheduled necropsy.

III. CONCLUSION

The oral LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate technical is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/16	[redacted]	1995	Acute Toxicity Study of MON 0139 by Oral Administration in Mice. [redacted] Data owner: Monsanto Monsanto Report No.: XX-95-205 Date: 1995-10-05 GLP: yes not published

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

Deviations: None

Dates of experimental work: 1995-08-08 - 1995-10-05

Executive Summary

The test substance, MON 0139, was evaluated for its acute oral toxicity potential in male and female Crj:CD-1(ICR) strain SPF mice when administered as a gavage dose at 5000 mg/kg body weight. No mortality occurred during the study. No clinical signs of toxicity were observed. There was a slight tendency toward retardation of body weight gain as compared with a control group for males from 7 days after administration. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute oral LD50 was calculated to be

LD_{50}, oral, mouse > 5000 g/kg body weight

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance MON 0139 is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Water for injection

3. Test animals:

Species:	Mouse
Strain:	Crj:CD-1(ICR)
Source:	Charles River Japan Inc.
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, sterilized by radiation (Oriental Yeast Co., Ltd.), *ad libitum* except during fasting prior to dosing	
Water:	Tap water; *ad libitum*	
Housing:	Plastic cages with wood chip bedding in groups of 5 (groups of 5 or 6 during quarantine/acclimation)	
Environmental conditions:	Temperature:	23 ± 3ºC
	Humidity:	50 ± 20%
	Air changes:	11 – 13 per hour
	Light cycle:	12 hour illumination per day

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs of toxicity were observed.

C. 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 5.2-5). In females, no compound-related changes were observed in the 5000 mg/kg body weight group.

Table 5.2-5 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

D. NECROPSY

No abnormalities were observed.

III. CONCLUSION

The oral LD_{50} of the test material (MON 0139) in mice was >5000 mg/kg body weight. Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, MON 0139 is not to be classified for acute oral toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.1/17	Moore, G.E.	1999	NUP5a99 62% glyphosate MUP: Acute oral toxicity study in rats – Limit test Product safety Labs, New Jersey, USA Study No.: 7907 Date: 1999-09-16 GLP: yes unpublished
Guideline:			US EPA Health Effects Test Guidelines, OPPTS 870.1100 (1998)
Deviations:			None
Dates of experimental work:			1999-08-03 – 1999-08-17

Executive Summary

NUP5a99 62% glyphosate MUP was administered to ten healthy rats by oral gavage at the dose of 5000 mg/kg bw. All animals survived and gained weight during the study. Following administration, most females exhibited anogenital staining and two females exhibited soft feces or diarrhea, but recovered by Day 2 and appeared active and healthy for the remainder of the study. Gross necropsy fmdings at terminal sacrifice were unremarkable.

Based on the results of this study, the single dose acute oral LD50 ofthe test substance is > 5000 mg/kg of body weight.

LD_{50}, oral, male and female rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance NUP5a99 62% glyphosate MOP does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	Ace Animals, me., Boyertown, PA
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS

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

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

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 Harmonized System (GHS) classification criteria, NUP5a99 62% glyphosate MOP is not to be classified for this endpoint.

IIA 5.2.2 Acute percutaneous toxicity

In the 2001 EU glyphosate evaluation glyphosate and its salts were found to to have a low acute dermal toxicity. As a result, the LD50 endpoints for rat and rabbit were set >5000 mg/kg bw for glyphosate and glyphosate salts Since then several new acute dermal toxicity studies have been conducted (see Table 5.2-6). There were no mortalities observed in the new studies at doses up to and including 5000 mg/kg bw. Therefore, the more recent studies support a refined endpoint of > 5000 mg/kg bw for acute dermal toxicity . According to EU and GHS classification criteria glyphosate does not need to be classified for acute dermal toxicity.

Table 5.2-6: 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
Studies from the 2001 evaluation	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted], 1995a (Sanachem)	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)	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] 1994a (Herbex)	Rat, Sprague Dawley	5/sex/2000	95	none	> 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
Studies from the 2001 evaluation	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1994c (ALkaloida)	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)	Rat, Sprague-Dawley	5/sex/2000	> 97	none	> 2000 (limit test)	No findings
	Annex B-5.2.2.1.1, Glyphosate Monograph [redacted] 1991c (FSG)	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] 1990b (AGC)	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 Cuthbert & [redacted] 1989b (CHE)	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] 2009b (HAG)	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] 1995c (ALS)	Rat, SD	5/sex/2000	95.68	water	> 2000 (limit test)	No findings
	IIA 5.2.2/04 [redacted] 2009b (EXC)	Rat, HanRcc: WIST	5/sex/2000	96.66	water	> 2000	No mortalities, no signs of systemic toxicity; in 4 females slight local signs (erythema, scaling and scabs) at the application sites

	Reference (Data owner)	Species Strain	Number of animals / Dose levels (mg/kg bw)	Purity (%)	Vehicle	LD_{50} (mg/kg bw)	Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.2.2/05 [redacted] 2009b (HAG)	Rat, CD	5/sex/2000	98.8	water	> 2000	No findings
	IIA 5.2.2/06 [redacted] 2010e (HAG)	Rat, CD	5/sex/2000	96.4	water	> 2000	No findings
	IIA 5.2.2/07 [redacted] 2010f (HAG)	Rat, CD	5/sex/2000	97.3	water	> 2000	No findings
	IIA 5.2.2/08 [redacted], 2005b (HAG)	Rat, Sprague Dawley	5/sex/5000	97.23	water	> 5000	No findings
	IIA 5.2.2/09 [redacted] 2008b (HAG)	Rat, Wistar Hannover	5/sex/2000	98.05	water (for moisto-ning)	> 2000	No findings
	IIA 5.2.2/10 [redacted] 2007e (NUF)	Rat, HanRcc: WIST	5/sex/2000	95.1	PEG 300	> 2000 (limit test)	No findings
	IIA 5.2.2/11 [redacted] 1996b (SYN)	Rat	5/sex/2000	95.6	Moiste-ned with deionised water	> 2000	Slight erythema in 1♂, small scabs in 1 ♀
	IIA 5.2.2/12 [redacted] 2007b (SYN)	Rat	5/sex/5000	96.1	Moiste-ned with purified water	> 5000	No findings
	IIA 5.2.2/13 [redacted] 2011a (SYN)	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)	Rabbit, NZW	5/sex/5000	97.8	Moiste-ned with saline	> 5000	Mortality (1 ♀); anorexia, diarrhea, soft stool

Table 5.2-7: 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] 1995b (Sanachem)	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] 1989b (I.Pi.Ci.)	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] 1987a (MON / CHE)	Rabbit NZW	5/sex/5000	Ammonium salt 90.8	Moistened 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] 1987b (MON / CHE)	Rabbit NZW	5/sex/5000	Sodium salt 80.3	Saline (1mL/g)	> 5000 (limit test)	Soft stool
	Annex B-5.2.2.2.2, Glyphosate Monograph [redacted] 1981b (MON / CHE)	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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/01	[redacted]	1989	Acute dermal toxicity (limit) test in rats Inveresk Research International, Musselburg, [redacted] Report No.: 5884 Date: 1989-06-21 GLP: yes not published

Guideline: OECD, EEC, EPA[2]

Deviations: None

Dates of experimental work: 1989-06-06 - 1989-06-21

Executive Summary

The acute dermal toxicity potential of the test material, Glyphosate Technical, was investigated in five male and five female Sprague-Dawley rats. The test substance was administered dermally in a single application under occlusion at a dose level of 2000 mg/kg bw. Clinical signs noted at 30 minutes to 1 day after dosing included piloerection and reduced activity. Scab formation was noted at the test sites 2 to 14 days after dosing. No mortality occurred during the study, and the gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Body weight gains with the exception of one animal, which lost weight, were acceptable. The acute dermal LD_{50} was

LD_{50}, dermal, rat > 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Water

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley
Source:	Charles River (UK) Limited, Manston Road, Margate, Kent.
Age:	8-10 weeks
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, *ad libitum*

[2] 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.

Water:	Tap water, *ad libitum*
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} of the test material, Glyphosate technical, in Sprague-Dawley Rats was estimated to be greater than 2000 mg/kg bw.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/02	[redacted]	2009b	Glyphosate – Acute Dermal Toxicity Study in Rats. [redacted] Report No.: 12171-08 Date: 2009-03-11 GLP: yes not published

Guideline: 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.

Dates of experimental work: 2008-12-04 - 2008-12-18

Executive Summary

The test substance, Glyphosate, was evaluated for its dermal toxicity potential and relative skin irritancy when a single dose of 5050 mg/kg bw was applied to the intact skin of albino rats. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain in animals surviving to termination, with the exception of 2 animals that lost or failed to gain weight during the study. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5050 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley
Source:	Texas Animal Specialties, Humble, TX, US
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

B: 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 CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. 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..

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The dermal LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/03	[redacted]	1995c	HR-001: Acute dermal toxicity study in rats. The Institute of Environmental Toxicology, [redacted] Data owner: Arysta LifeScience Report No.: 94-0154 Date: 1995-03-14 GLP: yes not published

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

Deviations: None

Dates of experimental work: 1995-02-09 – 1995-02-23

Executive Summary

HR-001 was singly administered to specific pathogen free SD rats (Crj:CD) in order to investigate its acute dermal toxicity at a dose level of 2000 mg/kg using 5 males and 5 females. There were no deaths in either sex. Neither clinical signs nor gross abnormalities at necropsy were noted in any animals. No body weight losses were recorded at 7 and 14 days after the administration when compared with the body weights of the day of administration. Based on the results mentioned above, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, female and male rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Specific pathogen free SD rats (Crj:CD)
Source:	Tsukuba Breeding Center of Charles River Japan, Inc.
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, *ad libitum*

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	

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were noted in any animals of 0 and 2000 mg/kg groups.

C. BODY WEIGHT
All animals gained their body weights on days 7 and 14 after administration.

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

III. CONCLUSION

The oral LD_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/04	[redacted]	2009b	Glyphosate Technical: Acute Dermal Toxicity Study in Rat. [redacted] Report No.: C22875 Date: 2009-04-02 GLP: yes not published

Guideline: 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).

Dates of experimental work: 2009-01-13 - 2009-02-03

Executive Summary

The test substance, glyphosate, was evaluated for its acute dermal toxicity potential when applied dermally to an area of clipped skin of male and female HanRcc: WIST (SPF) rats at a dose level of 2000 mg/kg bw. No mortality occurred during the study. No clinical signs were observed during the course of the study. No local dermal signs were observed in any of the treated male animals. In four female animals, slight erythema, scaling and cabs were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute dermal LD_{50} was calculated to be

LD_{50}, dermal, male and female rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rat
Strain:	HanRcc: WIST (SPF)
Source:	Harlan Laboratories Ltd., Laboratory Animal Services, Fuellinsdorf / Switzerland

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 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, 4132 Muttenz / Switzerland) during treatment and observation.
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 30 - 70% Air changes: 10 - 15/hour 12-hour light/dark cycle

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
No systemic clinical signs were observed during the course of the study.

C. 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.

D. NECROPSY

No macroscopic findings were recorded at necropsy.

E. 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 11.

III. CONCLUSION

The dermal LD_{50} 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 dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/05	[redacted]	2009b	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. [redacted] Data owner: Helm AG Report No.: 23912 Date: 2009-06-16 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2009-02-04 - 2009-03-04

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Glyphosate TC
   Identification: Glyphosate technical grade
   Description: White powder

Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	2010-08-01

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

3. **Test animals:**

Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/06	[redacted]	2010e	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. [redacted] Data owner: Helm AG Report No.: 24876 Date: 2010-01-06 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2009-10-15 - 2009-11-12

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #:	2009051501
Purity:	96.4%
Stability of test compound:	2011-05-15

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

3. Test animals:

Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/07	[redacted]	2010f	Acute Dermal Toxicity Study of Glyphosate TC in CD Rats. [redacted] Data owner: Helm AG Report No.: 24604 Date: 2010-02-19 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2009-10-26 - 2009-11-12

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification: Glyphosate technical grade

Description: White powder

Lot/Batch #:	20090506
Purity:	97.3%
Stability of test compound:	May 2011

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

3. Test animals:

Species:	Rat albino
Strain:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/08	[redacted]	2005b	Glyphosate Acid Technical: Acute Dermal Toxicity Study in Rats – Limit Test. [redacted] Report No.: 15275 Date: 2005-04-04 GLP: yes not published

Guideline:	US EPA OPPTS 870.1200 (1998) OECD 402 (1987).
Deviations:	There were no deviations from the study plan.
Dates of experimental work:	2004-05-05 - 2004-05-19

Executive Summary

The test substance, Glyphosate Acid Technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 5000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Acid Technical is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. **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.

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Rat albino
Strain:	Sprague-Dawley derived
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, *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

B: 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 CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate Acid Technical is not to be classified for acute dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/09	[redacted]	2008b	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 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2007-09-11 - 2008-06-11

Executive Summary

The test substance, Glyphosate Technical, was evaluated for its acute dermal toxicity potential in albino rats when administered as single dose of 2000 mg/kg bw. No mortality occurred during the study and no clinical signs or skin reactions were observed. All animals gained the expected body weight, except for two females on the second week of the observation period. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. Hence, the acute dermal LD_{50} was determined to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. **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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat albino
Strain:	Wistar Hannover
Source:	Bioagri Laboratórios, DF, Brazil
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

B: 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 CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The dermal LD_{50} 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 dermal toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/10	[redacted]	2007e	GLYPHOSATE TECHNICAL (NUP05068): Acute dermal toxicity study in rats [redacted] Data owner: Nufarm [redacted]Study No.: B02283 Date: 2007-03-01 GLP: yes unpublished

Guideline:	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
Dates of experimental work:	12-DEC-2006 to 02-JAN-2007

Executive Summary

Glyphosate Technical (NUP 05068) diluted in vehicle (PEG 300) at a concentration of 0.33 g/mL (dosage volume 6 mL/kg) was applied dermally at 2000 mg/kg bw to 5 male and 5 female rats.
The application period was 24 hours. The animals were examined daily for mortality, viability and clinical signs. Body weights were recorded on days 1 (prior to administration), 8 and 15. No deaths occurred during the study. No clinical signs nor body weight changes were observed during the course of the study. No macroscopic findings were observed at necropsy. The Glyphosate Technical (NUP 05068) dermal LD50 (rat)is > 2000 mg/kg body weight.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical (NUP 05068) glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	Solid
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Stable under storage conditions.

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

3. Test animals:

Species:	Rat
Strain:	HanRcc:WIST (SPF)
Source:	[redacted] Laboratory Animal Services CH-4414 FOliinsdorf I Switzerland
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No systemic or local signs of toxicity were observed during the study period.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

D. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

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 LD_{50} (rat) > 2000 mg/kg body weight.

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

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/11	[redacted]	1996b	Glyphosate Acid: Acute Dermal Toxicity Study In The Rat. [redacted] Data owner: Syngenta Report No.: CTL/P/4664 Date: 1996-08-23 GLP: yes not published
Guideline:			OECD guideline 402 (1987), OPPTS 870.1200 (1998): 92/69/EEC B.3 (1992)
Deviations:			None
Dates of experimental work:			1995-01-10 to 1995-08-23

Excecutive summary

In an acute dermal toxicity study, a group of five male and five female, young adult Alpk:AP_fSD (Wistar-derived) rats were given a single dermal application of 2000 mg/kg bw of glyphosate acid in deionised water and observed for 15 days.

None of the animals died. There were no significant signs of systemic toxicity and practically no signs of skin irritation. Most animals had exceeded their initial weight by the end of the study. There were no treatment-related findings at examination *post mortem*.

The acute dermal LD_{50} of glyphosate acid is in excess of 2000 mg/kg to male and female rats (limit test, no mortality).

I. MATERIALS AND METHODS

A: MATERIALS:

1. 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

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rat
Strain:	Alpk:AP_fSD (Wistar-derived)
Age/weight at dosing:	Young adult / 250-274 g (males), 203-216 g (females)
Source:	Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
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 *ad libitum*.
Water:	Mains water *ad libitum*
Environmental conditions:	Temperature: 21±2 °C Humidity: 40-70 % Air changes: Approximately 25-30/hour Photoperiod: 12 hours dark / 12 hours light

B: 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:AP_fSD (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/cm^2 for males and 16.2-17.3 mg/cm^2 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 LD_{50} was estimated (limit test, no mortalities).

II. 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.

Bodyweight: 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.

III. CONCLUSION:

The acute dermal LD_{50} of glyphosate acid is in excess of 2000 mg/kg to male and female rats (limit test, no mortality).

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/12	[redacted]	2007b	Glyphosate technical material: Acute dermal toxicity study in rats. [redacted] Data owner: Syngenta Report No.: B02766 Date: 2007-02-08 GLP: yes not published

Guideline:	OECD 402 (1987), JMAFF 12 NohSan 8147 (2000), US EPA Health Effects Test Guidelines, OPPTS 870.1200 (1998)
Deviations:	None
Dates of experimental work:	2006-11-30 to 2006-12-22

Excecutive summary

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.

The animals were examined daily during the acclimatization period and mortality, viability and clinical signs were recorded. All animals were examined for clinical signs once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on day 1 and once daily during test days 2-15. Local signs were noted once daily from test day 2 to 15. Mortality/viability was recorded once at approximately 30 minutes, 1, 2, 3 and 5 hours after treatment on test day 1 (with the clinical signs) and twice daily during days 2-15. Body weights were recorded on day 1 (prior to administration) and on days 8 and 15. All animals were necropsied and examined macroscopically.

No deaths occurred and no systemic signs or local signs of irritation were noted during the course of the study. The body weights of the animals were within the range commonly recorded for this strain and age. No macroscopic findings were observed at necropsy.

The acute dermal LD_{50} 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).

I. MATERIALS AND METHODS

A: MATERIALS:

1. 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).

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

3. 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] Ltd, Laboratory Animal Services, CH-4414 Füllinsdorf, Switzerland
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

B: 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 cm^2 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 acclimatization 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).

II. 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.

III. CONCLUSION:

The acute dermal LD_{50} 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).

Annex point	Author(s)	Year	Study title
IIA, 5.2.2/13	[redacted]	2011a	Glyphosate technical: Acute dermal toxicity study in rats; Final report amendment 1. [redacted] Data owner: Syngenta Report No.: 10/218-002P Date: 2011-04-13 GLP: yes not published

Guideline:	OECD 402 (1987): OPPTS 870.1200 (1998): EC 440/2008 (2008)
Deviations:	None
Dates of experimental work:	2010-09-28 to 2010-10-20

Excecutive summary

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 to a shaved area of the back (approximately 10% area of the total body surface).

The test item was applied as supplied. The application period was 24 hours, followed by a 14-day observation period.

Clinical observations along with a check of viability and mortality were performed on all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. Rats were killed and given a gross macroscopic examination at the end of the 2-week observation period (Day 14).

No mortality occurred. No adverse clinical signs were observed after treatment with the test item or during the 14 day observation period. The body weights were within the range commonly recorded for this strain and age. There were no treatment related macroscopic findings observed in any animals.

The acute dermal LD_{50} 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).

I. MATERIALS AND METHODS

A: MATERIALS:

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

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

3. Test animals:

Species:	Rat
Strain:	RjHan:(WI) Wistar
Age/weight at dosing:	Young adult / 220-259 g
Source:	JANVIER S.A.S., France
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

B: 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).

II. 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.

III. CONCLUSION

The acute dermal LD_{50} 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).

IIA 5.2.3 Acute inhalation toxicity

In the 2001 EU glyphosate evaluation the acute inhalation toxicity was low with LC_{50} values above the limit test dose of 5 mg/L air per 4 hours obtained for the acid and the isopropylammonium salt (IPA). It has to be noted that due to the physico-chemical properties (vapour pressure = $1.31.x\ 10^{-5}$ Pa) of the test materials, it is difficult to achieve inhalation concentrations up to the limit dose of 5 mg/L. Toxic symptoms after inhalative exposure included irritation of the upper respiratory tract, hyperactivity, loss of hair, ruffled fur and a slight decrease in body weight but were not consistently observed throughout the studies. Since the 2001 EU glyphosate evaluations several new acute inhalation toxicity studies were conducted. The new generated data confirm the low acute toxicity of glyphosate and its salts (see Table 5.2-8 and Table 5.2-9 below).

Table 5.2-8: 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
Studies from the 2001 evaluation	Annex B-5.2.3.1, Glyphosate Monograph [redacted] 1995 (Herbex)	Rat, Sprague Dawley	5/sex/5.35	95	Compressed air; 4 h nose-only	> 5.35	Wet fur, hunched posture, piloerection, incidents of decreased respiratory rate, ptosis, brown stained fur (head)
	Annex B-5.2.3.1, Glyphosate Monograph [redacted] 1994d (Alkaloida)	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 [redacted] 1989 (CHE)	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 [redacted] 2010a (HAG)	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 [redacted] 1995 (ALS)	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 [redacted] 2009 (EXC)	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 [redacted], 2009c (HAG)	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 [redacted], 2010g (HAG)	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)	Purity (%)	Vehicle	LC_{50} (mg/L air)	Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.2.3/06 [redacted] 2009 (HAG)	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] 2005c (HAG)	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)	Rat, Wistar Hannover	5/sex/5.21	98.05	4 h (MMAD: 18.2-19.9µm)	> 5.21	Wheeze and dyspnoea
	IIA 5.2.3/09 [redacted] 2007 (NUF)	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)	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)	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 5.2-9: Summary of acute inhalation toxicity studies with glyphosate salts

	Reference (Data owner)	Species Strain	Number of animals / Dose level (mg/L)	Purity (%)	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)	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
	Annex B-5.2.3.2, Glyphosate Monograph [redacted] 1989 (I. Pi. Ci.)	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)	Rat, Sprague Dawley	5/sex/1.9 (max. attainable conc.)	Ammo nium salt 85.5	Aerosol / vapor; 4 h whole body	> 1.9	Hyperactivity, perinasal encrustion
	Annex B-5.2.3.2, Glyphosate Monograph [redacted] 1987 (MON / CHE)	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)	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)	Rat. Sprague-Dawley	K-salt of glyphosate 5/sex/2.21 5/sex/5.27	K-salt 57.8% (= 47.2% glypho -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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/01	[redacted]	2010a	Acute Inhalation Toxicity Study of Glyphosate TC In Rats [redacted] Data owner: Helm AG Report No.: 24603 Date: 2010-06-03 GLP: yes not published

Guideline: EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300

Deviations: None

Dates of experimental work: 2009-10-26 - 2010-02-16

Executive Summary

The test substance, glyphosate, was evaluated for its acute inhalation toxicity potential in rats when administered for a single 4-hour period using a dynamic nose-only exposure chamber at an actual concentration of 5.18 mg/L.

No mortality occurred during the study. Clinical signs included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no pathological findings. The acute inhalation LC_{50} was calculated to be

LC_{50}, inhalation, rat > 5.18 mg/L

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: none

3. Test animals:

Species:	Rat
Strain:	CD/Crl:CD (SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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

B: 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 pm) 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 5.2-10: Details of test atmosphere

Mean achieved actual concentration (HPLC)	Actual concentration (gravimetric method)	MMAD	GSD	Respirable amount particle size ≤ 4 µm	
(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 euthanized and all study animals were subjected to gross necropsy.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
Clinical signs of toxicity included slight tremor and slight dyspnoea immediately until 3 hours after end of exposure.

C. BODY WEIGHT
All animals gained the expected body weight.

D. NECROPSY
No pathological findings were noted at necropsy.

III. CONCLUSION

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 Harmonized System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/02	[redacted]	1995	HR-001: Acute inhalation toxicity study in rats. [redacted] Data owner: Arysta LifeScience Report No.: 94-0155 Date: GLP: yes not published

Guideline:	U.S. EPA FIFRA Guideline Subdivision F
Deviations:	None
Dates of experimental work:	1995-04-05 – 1995-04-20

Executive Summary

Five Fischer (F344/DuCrj) rats of each sex were exposed to glyphosate mist for A-hour in a whole-body exposure chamber followed by clinical observation for 14 days. Body weight of each aniumal was measured prior to exposure (day 0) and on days 7 and 14.
After the end of the observation period, all animals were euthanized and necropsied.
Mean actual atmospheric concentration of HR-001 was 5.48 mg/L. Mean values of mass median aerodynamic diameters and geometric standard deviations were 4.8 and 1.7 µm. More than 91% of the test substance consisted of particles with an aerodynamic diameter less than 10 µm or less.
Clinical observation of animals during the exposure was impossible due to heavy foggy test substance mist in the chamber. There were no deaths in either sex during exposure or the subsequent 14-day observation period. After the termination of exposure, wetted fur in the perioral and in periocular regions, and red adhesive materials in the periocular and in nasorostral regions were observed. These signs were

respectively slight in degree and disappeared by day 4 in males and day 5 in females. All animals gained their body weights on day 7 and 14 when compared with those on day 0. No abnormalities were detected in any animal of either sex at final necropsy after the end of observation period.
The acute inhalation LC_{50} was calculated to be

LC_{50}, inhalation, rat > 5.48 mg/L

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate TC
Description:	Whitish crystals
Lot/Batch #:	T-941209
Purity:	97.56%
Stability of test compound:	Not mentioned in the report.

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	F344/DuCrj
Source:	Charles River Japan
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

B: 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 5.2-11.

Table 5.2-11: particule size distribution

Exposure group mg/l	Time of sampling (min)	Analytical concentration (mg/l)	Particule size	
			MMAD[3] (µm)	σg[4]
5.48	60	6.54	5.0	1.6
	120	4.80		
	180	5.11	4.6	1.8
	Mean	5.48	4.8	1.7
	S.D.[5]	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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no deaths in either sex at the tested concentration 5.48 mg/l.

[3] Mass median aerodynamic diameter
[4] geometric standard deviation
[5] Standard deviation

B. CLINICAL OBSERVATIONS

No notable serious changes were observed as clinical signs. Wetted and soiled fur in the periocular 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 tha acute inhalation toxicity study.

C. BODY WEIGHT

All animals gained weights, reflecting their good healthy conditions.

D. NECROPSY

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

III. CONCLUSION

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 Harmonized System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/03	[redacted]	2009	Glyphosate Tech: Acute Inhalation Toxicity [redacted] Data owner: Excel Report No.: 2743/0001 Date: 2009-06-22 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2009-05-12 - 2009-06-04

Executive Summary

The test substance, glyphosate, was evaluated for its acute inhalation toxicity potential in male and female HsdRccHan™: WIST rats by exposure to the dose level of 5.04 mg/L via an aerosol atmosphere. No mortality occurred during the study. Clinical signs included increased respiratory rate. There was no effect on body weight gain. The gross necropsy conducted at termination of the study demonstrated no observable abnormalities. The acute inhalation LC_{50} was calculated to be

LC_{50}, oral, male and female rat > 5.04 mg/L

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Tech is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Tech
Description:	White powder
Lot/Batch #:	GI-1045
Purity:	96.66 %
Stability of test compound:	Expiration in July 2010.

2. Vehicle and/or positive control:

Not relevant

3. Test animals:

Species:	Rat
Strain:	HsdRccHan™ : WIST
Source:	Harlan UK Limited, Oxon, UK
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

B: 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.

II. RESULTS AND DISCUSSION

A. TEST ATMOSPHERE

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

Table 5.2-12: Details of test atmosphere

Mean Achieved Atmoshpere Concentration (mg/L)	Mean Mass Median Aerodynamic Diameter (µm)	Inhalable Fraction (% <4 µm)	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 µm. 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 µm.

B. MORTALITY

There were no mortalities during the study.

C. 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.

D. BODY WEIGHT

Normal bodyweight development was noted during the course of the study.

E. NECROPSY

No macroscopic abnormalities were detected at necropsy.

III. CONCLUSION

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 Harmonized System (GHS) classification criteria, glyphosate tech is not to be classified for acute inhalation toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/04	[redacted]	2009c	Acute Inhalation Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 23911 Date: 2009-11-09 GLP: yes not published

Guideline: 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.

Dates of experimental work: 2009-02-04 - 2009-07-30

Executive Summary

The test substance, Glyphosate TC, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.12 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by laser measurement and determined as $d_{[50]}$ = 6.62 µm. No finer dust concentration of the test item could be generated. The test concentration 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. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be

LC_{50}, inhalation, rat > 5.12 mg/L air/4 hours (actual concentration)

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	98.8%
Stability of test compound:	2010-08-01

2. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
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

B: 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.

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY

There were no mortalities during the study.

C. 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.

D. BODY WEIGHT

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

E. NECROPSY

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

III. CONCLUSION

The acute inhalation LC_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute inhalation toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/05	[redacted]	2010g	Acute Inhalation Toxicity Study of Glyphosate TC in Rats. [redacted] Data owner: Helm AG Report No.: 24875 Date: 2010-06-03 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2009-10-15 - 2010-02-18

Executive Summary
The test substance, Glyphosate TC, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined by HPLC, was 5.02 mg/L air for 4 hours. In the inhalation chamber, close to the animals' noses, the generated dust had a mass median aerodynamic diameter (MMAD) of 4.197 ± 2.64 µm as determined with a cascade impactor. No smaller MMAD could be obtained with the test item. The analysis of the particle size distribution of the particle diameter (volume) in the exposure air was carried out by laser measurement and determined as $d_{[50]}$ = 37.15 µm. The particle size distribution of the particle size of the delivered test item was $d_{[50]}$ = 14.5 µm. The test concentration revealed slight ataxia, slight tremor and slight dyspnoea immediately until 3 hours after the end of exposure. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be

LC_{50}, inhalation, rat > 5.02 mg/L air/4 hours (actual concentration)

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate TC is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate TC

Identification:	Glyphosate technical grade
Description:	White powder
Lot/Batch #:	20080801
Purity:	96.4%
Stability of test compound:	May 2011

2. Test animals:

Species:	Rat albino
Strain / Stock:	CD / Crl:CD(SD)
Source:	Charles River Laboratories, Sulzfeld, Germany
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), *ad libitum* except for approx. 16 h before dosing
Water:	Tap water, *ad libitum*
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

B: 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.

II. RESULTS AND DISCUSSION

A. 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 5.2-13: 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.

B. MORTALITY

There were no mortalities during the study.

C. 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.

D. BODY WEIGHT

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

E. NECROPSY

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

III. CONCLUSION

The acute inhalation LC_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate TC is not to be classified for acute inhalation toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/06	[redacted]	2009	Glyphosate – Acute Inhalation Toxicity Study in Rats. [redacted] Data owner: Helm AG Report No.: 12107-08 Date: 2009-03-09 GLP: yes not published

Guideline:	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.
Dates of experimental work:	2008-11-07 - 2008-11-21

Executive Summary

The test substance, Glyphosate, was evaluated for its acute inhalation toxicity potential. Five male and five female rats were exposed for 4 hours to an aerosol generated from the undiluted test substance at a level of

2.24 mg/L. The exposure concentration was determined gravimetrically. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.6 µm. There was no mortality during the study. Clinical sings included piloerection and activity decrease, which were no longer evident by Day 4. Body weights were unaffected by the exposure. The gross necropsy revealed no observable abnormalities.
The acute inhalation LC_{50} was determined to be

LC_{50}, inhalation, rat > 2.24 mg/L air/4 hours (actual concentration)

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Test animals:

Species:	Rat albino
Strain / Stock:	Sprague-Dawley
Source:	Texas Animal Specialties, Humble, TX, US
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

B: 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.

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY
There were no mortalities during the study.

C. CLINICAL OBSERVATIONS
The only prominent in-life observations were piloerection and activity decrease. Animals were asymptomatic by Day 4.

D. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/07	[redacted]	2005c	Glyphosate Acid Technical: Acute Inhalation Toxicity Study in Rats – Limit Test. [redacted] Data owner: Helm AG Report No.: 15276 Date: 2005-04-04 GLP: yes not published
Guideline:			OPPTS 870.1300 (1998), OECD 403 and JMAFF 59 NohSan No. 4200 (1985).
Deviations:			There were no deviations from the Study Plan.
Dates of experimental work:			2003-05-17 - 2003-05-24

Executive Summary
The test substance, Glyphosate Acid Technical, was evaluated for its acute inhalation toxicity potential. The test substance was administered to albino rats for a single 4-hour period using a dynamic nose-only exposure chamber. The exposure concentration, which was determined gravimetrically, was 2.04 mg/L air for 4 hours. The analysis of the particle size distribution was carried out by a cascade impactor and the mass median aerodynamic diameter (MMAD) was estimated to be 2.5 µm. All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behaviour. No mortality occurred during the study and no pathological findings were noted at necropsy. All animals gained the expected body weight.

The acute inhalation LC_{50} was determined to be

LC_{50}, inhalation, rat > 2.04 mg/L air/4 hours (actual concentration)

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Test animals:

Species:	Rat albino
Strain / Stock:	Sprague-Dawley derived
Source:	Ace Animals, Inc., Boyertown, PA, US
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

B: 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 CO_2. All study animals were subjected to gross necropsy and all abnormalities were recorded.

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY
There were no mortalities during the study.

C. CLINICAL OBSERVATIONS
All animals appeared active and healthy upon removal from the exposure chamber and over the entire 14-day observation period.

D. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

E. NECROPSY
The gross necropsy conducted at termination of the study demonstrated no observable abnormalities.

III. CONCLUSION

The acute inhalation LC_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/08	[redacted]	2008	Acute Inhalation Toxicity Test of Glyphosate Technical in Rats *(Rattus norvegicus)*. [redacted] Data owner: Helm AG Report No.: RF-3996.309.377.07 Date: 2008-09-11 GLP: yes not published

Guideline: OECD guideline 403

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

Dates of experimental work: 2008-06-06 - 2008-06-20

Executive Summary

The test substance, Glyphosate Technical, was evaluated for its acute inhalation toxicity potential. One group of rats (five/sex) was exposed nose-only for a 4-hour exposure period to the aerosolized test item, using a total airflow of 10 L/min. The aerodynamic particle size distribution determined with a cascade impactor indicated that 4.72 to 5.15 % of the aerosol generated was within the respirable size range. The mass median aerodynamic diameter (MMAD) ranged from 18.555 to 19.901 µm. The mean actual concentration determined gravimetrically was 5.211 mg/L. No mortality occurred during the study and no pathological findings were noted at necropsy. 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. All animals gained the expected body weight, except for the males on the first post-exposure day. The acute inhalation LC_{50} was determined to be

LC_{50}, inhalation, rat > 5.211 mg/L air/4 hours (actual concentration)

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance Glyphosate Technical is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Test animals:

Species:	Rat albino (*Rattus norvegicus*)
Strain / Stock:	Wistar Hannover
Source:	Bioagri Laboratórios, DF, Brazil
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

B: 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 aerosolized. 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 euthanized 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.

II. RESULTS AND DISCUSSION

A. 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 4.72 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.

B. MORTALITY

There were no mortalities during the study.

C. 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.

D. 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.

E. NECROPSY

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

III. CONCLUSION

The acute inhalation LC_{50} 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 Harmonized System (GHS) classification criteria, Glyphosate Technical is not to be classified for acute inhalation toxicity.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/09	[redacted]	2007	Glyphosate Technical (NUP05068) : 4-Hour acute inhalation toxicity study in rats [redacted] Data owner: Nufarm Report No.: B02327 Date: 2007-04-02 GLP: yes unpublished

Guideline: 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).

Dates of experimental work: 2006-12-14 to 2006-12-28

Executive Summary

A group of five male and five female albino rats [HanRcc:WIST(SPF)] was exposed by nose-only, flow-past inhalation to Glyphosate Technical (NUP 05068) at a gravimetrically determined mean aerosol concentration of 3.252 mg/L air (s.d. ± 0.053, n = 4). This concentration was considered to represent the highest technically achievable aerosol concentration suitable for acute inhalation toxicity testing in rodents.

Two gravimetric measurements of particle size distribution during the exposure produced mass median aerodynamic diameters and geometric standard deviations (GSD) of 2.95 µm (GSD 2.97) and 3.05 µm (GSD 2.73). All animals were observed for clinical signs and mortality during and following the inhalation exposure, i.e. over a 15-day observation period. Body weights were recorded prior to exposure on test day 1, and during the observation period on test days 4, 8 and 15. On day 15, all animals were sacrificed and necropsied. The ranges of temperature, relative humidity, oxygen content, particle size and airflow measured during the exposure were considered to be satisfactory for a study of this type. There were no deaths and no macroscopic pathology findings. Clinical signs consisted of salivation in two male animals, and transient effects on breathing, i.e. deep respiration and/or rattling breath sounds, in these two and another male, as well as two female animals. Two days after the exposure (test day 3) until the scheduled necropsy day (test day 15) all animals were free from clinical signs. 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. The clinical signs and the transient losses in body weight were attributed to the treatment with the test item, although slight physical stress during restraint in the exposure tubes may have contributed to the effect on body weight.

In conclusion, the LC_{50} of Glyphosate Technical (NUP 05068) obtained in this study was estimated to be greater than 3.252 mg/L air (gravimetrically determined mean aerosol concentration).

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical (NUP 05068)
Description:	White powder
Lot/Batch #:	200609062
Purity:	95.1%
Stability of test compound:	Stable under storage conditions.

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat

Strain:	HanRcc:WIST (SPF)
Source:	RCC Ltd, Laboratory Animal Services CH-4414 FOliinsdorf I Switzerland
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

B: 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 nhalation 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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).

C. 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.

D. NECROPSY

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

III. CONCLUSION

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 Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/10	[redacted].	1996	Glyphosate Acid: 4-Hour Acute Inhalation Toxicity Study In Rats [redacted] Data owner: Syngenta Report No.: CTL/P/4882 Date: 1996-04-29 GLP: yes not published

Guideline:	OECD 403 (1981): OPPTS 870.1300 (1998): 92/69/EEC B.2 (1992) + amendment 93/21/EEC (1993)
Deviations:	None
Dates of experimental work:	1995-10-03 to 1996-04-29

Excecutive summary

In an acute inhalation toxicity study, groups of five male and five female Alpk:APfSD (Wistar-derived) rats was exposed nose-only for a single four-hour period to glyphosate acid at target particulate concentrations of 5 mg/L and 2 mg/L. The particle size distribution of the test atmosphere was analysed at frequent intervals during the exposure period. Following exposure, the animals were retained without treatment for 14 days. Clinical observations and bodyweights were recorded and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*.

The achieved test atmosphere had the following characteristics:

Table 5.2-14: Details of test atmosphere

Target concentration mg/L	Achieved particulate concentration mg/L	MMAD* µm	GSD+
2	2.47- ± 0.15	3.57, 3.03	1.94, 1.90
5	4.43 + 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 LC_{50} of glyphosate acid exceeded 2.47 mg/L for male rats and exceeded 4.43 mg/L for female rats.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	Technical; white solid
Lot/Batch #:	P25
Purity:	95.6 % w/w
Stability of test compound:	Confirmed by Sponsor

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD
Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
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

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 22 November 1995End: 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:

$$\text{Concentration (mg/L)} = \frac{\text{post wt (mg) - pre wt (mg)}}{\text{time (minutes) x airflow (L/minute)}}$$

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 5.2-15: 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	Gravi-metric	Analysed	Gravi-metric	Analysed	Gravi-metric	Analysed	Grav-imetric
Particles > 9.8 µm (% w/w)	0.9	0.7	5.1	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: $\frac{\text{weight trapped at each size range x 100}}{\text{Total weight trapped}}$

Statistics: The acute inhalation LC_{50} was estimated.

II. 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 5.2-16: 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 andwere 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/11	[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: yes not published

Guideline:	OECD 403 (1981): OPPTS 870.1300 (1998): 440/2008 B.2 (2008
Deviations:	None
Dates of experimental work:	2011-03-17 to 2011-06-06

Excecutive summary

In an acute inhalation toxicity study, a group of young adult Wistar RjHan: (WI) strain rats, (5 males and 5 females) was exposed to a target aerosol concentration of 5 mg/L Glyphosate Technical (96.9% w/w 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. Aerosol concentrations were measured gravimetrically. The particle size distribution of the test aerosol was determined regularly during the exposure period.

Clinical observations and bodyweights were recorded throughout the study and at the end of the scheduled period the animals were killed and subjected to a gross examination *post mortem*.

The mean achieved atmosphere concentration was 5.04 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was 3.65 µm ±2.24 (GSD [Geometric Standard Deviation]).

One male rat died following a 4 hour exposure to 5.04 mg/L Glyphosate Technical on Day 4.

Wet fur and fur staining were commonly recorded on the day of exposure and on the day after exposure. These observations were considered to be related to the restraint and exposure. Significant clinical signs were recorded on the day of exposure and the following day included laboured and noisy respiration, respiratory rate increase, gasping respiration, sneezing, activity decreased, thin body appearance (weak/wasted). The majority of the animals recovered from Day 3.

Normal bodyweight gain was noted for all surviving animals from Day 1, with the exception of one male where a slight bodyweight loss was recorded during the first week of the observation period.

No macroscopic findings were seen at necropsy. A specific cause of death was not determined for the single male that died.

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 LC_{50} of Glyphosate Technical, in Wistar RjHan: (WI) strain rats is therefore considered to be greater than 5.04 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control:

None

3. Test animals:

Species:	Rat
Strain:	RjHan:WI
Source:	Janvier (CS 4105, Le Genest St Isle, 53941, St Berthevin, Cedex, France
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

B: 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 (***Pauluhn, 1994***).

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) (***Silver, 1946***), 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 4µm (considered to be the inhalable portion) was determined.

Table 5.2-17: Summary of main study test atmosphere characteristics

Parameter	Target concentration 5 mg/L	
Mean achieved concentration (mg/L)	5.04 ± 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	1.44	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°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.

II. 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.

III. CONCLUSION

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 RjHan: (WI) strain rats is considered to be greater than 5.04 mg/L.

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/12	[redacted]	1999	NUP5a99 62% glyphosate MUP: Acute inhalation toxicity study in rats – Limit test [redacted] Data owner: Nufarm Study No.: 7909 Date: 1999-09-16 GLP: yes unpublished

Guideline: Health Effects Test Guidelines, OPPTS 870.1300 (1998)

Deviations: None

Dates of experimental work: August 6-20, 1999

Executive Summary

After establishing the desired generation procedures during pre-test trials, ten healthy rats were exposed to 2.08 mg/L for 4 hours. Chamber concentration and particle size distributions of the test substance were determined periodically during the exposure period. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily for 14 days. Bodyweights were recorded prior to exposure and again on Days 7 and 14 (termination). Necropsies were performed on all animals at terminal sacrifice. All animals survived exposure to the test atmosphere and gained bodyweight over the l4-day observation period. The gravimetric chamber concentration was 2.08 mg/L. Based on graphic analysis of the particle size distribution as measured with an Andersen Cascade Impactor, the mass median aerodynamic diameter was estimated to be 2.6 microns. 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. Gross necropsy findings at terminal sacrifice were unremarkable. Based on the results of this study, the single exposure acute inhalation LC50 of NUP5a99 62% glyphosate MUP is > 2.08 mg/L, the maximum achievable concentration.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley derived, albino
Source:	Ace Animals, me., Boyertown, PA
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

B: 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 ≤ 4 /µm). 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

The single exposure acute inhalation LC50 ofNUP5a99 62% glyphosate MUP is > 2.08 mg/L.

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

Annex point	Author(s)	Year	Study title
IIA, 5.2.3/13	[redacted]	2004	An Acute Nose-Only Inhalation Toxicity Study in Rats with MON 78623 [redacted] Data owner: Monsanto Monsanto Report No.: SB-2003-116 Date: 2004-02-06 GLP: yes not published

Guideline:	EC method B.2, OECD 403, EPA Health Effects Test Guidelines, OPPTS 870.1300, JMAFF 12 Nohsan No. 8147
Deviations:	None
Dates of experimental work:	2003-10-22 – 2003-12-29

Executive Summary
The four-hour nose-only inhalation toxicity of MON 78623 was evaluated in Sprague Dawley rats. Two limit tests were performed in which one group of five male and five female rats each received a four-hour nose-only inhalation exposure to a time-weighted average aerosol concentration (analytically determined) of 2.21 or 5.27 mg/L. For the first exposure (2.21 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9 µ and 2.18, respectively. The percentage of particles ≤ 4.0 µ was determined to be 67%. Since there was no mortality, a second limit test was conducted at a greater target concentration. For the second exposure (5.27 mg/L) the mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 3.8 µ and 2.20, respectively. The percentage of particles ≤ 4.0 µ was determined to be 53%. Following each exposure, the limit test rats were observed daily and weighed weekly. A gross necropsy examination was performed on all limit test animals at the time of scheduled euthanasia (day 14).

No mortality occurred for the 2.21 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and dark material around the facial area. Body weight gain was noted for all animals during the test period. No gross internal findings were observed at necropsy on study day 14.

No mortality occurred for the 5.27 mg/L dose level. The most notable clinical abnormalities observed during the study included transient incidences of congested breathing and few feces. 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 during the test period and all animals exceeded their initial body weight at study termination. No gross internal findings were observed at necropsy.

The acute inhalation LC_{50} was calculated to be

LC_{50}, inhalation, rat > 5.27 mg/L

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance MON 78623 is not to be classified for acute inhalation toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rat
Strain:	Sprague Dawley
Source:	Harlan Sprague Dawley Inc., Indianapolis, IN, US
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), *ad libitum* (except during acclimatization to the exposure tubes and during the exposure)
Water:	Tap water, *ad libitum* (except during acclimatization 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

B: 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 analyzed 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 ≤ 4.0 µ were then determined. The results were as follows:

Table 5.2-18: Details of Test Atmosphere

Mean Achieved Actual Concentration (analytical method)	MMAD	GSD	Respirable Amount Particle Size ≤ 4 µm
(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 aerosolized at the following levels:

Table 5.2-19: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. 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.

D. 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.

III. CONCLUSION

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 Harmonized System (GHS) classification criteria glyphosate is not to be classified for acute inhalation toxicity.

IIA 5.2.4 Skin irritation

In the 2001 EU glyphosate evaluation glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. In addition to several studies, that were not previously evaluated (see Table 5.2-20) two recently performed studies on the skin irritating potential of glyphosate acid supported the previous findings ([redacted] 2007a, [redacted] 2009a). Thus, all results lead to the conclusion that glyphosate and its salts do not require classification for skin irritation.

Table 5.2-20: 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] 1994e (Alkaloida)	Rabbit NZW	4	97.2	0.5 g; intact + abraded skin	Very slight irritation
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1991a (I. Pi. Ci.)	Rabbit NZW	3 ♂	98	0.5 g moistened with saline; intact skin	Very slight irritation
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1991d (FSG)	Rabbit NZW	2 ♂, 1 ♀	96.8	0.5 g; intact skin	Non irritant
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1990c (AGC)	Rabbit NZW	3 ♂	98.1	0.5 g moistened with saline; intact + abraded skin	Non irritant

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] 1989c (CHE)	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] 2007d (NUF)	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] 2009a (HAG)	Rabbit Himalayan	3 ♂	96.4	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/03 [redacted] 1995a (ALS)	Rabbit NZW	6 ♀	97.56	0.5 g moistened with 0.5 mL water; intact skin	Non irritant
	IIA 5.2.4/04 [redacted] 2009c (HAG)	Rabbit Himalayan	3 ♂	98.8	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/05 [redacted] 2010a (HAG)	Rabbit Himalayan	3 ♂	97.3	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/06 [redacted] 2009c (HAG)	Rabbit NZW	1 ♂, 2 ♀	96.4	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/07 [redacted] 2005d (HAG)	Rabbit, NZW	3 ♂	97.23	0.5 g moistened with water; intact skin	Slightly irritating
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/08 [redacted] 2008a (HAG)	Rabbit, NZW	3 ♀	98.05	0.5 g moistened with water; intact skin	Non irritant
	IIA 5.2.4/09 [redacted] 1988b (MON)	Rabbit NZW	3 ♂, 3 ♀	97.76	0.5 g moistened with saline; intact skin; semi-occluded	Non irritant
	IIA 5.2.4/10 [redacted] 1979b (MON)	Rabbit	3 ♂, 3 ♀	98.5	0.5 g moistened with water; intact skin; occluded	Primary dermal irritation index 0.1
	IIA 5.2.4/11 [redacted] 1996c (SYN)	Rabbit, NZW	6 ♀	95.6	0.5 g moistened with 0.5 mL water; intact skin	Non irritant

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2.4/12 [redacted] 2007c (SYN)	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] 2011b (SYN)	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 5.2-21: 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
Studies from the 2001 evaluation	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1994b (MON / CHE)	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.5 mL (pure)	Slight irritation
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1994b (Herbex)	Rabbit NZW	2 ♂, 1 ♀	IPA 360 g/L	0.5 mL; intact skin	Non irritant
	Annex B-5.2.5.1, Glyphosate Monograph [redacted] 1989c (I.Pi.Ci.)	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] 1987c (MON/CHE)	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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/01	[redacted]	2007a	Glyphosate Technical (NUP 05068): Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application) [redacted] [redacted]Study No.: B02294 Data owner: Nufarm Date: 2007-03-01 GLP: yes unpublished

Guideline:	OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), JMAFF guideline 2-1-4 (2005)
	Yes
Deviations:	The test patch used had a surface of 16 cm^2 instead of 6 cm^2.
Dates of experimental work:	2007-01-04 - 2007-01-15

Executive Summary
In a primary dermal irritation study, young adult New Zealand albino rabbits (1 male, 2 females) were dermally exposed to glyphosate technical (NUP 05068). The clipped, intact skin of the left flank was exposed to 0.5 g of the solid test item, moistened with about 0.5 mL water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72 hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72 hours after removal of the test substance.
No skin reactions were observed at the application site of any animal at any observation time point. 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.
Based on the scores for erythema and according to 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF

Source:	Harlan Netherlands BV, NL-5961 NM Horst, and NL-5960 AD Horst, The Netherlands
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 blooks 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

B: 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-cm^2 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT
All rabbits showed the expected body weight gain.

D. NECROPSY
No necropsy was performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/02	[redacted]	2009a	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC In Rabbits [redacted] Data owner: Helm AG Report No.: 24877 Date: 2009-11-27 GLP: yes unpublished

Guideline:	OECD 404 (2002); Commission Directive 2004/73/EC B.4 (2004), OPPTS 870.2500 (1998)
Deviations:	None
Dates of experimental work:	2009-10-15 - 2009-10-23

Executive Summary

In a primary dermal irritation study, young male adult Himalayan albino rabbits were dermally exposed to glyphosate TC. The clipped, intact skin was exposed to 0.5 g of the solid test item, moistened with purified water, for 4 hours under semi-occlusive conditions. The rabbits were observed for 72 hours. Skin irritation was scored using the Draize scheme 1, 24, 48 and 72 hours after removal of the test substance.

No skin reactions were observed at the application site of any animal at any observation time point. 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.

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Löhndorf/Post Wankendorf, Germany
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

B: 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-cm^2 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT
All rabbits showed the expected body weight gain.

D. NECROPSY
No necropsy was performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/03	[redacted]	1995a	HR-001: Primary Dermal irritation study in rabbits. [redacted] Report No.: IET 95-0035 Data owner: Arysta LifeScience Date: 1995-06-28 GLP: yes unpublished

Guideline:	U.S. EPA FIFRA Guideline Subdivision F MAFF 59 NohSan No. 4200 (1985) Draize method
Deviations:	None
Dates of experimental work:	1995-04-28 – 1995-05-19

Executive Summary

HR-001 was applied to the skin of six female specific pathogen free New Zealand White rabbits for 4 hours to evaluate its primary dermal irritating potential. Observation of primary dermal irritation was perfrmed according to the Guideline of MAFF in Japan and the method of Draize.

No signs of erythema, escar, edema and any other evidence of irritation were observed in either the test substance treated site or the negative control site at any time point of 1, 24, 48, and 72 hours after the patch removal.

Based on these results and according to 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 is also not classified for skin irritation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	Minowa Breeding Center of Kitayame Labes, Co., Ltd.
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
Clinical signs were not observed.

C. BODY WEIGHT
All rabbits showed the expected body weight gain.

D. NECROPSY
No necropsy was performed.

E. SKIN OBSERVATIONS

No signs of erythema, eschar, edema and any other evidence of irritation were observed in either the test sunstance treated site or the negative control site at any time during the observation period.
The observation period was therefore completed after 72 hours.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/04	[redacted]	2009c	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 23913 Date: 2009-04-30 GLP: yes unpublished

Guideline:	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
Dates of experimental work:	2009-02-04 – 2009-02-13

Executive Summary

The test substance, glyphosate, was evaluated for its potential to cause irritant/corrosive effects. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. **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.

2. Vehicle and/ or positive control: Deionised water

3. 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. 4 - 5 months
Sex:	Male animals
Weight at dosing:	Animal no. l : 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

B: 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 cm^2).
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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/05	[redacted]	2010a	Acute Dermal Irritation/Corrosion Test (Patch Test) of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 24605 Date: 2010-01-06 GLP: yes unpublished

Guideline:	OECD 404 (2002) US EPA OPPTS 870.2500
Deviations:	No deviations from the Study Plan
Dates of experimental work:	2009-10-26 - 2009-11-06

Executive Summary

The test substance, glyphosate, was evaluated for its potential to cause irritant/corrosive effects. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG Branch Lohndorf 24601 Lohndorf/Post Wankendorf, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/06	[redacted]	2009c	Glyphosate – Acute Dermal Irritation Study in Rabbits [redacted] Report No..: 12173-08 Date: 2009-03-11 GLP: yes unpublished
Guideline:			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
Dates of experimental work:			2008-11-03 - 2008-11-14

Executive Summary
The test substance, glyphosate, was evaluated for its potential to cause irritant/corrosive effects. Therefore a primary dermal irritation study was conducted on three albino rabbits. There was one intact test site per animal. Each test site was treated with 500 mg of test substance moistened with 0.2 mL of deionized water and covered with a semi-permeable dressing.
The test substance was maintained in contact with the skin for 4 hours. Observations for dermal irritation and defects were made at 1, 24, 48 and 72 hours after removal of the dressings. Irritation scores were derived from the respective erythema and edema scores through the 72-hour observations for each animal resulting in a Primary Irritation Index of 0.0.
Based on the PII of 0.0, the test substance is rated

non-irritating

Based on the scores at the 72-hour observation only, the test substance is assigned to Toxicity Category IV.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Albino rabbit
Strain:	New Zealand White
Source:	Nichols Rabbitry Inc.; Lumberton, TX
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/07	[redacted]	2005d	Glyphosate Acid Technical – Primary Skin Irritation Study in Rabbits [redacted] Report No.: PSL 15278 Date: 2005-04-04 GLP: yes unpublished

Guideline:	US EPA OPPTS 870.2500 Equivalent to OECD 404 (2002).
Deviations:	None
Dates of experimental work:	2004-05-05 - 2004-05-08

Executive Summary
A primary skin irritation test was conducted with rabbits to determine the potential for Glyphosate Acid Technical to produce irritation after a single topical application. Under the conditions of this study, the test substance is classified as slightly irritating to the skin.
Five-tenths of a gram of the test substance was moistened with distilled water and applied to the skin of three healthy rabbits for 4 hours. Following exposure, dermal irritation was evaluated by the method of Draize *et al*.. 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. The Primary Dermal Irritation Index (PDII) calculated for this test substance was 0.1. Under the conditions of this study, Glyphosate Acid Technical is classified as

slightly irritating

to the skin.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	Robinson Services, Inc. Clemmons, NC
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

B: 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 l-inch x l-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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

III. CONCLUSION

Under the conditions of this study, Glyphosate Acid Technical is classified as slightly irritating to the skin.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/08	[redacted]	2008a	Acute Dermal Irritation/Corrosion Study in Rabbits with Glyphosate Technical [redacted] Report No.: RF-3996.311.476.07 Date: 2008-09-23 GLP: yes unpublished

Guideline:	OECD 404 (2002).
Deviations:	The experimental phase initiation and experimental phase conclusion dates were updated
Dates of experimental work:	2008-05-20 - 2008-05-24

Executive Summary
The acute dermal irritation/corrosion potential of GLYPHOSATE TECHNICAL was evaluated in three New Zealand White rabbits. The test was initially conducted using one single rabbit. Because no dermal reaction was observed in the initial test, two additional animals were tested to confirm the response. A

moistened gauze patch containing 0.5 g of the test item undiluted was applied over skin of each animal. After the 4-hr exposure period, the patches were removed and the animals examined at approximately 1, 24, 48 and 72 hours to verify the erythema, eschars and edema formation, and for behavioral and clinical alterations. Adjacent untreated shaved areas of the skin were used as the negative control. The test item 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification:	Glyphosate Technical
Description:	Solid
Lot/Batch #:	20070606
Purity:	98.05%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	*Granja Angolana's* rabbitry, Sao Roque/SP - Brazil
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 (*Guabi, Mogiana Alimentos S.A. - Brazil*)
Water:	Tap water, *ad libitum*
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

B: 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 visualization 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 cm^2) 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT
All animals presented gain in body weight during the observation period.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/09	[redacted]	1988b	Primary Dermal 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 GLP: yes Unpublished
Guideline:			US EPA 81-5

Deviations:	None
Dates of experimental work:	1998-04-11 to 1998-04-14

Executive Summary
Glyphosate was evaluated for potential primary dermal irritation using six New Zealand White rabbits. Each rabbit was administered 0.5 g of the test article (moistened with 0.5 mL of physiological saline) to two intact test sites. Each test site was semi-occluded for four hours following dose administration. Dermal irritation was scored according to the Draize method at 0.5, 24, 48 and 72 hours after patch removal. No dermal irritation was noted following test article application (scores of 0 for all animals at all time points).

Based on the scores for erythema and according to 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76%
Stability of test compound:	Stored at room temperature

2. Vehicle and/or positive control: Physiological saline

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Ace Animals, Inc., Boyertown, PA, US
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

B: 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 euthanized 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 ad 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
Not reported.

C. BODY WEIGHT
Not reported.

D. NECROPSY
No necropsy was performed.

E. SKIN OBSERVATIONS
No dermal irritation was noted following test substance application.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/10	[redacted]	1979b	Primary Dermal Irritation in Rabbits [redacted] Data owner: Monsanto Monsanto Report No.: BND-77-428 Date: 1979-08-06 GLP: no (pre-GLP) Unpublished

Guideline: Not specified

Deviations: Not specified

Dates of experimental work: Not specified

Executive Summary

Glyphosate was evaluated for potential primary dermal irritation using six New Zealand White rabbits. Each rabbit was administered 0.5 mL of the test article (applied as a 25% w/v solution in distilled water) to two intact test sites and two abraded sites. Each test site was semi-occluded for 24 hours following dose administration. Dermal irritation was scored according to the Draize method at 24 and 72 hours after patch removal. 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.

Based on the scores for erythema and according to 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	Fine white powder
Lot/Batch #:	XLI-180
Purity:	99%
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Marland Breeding Farms, Inc., Hewitt, NJ, US
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

B: 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

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
Not reported.

C. BODY WEIGHT
Not reported.

D. NECROPSY
No necropsy was performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/11	[redacted]	1996c	Glyphosate Acid: Skin Irritation to the Rabbit [redacted] Data owner: Syngenta Report No.: CTL/P/4695 Date: 1996-08-23 GLP: yes not published

Guideline:	OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)
Deviations:	None
Dates of experimental work:	Not specified

Excecutive summary
In a primary dermal irritation study, young adult, New Zealand White, female rabbits were dermally exposed to approximately 500 mg of glyphosate acid (95.6 % w/w) for 4 hours to an area (approximately 2.5 cm x 2.5 cm) on the left shorn flank, under a semi-occlusive dressing. Animals were observed for 72 hours. Irritation was scored by the method of ***Draize, 1959.***

There were no signs of skin irritation in any animal.

Glyphosate acid is non-irritant following a single four-hour application to rabbit skin.

I. MATERIALS AND METHODS

A: MATERIALS:

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	Animals 1-5: Interfauna, Wyton, Nr Huntingdon, Cambridgeshire, UK. Animal 9: Charles River UK Limited, Margate, Kent, UK.
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) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 17±2°C Humidity: 40-70% Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

B: 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 (***Draize 1959***) 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.

II. RESULTS AND DISCUSSION

There were no signs of ill-health and no signs of skin irritation in any animal during the study.

Table 5.2-22: 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

III. CONCLUSION:

Glyphosate acid is non-irritant following a single four-hour application to rabbit skin.

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/12	[redacted]	2007c	Glyphosate Technical Material: Primary Skin Irritation Study In Rabbits (4-Hour Semi-Occlusive Application) [redacted] Data owner: Syngenta Report No.: R61837/1010 Date: 2007-02-08 GLP: yes not published

Guideline: OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)

Deviations: None

Dates of experimental work: 2006-12-13 to 2006-12-22

Excecutive summary

In a primary dermal irritation study, Glyphosate Technical Material was applied by topical semi-occlusive application of 0.5 g to the intact left flank of each of three young adult New Zealand White rabbits (one male and two females). The duration of treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing. Irritation was scored according to the Draize classification criteria ***(Draize J H 1944 and 1959)***.

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. The primary irritation index was calculated by totaling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points. The primary irritation index was 0.00 (max. 8.0).

The application of Glyphosate Technical Material to the skin resulted in no signs of irritation. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals and no clinical signs were observed.

According to Draize classification criteria Glyphosate Technical Material is considered to be "not irritant" to rabbit skin (P.I.I. = 0.00).

I. MATERIALS AND METHODS

A: MATERIALS:

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	Harlan Netherlands BV, Kreuzelweg 53, NL-5961 NM Horst / The Netherlands, Postbus 6174, NL-5960 AD Horst / The Netherlands
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.

B: 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 cm^2 (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 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 ***(Draize 1959 and 1944)*** 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.

II. 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.

III. CONCLUSION:

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).

Annex point	Author(s)	Year	Study title
IIA, 5.2.4/13	[redacted]	2011b	Glyphosate technical: Primary skin irritation study in rabbits [redacted] Data owner: Syngenta Report No.: 10/218-006N Date: 2011-04-13 GLP: yes not published

Guideline:	OECD 404 (2002): OPPTS 870.2500 (1998): 2004/73/EC B.4 (2004)
Deviations:	None
Dates of experimental work:	2010-10-18 to 2011-04-13

Excecutive summary

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). The test substance was applied to an area of the intact shaved flank (2.5 cm x 2.5 cm), under a semi-occlusive dressing. After 4 hours, the dressing was removed and the skin was flushed with lukewarm tap water to clean the application site.

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 (***Draize, 1959; Draize et al 1944)***. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing and the primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing by the number of data points.

Initially, a single animal was treated. As neither a corrosive effect nor a severe irritant effect was observed after the 1-hour exposure, the test was completed using the 2 remaining animals.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The body weights of all rabbits were considered to be within the normal range of variability.

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.

According to the Draize classification criteria, glyphosate technical is considered to be a "mild-irritant" to rabbit skin (P.I.I. = 0.11).

I. MATERIALS AND METHODS

A: MATERIALS:

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	S&K-Lap Kft., 2173 Kartal, Császár út 135, Hungary
Housing	Individually in metal cages
Acclimatisation period	5 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: 17-20°C Humidity: 30-70% Air changes: 15-20/hour Photoperiod: 12 hours light/12 hours dark

B: 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 (***Draize, 1959; Draize et al 1944)*** 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.

II. 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 5.2-23: Individual and mean skin irritation scores of Glyphosate technical

Time	Erythema			Oedema		
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

III. CONCLUSION:

According to the Draize classification criteria, glyphosate technical is considered to be a "mild-irritant" to rabbit skin (P.I.I. = 0.11).

IIA 5.2.5 Eye irritation

In the 2001 EU glyphosate evaluation undiluted glyphosate acid was found to be strongly irritating to rabbit eyes, requiring classification as severely irritating and labelling as **R41 – 'Risk of serious damage to eyes'** according to EU classification criteria. The corresponding GHS classification is **'Irreversible effects on the eye/serious damage to eyes (Category 1)'**. Recently performed studies on the eye irritating potential of glyphosate acid supported the previous findings ([redacted] 2007, [redacted] 2009b). There was markedly less eye irritation observed with the salts used in glyphosate based formulations. Glyphosate salts in formulations are of a more neutral pH than glyphosate acid which is not the form used in commercial products. Thus, the glyphosate salts need not to be classified for eye irritation.

Table 5.2-24: 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] 1994c (Herbex)	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] 1994f (Alkaloida)	Rabbit NZW	4 ♀	97.2	100 mg (pure)	Significant ocular lesions, especially chemosis irritant
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1991e (FSG)	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] 1991b (I. Pi. Ci.)	Rabbit NZW	3 ♂	98	100 mg (pure)	Several ocular effects (score 1 & 2) that were reversible within 3 days slightly irritant

	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] 1990d (AGC)	Rabbit NZW	3 ♀	98.1	100 mg	Corneal opacity not reversible within 7 days (2♀), iris lesions not reversible within 6 days (1♀), conjunctivcal redness not reversible within 6 days (3 ♀) slightly irritant
	Annex B-5.2.6.1, Glyphosate Monograph [redacted] 1989d (CHE)	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 5.2.5 [redacted] 1996 (CHE)	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)
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/01 [redacted] 2007b (NUF)	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. irritating
	IIA 5.2.5/02 [redacted] 2009b (HAG)	Rabbit Himalayan	3 ♂	96.4	100 mg (eyes were rinsed 1 h post-application)	slight and fully reversible ocular changes (≤ 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] 1995b (ALS)	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] 2009c (EXC) (Expert statement)	Rabbit	Not applicable	96.66	Test solution: 1% in purified water	Not performed since pH of test item solution was < 2 (corrosive)

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Amount applied Exposure conditions	Effects / Result
Studies not reviewed in the 2001 evaluation	IIA 5.2.5/05 [redacted] 2009d (HAG)	Rabbit Himalayan	3 ♂	98.8	100 mg (eyes were rinsed 1 h post-application)	Non irritant
	IIA 5.2.5/06 [redacted] 2010b (HAG)	Rabbit Himalayan	3 ♂	97.3	100 mg (eyes were rinsed 1 h post-application)	Non irritant
	IIA 5.2.5/07 [redacted] 2009d (HAG)	Rabbit NZW	2♂, 1 ♀	96.4	0.1 mL (93.2 mg)	Irritant
	IIA 5.2.5/08 [redacted] 2005e (HAG)	Rabbit NZW	3 ♂	97.23	0.1 mL (60 mg)	Irritant
	IIA 5.2.5/09 [redacted] 2008b (HAG)	Rabbit NZW	1 ♂ 2 ♀	98.05	100 mg	Severely irritating
	IIA 5.2.5/10 [redacted] 1988c (MON)	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)	Rabbit NZW	6 ♀	95.6	100 mg	Moderately irritant (according to Kay & Chalandra)
	IIA 5.2.5/12 [redacted] 2007d (SYN)	Rabbit NZW	1 ♂ 2 ♀	96.1	100 mg	Slight conjunctival redness, conjunctival chemosis, reddening of the sclera and discharge were observed. These effects were reversible and were no longer evident 7 days after treatment.
	IIA 5.2.5/13 [redacted] 2011b (SYN)	Rabbit NZW	1 ♂	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 5.2-25: Summary of eye irritation studies with glyphosate salts

Reference (Data owner)		Species Strain	Number and /or sex of animals /	Purity [%]	Amount applied / Exposure conditions	Results
Studies from the 2001 evaluation	Annex B-5.2.6.2, Glyphosate Monograph [redacted] 1994c (MON / CHE)	Rabbit NZW	1 ♂, 5 ♀	IPA 62%	0.1 mL (pure) (pre-treatement 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] 1989d (I.Pi.Ci.)	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] 1987e (MON/CHE)	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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/01	[redacted]	2007b	Glyphosate Technical (NUP 05068): Primary Eye Irritation Study In Rabbits [redacted] [redacted] Study No.: B02305 Data owner: Nufarm Date: 2007-03-06 GLP: yes unpublished

Guideline:	OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), JMAFF guideline 2-1-5 (2005)
Deviations:	None
Dates of experimental work:	2007-01-17 - 2007-02-26

Executive Summary

In an eye irritation study, 0.1 g of the undiluted test substance was instilled into the left conjunctival sac of three young adult New Zealand albino rabbits. Animals were observed for 14 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72 hours and 7, 10 and 14 days after test item instillation.
Application of glyphosate technical (NUP 05068) into the rabbit eye resulted in the earlyand transient onset of the ocular changes of 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. The individual mean irritation scores (24 to 72 hours) of the three rabbits were as follows:

- for corneal opacity: 1.67, 2.0 and 0.67;
- for iris lesions: 0.0, 0.0, 0.0
- for conjunctival redness: 2.67, 2.22, 2.0
- for chemosis of the conjunctiva: 2.0, 2.0, 1.0.

Based on the study results, the test substance glyphosate technical (NUP 05068) is irritating to the rabbit eye. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, SPF
Source:	Harlan Netherlands BV, NL-5961 NM Horst, and NL-5960 AD Horst, The Netherlands
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-Kaiseraugust), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages with feed hoppers and drinking water bowls. Wood blooks 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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT
All rabbits showed the expected body weight gain.

D. NECROPSY
No necropsy was performed.

E. 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 5.2-26.

Table 5.2-26: 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/02	[redacted]	2009b	Acute Eye Irritation/Corrosion Test Of Glyphosate TC In Rabbits [redacted] Data owner: Helm AG Report No.: 24878 Date: 2009-11-27 GLP: yes unpublished

Guideline:	OECD 405 (2002); Commission Directive 2004/73/EC B.5 (2004), OPPTS 870.2400 (1998)
Deviations:	None
Dates of experimental work:	2009-10-15 - 2009-10-29

Executive Summary
In an eye irritation study, 0.1 g of the undiluted test substance was instilled into the right conjunctival sac of three young male adult Himalayan albino rabbits. Animals were observed for 8 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72 hours and 4, 5, 6, 7 and 8 days after test item instillation. Application of glyphosate TC into the rabbit eye resulted slight and fully reversible ocular changes, never exceeding Grade 1. All eye effects were reversible within 8 days after instillation. No signs of corrosion or staining were observed in any eye. The individual mean irritation scores (24 to 72 hours) of the three rabbits were as follows:

- for corneal opacity: 1.0, 1.0 and 1.0
- for iris lesions: 1.0, 0.7, 0.3
- for conjunctival redness: 1.0, 1.0, 1.0
- for chemosis of the conjunctiva: 0.7, 0.3, 0.0

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control:
None

3. Test animals:

Species:	Rabbit
Strain:	Himalayan
Source:	LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG, Löhndorf/Post Wankendorf, Germany

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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT
There were no effects on body weight noted.

D. NECROPSY
No necropsy was performed.

E. 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 5.2-27.

Table 5.2-27: 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	1	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**

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/03	[redacted]	1995b	HR-001: Primary Eye irritation study in rabbits. [redacted] Report No.: IET 95-0034 Data owner: Arysta LifeScience Date: 1995-06-29 GLP: yes unpublished

Guideline:	U.S. EPA FIFRA Guideline Subdivision F MAFF 59 NohSan No. 4200 (1985)
Deviations:	None
Dates of experimental work:	1995-05-09 – 1995-05-30

Executive Summary

HR-001 was applied to the eye of 12 female specific pathogen free New Zealand white rabbits to evaluateits primary eye irritating potential. This study was performed according to the method of Draize. Firstly 6 animals were assigned to Group A (the group without eye irrigation after application). Secondly, 3 animals each were assigned to Group B (the group with eye irrigation at 30 seconds after application) and Group C (the group with eye irrigation at 2 minutes after application), since the eye irritation was observed in Group A.

- Irritation of cornea: at 1 hour after application, all animals of Group A showed score 2, the easily discernible translucent area, details of iris slightly obscured. At 24 hours after application, one animal of Group A showed score 3, a nacreous area, no details of iris visible, size of pupil barely discernible. At 1 or 24 hours after application, all animals of Group B and one animal of Group C showed score 1, the scattered or diffuse areas of opacity, details of iris clearly visible. As for the area of cornea involved, Group A showed score 4, greater than the three quarters, up to whole area of the cornea. Group B showed score 2, greater than one quarter, but less than half, or score 3, greater than half, but less than three quarters. Group C showed score 2. These opacities disappeared by Day 16 in 3 of 6 animals of Group A, and the opacity (opacity score 1) remained until Day 21 in other 3 animals of Group A. In Group B, the opacity disappeared by Day 4 and in Group C by 48 hours after application.

- Irritation of iris: at 1 hour after application, al animals of Group A and C showed score 1, the congestion and/or markedly deepened rugae of iris. In Group B, one animal showed score at 1 hour and 2 animals showed score 2 at 24 hours after application. The irritation of iris disappeared by Day 10 in Group A, by 48 hours in Group B and by 24 hours in Group C after application.

- Irritation of conjunctivae: at 1 hour after application, all groups showed redness score 1, the definite hyperaemia of some blood vessels, and chemosis score 2, the obvious swelling with partial eversion of lids. In addition, Groups A and B showed chemosis score 3, i.e. the swelling with lids about half closed. Groups B and C showed discharge score 2, i.e. the discharge with moistening of the lids and hairs just adjacent to lids, and Groups A and C showed discharge score 3, i.e. the discharge with moistening of the lids and hairs and considerable area around the eye. By

24 or 48 hours after application, score 1 redness of conjunctivae in all animals of Group A and in one animal each of Groups B and C had changed to score 2, i.e. the diffuse and crimson colored redness. And score 3 by 24 hours after application. These conjunctival irritations gradually began to decrease thereafter, and disappeared by Day 16 in Group A and by Day 17 in Groups B and C.

- Corneal vascularisation: on Day 10, the corneal vascularisation was observed in 3 animals of Group A. This sign remained until Day 21 after application.

Based on the study results, the test substance glyphosate technical (HR-001) is irritating to the rabbit eye. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White, Kbl:NZW
Source:	Minow Breeding Center of Kitayama Labes, Co., Ltd.
Age:	11 weeks
Sex:	females
Weight at dosing:	2.378 kg (Group A), 2.357 kg (Group B) and 2.426 kg (Group C)
Acclimation period:	Eleven days
Diet/Food:	Pellet Diet GC4 (Oriental Yeast Co., Ltd.)
Water:	Water filtrated and sterilized, *ad libitum*
Housing:	Individually in stainless steel cages.
Environmental conditions:	Temperature: 24°C Humidity: 52.8 – 57.9% Air changes: 15/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1995-04-28 – 1995-05-30

Animal assignment and treatment:

12 female specific pathogen free New Zealand rabbits were given a single ocular instillation of 0.1 g of technical glyphosate. The dose was instilled in the conjunctival sac of left 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 test substance. The left eyes of animals in the eye treated groups were irrigating with water at 30 seconds (3 animals) or 2 minutes (3 animals) after application. The right eye remained untreated. All animals were observed for primary eye irritation 1, 24, 48 and 72 hours, 4, 7, 10, 13, 16, 19 and 21 days after instillation. The cornea, iris and conjunctive were examined with a hand slit-lamp during a subsequent 21-day observation period. Body weights were measured prior to application, and after the final observation.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. CLINICAL OBSERVATIONS
No clinical signs of systemic toxicity were observed during the study.

C. BODY WEIGHT
All rabbits showed the expected body weight gain.

D. NECROPSY
No necropsy was performed.

E. EYE OBSERVATIONS
Results of the no eye irrigating group are summarised in the Table 5.2-28.

Without eye irrigation

- *Irritation of cornea*
  At 1 hour after application, all animals showed score 2, the easily discernible translucent aera, 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 5.2-28: 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**	**---**
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**	**---**

Animal	Scoring [h]	Cornea		Iris	Conjunctiva		
		Opacity	Area		Redness	Chemosis	Discharge
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**	**---**
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 5.2-29: **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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/04	[redacted].	2009c	Expert Statement Glyphosate technical: Primary eye irritation study in rat [redacted] Data owner: Excel Report No.: C22897 Date: 2009-01-23 GLP: yes not published

Guideline:	OECD 405 (2002) Council Regulation (EC) No 440/2008 (2008)
Deviations:	None
Dates of experimental work:	2009-01-22

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 ≤ 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).*

III. CONCLUSION

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).

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/05	[redacted]	2009d	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 23914 Date: 2009-04-30 GLP: yes unpublished

Guideline: 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.

Dates of experimental work: 2009-02-02 - 2009-02-15

Executive Summary
Under the present test conditions, 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 cornea1 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 t o 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.
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: No vehicle used

3. 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. 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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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 t o 72 hours and in animal no. three 24 and 48 hours after instillation.

The fluorescein test performed 24 hours after instillation revealed cornea1 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/06	[redacted]	2010b	Acute Eye Irritation/Corrosion Test of Glyphosate TC in Rabbits [redacted] Data owner: Helm AG Report No.: LPT 24606 Date: 2010-01-06 GLP: yes unpublished

Guideline:	OECD 405 (2002) US EPA OPPTS 870.2400.
Deviations:	No deviations from the Study Plan.
Dates of experimental work:	2009-10-26 - 2009-11-12

Executive Summary

Under the present test conditions, 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 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 cornea1 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.
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: No vehicle was used

3. 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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/07	[redacted].	2009d	Glyphosate – Acute Eye Irritation Study in Rabbits [redacted] Data owner: Helm AG Report No.: 12172-08 Date: 2009-03-11 GLP: yes unpublished

Guideline:	US EPA OPPTS 870.2400 Equivalent to OECD 405 (2002).
Deviations:	Humidity was in the range of 33-92% instead of 30-70%. This deviation did not affect the study outcome
Dates of experimental work:	2008-11-10 - 2008-11-27

Executive Summary
An acute eye irritation study was conducted on three albino rabbits using test substance Glyphosate. The test substance, 0.1 mL by volume (93.2 mg), was placed into the conjunctival sac of the right eye of each animal selected for testing. All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation. There were no positive effects exhibited in any eyes on Day 10 after treatment. The test substance is rated

moderately irritating

and assigned to Toxicity Category II.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: No vehicle was used

3. Test animals:

Species:	Albino rabbit
Strain:	New Zealand White
Source:	Nichols Rabbitry Inc., Lumberton, TX
Age:	Approx. 3 months
Sex:	2 males and 1 female (nulliparous and non-pregnant)
Weight at dosing:	Males: 2.200-2.400 kg; Female: 2.300 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:	22 ± 3°C
	Humidity:	30 - 70%
	Air changes:	10 - 12/hour
	12-hour light/dark cycle	

B: STUDY DESIGN AND METHODS

In life dates: 2008-11-10 to 2008-11-27

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/08	[redacted]	2005e	Eye Irritation/Corrosion Effects in rabbits (Oryctolagus cuniculus) of Glyphosate 95 TC [redacted] Data owner: Helm AG Report No.: PSL 15277 Date: 2005-04-04 GLP: yes unpublished

Guideline: US EPA OPPTS 870.2400
OECD 405 (2002).

Deviations: No deviations from Study Plan.

Dates of experimental work: 2004-05-26 - 2004-06-05

Executive Summary
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: No vehicle was used.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand albino
Source:	Robinson Services, Inc Clemmons, NC
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, *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

B: 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 cornea1 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).

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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 cornea1 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).

III. CONCLUSION

Under the conditions of this study, Glyphosate Acid Technical is classified as severely irritating to the eye.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/09	[redacted]	2008b	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 GLP: yes unpublished

Guideline: OECD 405 (2002).

Deviations: The experimental phase initiation and experimental phase conclusion dates were updated.

Dates of experimental work: 2008-05-26 - 2008-06-17

Executive Summary

The acute eye irritation/corrosion potential of GLYPHOSATE TECHNICAL was evaluated in three New Zealand White rabbits. The test was initially conducted using one rabbit. Because some severe ocular reactions were observed in the initial test, only one additional animal was tested to confirm the response. Each animal received a 0.1 g dose of the test item undiluted in the conjunctival sac of the left eye. After application, 1/2 of the animals was examined at approximately 1,24,48,72 hours, and 7 and 14 days, and 1/2 of the animals was examined at approximately 1, 24,48, 72 hours, and 7, 14 and 21 days to verify the presence of lesions in the cornea, iris, eyelid and eyeball conjunctivae, and for behavioral and clinical alterations. The untreated right eye was used as a negative control. The test item applied in the eye of the rabbits produced corneal opacity, iritis (circumcorneal injection), conjunctival hyperemia, edema and secretion in 2/2 test eyes. Corneal opacity and conjunctival hyperemia were still noted at the final of the observation period in 1/2 test eyes. All initation signs had returned to normal by the 14-day time point following treatment to 1/2 test eyes. Fluorescein sodium dye detected treatment-related changes to the surface of the cornea in 2/2 test eyes. Additional ocular changes observed included: blepharitis and a small raised off area on the corneal surface. Based on the "UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008)" the ocular classification is "Category 1

Irreversible effects on the eye".

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: No vehicle was used

3. Test animals:

Species: Rabbit
Strain: New Zealand White
Source: *Granja Angolana's* rabbitry, Sao Roque/SP - Brazil

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 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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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 corned surface in the right inferior quadrant at the 21-day time point.

III. CONCLUSION

Based on the "UN GHS / EU CLP Criteria for Classification (UN, 2009; EC, 2008)" the ocular Classification for Glyphosate Technical is "Category l Irreversible effects on the eye".

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/10	[redacted]	1988c	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 GLP: yes unpublished

Guideline:	US EPA 81-4
Deviations:	None
Dates of experimental work:	1988-04-11 to 1988-05-02

Executive Summary

In an eye irritation study, 0.1 g of the undiluted test substance was instilled into the conjunctival sac of one eye of six young adult New Zealand rabbits. Animals were observed for 21 days. Eye irritation was scored using the Draize scheme 1, 24, 48 and 72 hours and 7, 14, and 21 days after test substance instillation.

Under the conditions of this study, Glyphosate produced corneal opacity and conjunctival irritation with blistering in all rabbits after test article instillation. Three rabbits exhibited pannnus on the cornea; one rabbit had prominent vascularization of the conjunctiva and another animal had a blood-like discharge. One rabbit was found dead 20 days after dose administration. This death was not considered treatment related. Corneal opacity persisted through study day 21 (termination) in three of five (3/5) animals. 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) of the six rabbits were as follows:

- for corneal opacity: 2.1;
- for iris lesions: 0.2;
- for conjunctival redness: 2.0;
- for conjunctival chemosis: 2.6.

Based on the study results, the test substance glyphosate is severely irritating to the rabbit eye. According to EU classification criteria the test substance glyphosate is to be classified as risk of serious damage to eyes (Xi, R41). This corresponds to Category 1 according to the OECD Globally Harmonized System (GHS) classification criteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	XLI-55
Purity:	97.76%
Stability of test compound:	Stored at room temperature

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Ace Animals, Inc., Boyertown, PA, US
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), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Individually in wire mesh cages
Environmental conditions:	Temperature: 20 – 23.9ºC
	Humidity: 40 – 60%
	Air changes: Not specified
	Light cycle: 12 hours light/dark cycle

B: 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.

II. RESULTS AND DISCUSSION

A. 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.

B. CLINICAL OBSERVATIONS
Not reported.

C. BODY WEIGHT
Not reported.

D. NECROPSY

Not reported.

E. 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 ad 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 5.2-30.

Table 5.2-30: 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	1	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**	
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**	

Animal No.	Scoring [h]	Cornea		Iris	Conjunctivae		
		Opacity	Area		Redness	Chemosis	Discharge
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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/11	[redacted]	1997	Glyphosate Acid: Eye Irritation to the Rabbit [redacted] Data owner: Syngenta Report No.: CTL/P/5138 Date: 1997-03-18 GLP: yes not published

Guideline: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)
Deviations: None.
Dates of experimental work: 1996-04-04 to 1997-03-18

Excecutive summary
In a primary eye irritation study, 100 mg of Glyphosate acid (95.6 % w/w was instilled into the conjunctival sac of the left eye of one of six young adult, New Zealand White albino female rabbits. 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. 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.

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.

The eyes were examined and the Draize scale (***Draize 1959***) 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 (***Kay and Calandra 1962***) was used to interpret and classify the numerical scores.

Corneal, iridial and conjunctival effects were seen in all animals for up to 4 days. 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.

Glyphosate acid is a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye.

I. MATERIALS AND METHODS

A. MATERIALS

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	Charles River UK Limited, Margate, Kent, UK.
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) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 17±2°C Humidity: 40-70% Air changes: Approximately 25/hour Photoperiod: 12 hours light / 12 hours dark

B: 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 (***Draize 1959***) 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 (***Kay and Calandra 1962***) was used to interpret and classify the numerical scores.

II. 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 5.2-31: 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**
after 1 hour	0	0	0	1	1	1	0	0	0	0	0	0	1	2	2	1	2	2	2	2	1	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
after 48 hours	1	1	1	2	1	2	1	1	1	1	1	1	2	2	2	2	2	2	1	1	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	1	1	1	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	2
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
after 8 days	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-	-	-	-	-	0	-

III. CONCLUSION:

Glyphosate acid is a moderate irritant (class 5 on a 1-8 scale) to the rabbit eye.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/12	[redacted]	2007d	Glyphosate Technical Material: Primary Eye Irritation Study In Rabbits [redacted] Data owner: Syngenta Report No.: B02788 Date: 2007-03-26 GLP: yes not published

Guideline: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2004)

Deviations: None.

Dates of experimental work: 2006-12-22 to 2007-03-26

Excecutive summary

In a primary eye irritation study, 0.1 g of Glyphosate Technical Material was instilled into the conjunctival sac of the left eye of each of three young adult New Zealand albino rabbits (1 male and 2 females). The animals were then observed for 7 days. The ocular reaction 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.

The instillation of Glyphosate Technical Material into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclerae, discharge and chemosis. These effects were reversible and were no longer evident 7 days after treatment, the end of the observation period. No abnormal findings were observed in the cornea or iris of any animal at any of the examinations. No corrosion was observed at any of the measuring intervals. No staining of the treated eyes by the test item was observed and no clinical signs were observed.

Thus, Glyphosate Technical Material did not induce significant or irreversible damage to the rabbit eye.

I. MATERIALS AND METHODS

A: MATERIALS:

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 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	Harlan Netherlands BV, Kreuzelweg 53, NL-5961 NM Horst / The Netherlands, Postbus 6174, NL-5960 AD Horst / The Netherlands
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.

B: 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.

II. 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 5.2-32: Eye irritation scores of Glyphosate Technical according to the numerical scoring system listed in the Commission Directive 2004/73/EC, 29 April 2004

Time	Cornea			Iris			Conjunctiva					
							Redness			Chemosis		
Animal number	**31M**	**32F**	**33F**	**31M**	**32F**	**33F**	**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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.5/13	[redacted]	2011b	Glyphosate technical: Acute eye irritation study in rabbits [redacted] Data owner: Syngenta Report No.: 10/218-005N Date: 2011-05-13 GLP: yes not published

Guideline: OECD 405 (2002): OPPTS 870.2400 (1998): 2004/73/EC B.5 (2008)

Deviations: None.

Dates of experimental work: 2010-12-10 to 2011-05-12

Excecutive summary

In a primary eye irritation study, 0.1 g of Glyphosate Technical (96.3% w/w Glyphosate technical) was instilled into the conjunctival sac of the left eye of one adult male New Zealand White rabbit. The untreated right eye served as the control. Immediately following instillation, an assessment of initial pain was made. Scoring of irritation effects was performed approximately 1 and 24 hours after test material instillation. Irritation was scored according to the Draize numerical evaluation (***Draize JH, 1977***).

Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation (Regulation (EC) No 440/2008).

Initial Pain Reaction (IPR) scores were taken after instillation into the eye and a score of 3 (on a 0-5 scale) was observed. Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit at 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 at one hour after the treatment. Fluorescein staining was positive at the 24 hour observation.

No clinical signs of systemic toxicity were observed in the animal during the study and no mortality occurred.

Under the conditions of this study, Glyphosate Technical is classified as corrosive to the eye.

I. MATERIALS AND METHODS

A: MATERIALS:

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	S&K-Lap Kft., 2173 Kartal, Császár út 135, Hungary
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

B: 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 (***Draize, 1977***).

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.

II. 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 5.2-33: 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

III. CONCLUSION

Under the conditions of this study, Glyphosate Technical is classified as corrosive to the eye.

IIA 5.2.6 Skin sensitisation

In the 2001 EU glyphosate evaluation neither glyphosate acid nor the salts have shown sensitizing effects in guinea pigs. However, only the acid and the IPA were tested under the more stringent conditions of the Magnusson-Kligman test. Since the previous evaluation several new studies for skin sensitizing effects have been conducted according to recent guidelines. The data all confirmed that glyphosate and glyphosate salts (as illustrated by the IPA salt) do not possess a skin sensitising potential, and need not to be classified.

Table 5.2-34: 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]1994d (Herbex)	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)	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)	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)	Guinea pig Dunkin Hartley	46 ♀	98.6	induction: 10% in water; challenge: 25% in water	MKT	Not sensitising

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2.6/01 [redacted] 2007c (NUF)	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] 2010b (HAG)	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
	IIA 5.2.6/03 [redacted] 1995c (ALS)	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] 2009d (EXC)	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] 2009d (HAG)	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] 2010h (HAG)	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] 2009e (HAG)	Guinea pig	15 ♂ 15 ♀	96.4	400 mg moistened induction and challange	Buehler Test	Not sensitising
	IIA 5.2.6/08 [redacted] 2005f (HAG)	Guinea pig	30 ♂ ♀	97.23	70 % in water induction and challange	Buehler Test	Not sensitising
	IIA 5.2.6/09 [redacted] 2008 (HAG)	Guinea pig	30 ♂	98.05	50 % inductions and challange	Buehler Test	Not sensitising

	Reference (Data owner)	Species Strain	Number and /or sex of animals	Purity [%]	Exposure conditions	Test Method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.2,6/10 [redacted] 2006 (NUF)	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
	IIA 5.2.6/11 [redacted] 1996d (SYN)	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)	Mouse CBA	4 ♀/group	96.1	Glyphosate acid dose levels: 0, 10, 25, 45 (% w/v) Hexylcinnamaldehyde positive control demonstrated sensitivity of study	LLNA	Not sensitising
	IIA 5.2.6/13 [redacted] 2011 (SYN)	Mouse CBA	4 ♀/group	96.3	Glyphosate acid dose levels: 0, 10, 25, 50 (% w/v) Hexylcinnamaldehyde positive control demonstrated sensitivity of study	LLNA	Not sensitising

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

Table 5.2-35: Summary of skin sensitisation studies with glyphosate salts

	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] 1994d (MON / CHE)	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)	Guinea pig	16	IPA 53.8%	Induction and challange: 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 confirmatory studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/01	[redacted]	2007c	Glyphosate Technical (NUP 05068): Contact Hypersensitivity in Albino Guinea Pigs, Maximisation Test [redacted] [redacted] Study No.: B02316 Data owner: Nufarm Date: 2007-03-06 GLP: yes unpublished

Guideline:	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), JMAFF guideline 2-1-6 (2005)
Deviations:	No
Dates of experimental work:	2007-01-10 - 2007-02-15

Executive Summary
Glyphosate technical (NUP 05068) was tested for its sensitizing effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 3% dilution of the test item in PEG 300 and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 h under occlusion with the test item at 50% one week after the intradermal induction.
Two weeks after induction the animals were challenged by epidermal application of the test item at 25% under occlusive dressing.
The study was performed using one control group consisting of 10 animals, and one test group consisting of 20 animals.
None of the animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment.
Based on the study results, glyphosate technical (NUP 05068) is not to be classified according to EU classification criteria and OECD Globally Harmonized System (GHS) criteria for skin sensitisation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

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

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(HA)BR, SPF

Source:	Charles River Deutschland GmbH, D-88353 Kissleg, Germany
Age:	5 - 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 ± 3°C
	Humidity: 30 - 70%
	Air changes: 10 - 15/hour
	12 hours light/dark cycle

B: 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 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No signs of systemic toxicity were observed.

C. 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.

D. NECROPSY
No necropsy was performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/02	[redacted]	2010b	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 GLP: yes unpublished

Guideline:	OECD 406 (1992); Commission Directive 96/54/EC B.6 (1996), OPPTS 870.2600 (1998)
Deviations:	None
Dates of experimental work:	2009-10-15 - 2009-11-28

Executive Summary

Glyphosate TC was tested for its sensitizing effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 0.01% dilution of the test item in physiological saline and an emulsion of Freund's Complete Adjuvant (FCA)/physiological saline. The epidermal induction was conducted for 48 h under occlusion with the test item at 50% one week after the intradermal induction.
Two weeks after induction the animals were challenged by epidermal application of the test item at 25% under occlusive dressing. The study was performed using a control group consisting of five animals, one test group consisting of ten animals and a positive control group consisting of 20 animals.
None of the vehicle control or test animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment. 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).
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	Charles River Laboratories GmbH, Kißlegg, Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No signs of systemic toxicity were observed.

C. BODY WEIGHT
All animals showed the expected gain in body weight.

D. NECROPSY
No necropsy was performed.

E. 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).

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/03	[redacted]	1995	HR-001: Dermal sensitisation study in Guinea pigs. [redacted] Study No.: IET 95-0036 Data owner: Arysta LifeScience Date: 1995-06-28 GLP: yes Unpublished

Guideline: U.S. EPA FIFRA Guideline Subdivision F

Deviations: None

Dates of experimental work: 1995-04-19 – 1995-05-13

Executive Summary

HR-001 was applied to the skin of female Hartley strain guinea pigs to evaluate its dermal sensitizing potenital. This study was performed according to the guinea pig maximization test. DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance. Animals were assigned to the following 4 groups:

- 20 animals to the test substance treatment group, treated with the test substance both at the induction and challenge,
- 20 animals to the negative control group for the test substance, treated with the test substance at the challenge but not at the induction,
- 10 animals to the DNCB treatment group treated with DNCB both at induction and challenge, and
- 10 animals to the negative control group for DNCB, treated with DNCB at the challenge but not at the induction.

Concentrations of 5%, 25% and 25% of the test substance, and concentrations of 0.1%, 1% and 0.5% of DNCB were selected as the dose for intradermal induction, topical induction, and challenge, respectively. Skin reaction to the challenge was observed 24 and 48 hours after removal of the patch and dermal sensitization rates were calculated.

All 20 animals in the test substance treatment group exhibited the reaction of 0 (no reaction).

All 20 animals in the negative control group for the test substance also exhibited score 0. Thus the sensitizing rate, i.e. the percentage of animals positively sensitized, was 0% in the test substance treatment group.

The DNCB treatment group was evaluated on 9 animals group because one animal died at Day 12 after intradermal induction. All 9 animals exhibited a reaction of score 3 (intense redness and swelling). On the other hand, all 10 animals in the negative control group for DNCB exhibited a score of 0. Thus the sensitizing rate of DNCB was greater than 100%. This was sufficient to assure the reliability of this study.

Based on the study results, glyphosate technical (HR-001) is not to be classified according to EU classification criteria and OECD Globally Harmonized System (GHS) criteria for skin sensitisation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

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

3. Test animals:

Species:	Guinea pig
Strain:	Hartley, Crj:Hartley
Source:	Atsugi Breeding Center of Charles River Japan Co., Japan
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 sterilized 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

B: 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 temoval 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
One animal died in the DNCB treatment group. At necroscpy 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 groupdid not show any abnormality in the health condition and the skin reactions were clearly observed.

B. CLINICAL OBSERVATIONS
No signs of systemic toxicity were observed.

C. BODY WEIGHT
No abnormal body weight changes were noted in any animal of the four groups.

D. NECROPSY
No necropsy was performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/04	[redacted]	2009d	Glyphosate Technical: Contact Hypersensitivity in albino guinea pigs – Maximization-Test [redacted] Data owner: Excel Report No.: C22908 Date: 2009-05-15 GLP: yes not published

Guideline:	OECD 406 (1992) Commission Regulation (EC) No 440/2008 (2008); method B.6
Deviations:	None
Dates of experimental work:	2009-01-14 to 2009-02-27

Executive Summary

Glyphosate Technical was tested for its sensitizing effect on the skin of guinea pig in the Maximization-Test. No mortality occurred during the study, hence no necropsies were performed. No clinical signs of systemic toxicity were observed in the animals. No positive/skin reactions were observed in the animals in the challenge procedure. There was no effect on body weight gain. The Glyphosate Technical in the Maximization-Test was concluded to be

Not sensitising to the skin

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate technical is not to be classified for skin sensitisation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Guinea pig
Strain:	Albino Dunkin Hartley, CRL:(AH)BR, SPF
Source:	Charles River Deutschland GmbH, Kisslegg, Germany
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)
Diet/Food:	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.*
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

B: 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 sensitization 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 sensitizing 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.

II. RESULTS AND DISCUSSION

A. 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.

B. CLINICAL OBSERVATIONS

No signs of systemic toxicity were observed in the animals.

C. 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.

D. NECROPSY

No necropsies were performed.

E. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/05	[redacted]	2009d	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 GLP: yes unpublished

Guideline:	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.
Dates of experimental work:	2009-02-04 - 2009-03-28

Executive Summary

The purpose of this study was to determine the potential of Glyphosate TC to produce skin sensitisation reactions in guinea pigs in a test model according to MAGNUSSON and KLIGMAN. A 0.5% suspension of Glyphosate TC in aqua ad iniectabilia chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema 24 and 48 hours after administration. 2 mL of a 50% suspension of Glyphosate TC in aqua ad iniectabilia/animal chosen for the 2nd (topical) induction stage was not irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with sodium laurylsulfate on the day before the 2nd induction in order to induce a local irritation. The challenge with 2 mL of a 50% suspension of Glyphosate TC in aqua ad iniectabilia/animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties. The vehicle control revealed no skin reactions. Animals of the same strain treated with 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). The body weight gain of the animals treated with Glyphosate TC was within the range of the vehicle control at study termination. The significant difference in body weight between test item-treated and control animals at start of the study is regarded to be without any biological relevance. Behaviour of the animals remained unchanged. Under the present test conditions, Glyphosate TC was found to be

not sensitising

to guinea pigs in a test model according to MAGNUSSON and KLIGMAN.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: *Aqua ad iniectabilia/* Benzocaine

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	Charles River Deutschland GmbH Stolzenseeweg 32 - 36 88353 Kißlegg Germany
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 ± 3°C Humidity: 55 ± 15% 12-hour light/dark cycle

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities during the study.

B. 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.

C. BODY WEIGHT

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

III. CONCLUSION

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to MAGNUSSON and KLIGMAN.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/06	[redacted]	2010h	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 24607 Date: 2010-04-19 GLP: yes unpublished
Guideline:			OECD 406 US EPA OPPTS 870.2600.
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.

Dates of experimental work: 2009-10-26 - 2010-01-30

Executive Summary
The purpose of this study was to determine the potential of Glyphosate TC to produce skin sensitisation reactions in guinea pigs in a test model according to MAGNUSSON and KLIGMAN. A 0.5% concentration of Glyphosate TC in aqua ad iniectabilia chosen for the 1st (intracutaneous) induction stage revealed a discrete or patchy erythema in all 10 animals 24 and 48 hours after adminstration. 2 mL of a 50% concentration of Glyphosate TC in aqua ad iniectabilia/animal chosen for the 2nd (topical) induction stage was non-irritating to the shaved skin in the preliminary experiment. Hence, in the main study the skin was coated with sodium laurylsulfate on the day before stage 2 induction in order to induce a local irritation. The challenge with 2 mL of a 25% concentration of Glyphosate TC in aqua ad iniectabilia/animal revealed no skin irritation in any animal and, thus, the test item had no sensitising properties.The vehicle control revealed no skin reactions. Animals of the same strain treated with 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). The animals gained the expected weight within the test period. Behaviour of the animals remained unchanged. Under the present test conditions, Glyphosate TC was found to be

not sensitising

to guinea pigs in a test model according to MAGNUSSON and KLIGMAN.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: *Aqua ad iniectabilia*/ Benzocaine

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin-Hartley
Source:	Charles River Deutschland GmbH Stolzenseeweg 32 - 36 88353 Kißlegg Germany
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. BODY WEIGHT
Body weight gain was unaffected by the administration of the test substance.

III. CONCLUSION

Under the present test conditions Glyphosate TC revealed no sensitising properties in guinea pigs in a test model according to MAGNUSSON and KLIGMAN.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/07	[redacted]	2009e	Glyphosate – Skin Sensitization Study in Guinea Pigs. Buehler Test. [redacted] Data owner: Helm AG Report No.: 12174-08 Date: 2009-03-11 GLP: yes unpublished

Guideline: 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

Dates of experimental work: 2008-11-30 - 2009-01-02

Executive Summary
A skin sensitization study was conducted on 15 male and 15 female short-haired albino guinea pigs to determine if test substance Glyphosate produced a sensitizing reaction. Males and females were assigned to each of two groups, designated Groups I (5/sex) and II (10/sex). Group I animals remained untreated during the induction phase of the study and served as a naive control group. Group II animals, the test group, were treated with 400 mg of test substance moistened with 2 mL of deionized water. The animals were treated once weekly for three weeks, for a total of three treatments. After a two week rest period, all animals (Groups I and II) were challenged at a virgin test site with an application of 400 mg of test substance moistened with 2 mL of deionized water. The test substance 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. 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 sensitizing.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water/ alpha-Hexylcinnamaldehyde

3. Test animals:

Species:	Guinea Pig
Strain:	Hartley-Albino
Source:	Charles River Laboratories; Wilmington, MA
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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 sensitizing reaction in guinea pigs.

III. CONCLUSION
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 sensitizing.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/08	[redacted]	2005f	Glyphosate acid technical – Dermal Sensitization in Guinea Pigs (Buehler Method) [redacted] Data owner: Helm AG Report No.: PSL 15279 Date: 2005-04-04 GLP: yes unpublished

Guideline:	US EPA OPPTS 870.2600 OECD 406.
Deviations:	No deviations from the Study Plan
Dates of experimental work:	2004-05-03 - 2004-06-03

Executive Summary
A dermal sensitization test was conducted with guinea pigs to determine the potential for Glyphosate Acid Technical to produce sensitization after repeated topical applications. A 70% w/w mixture of the test substance in distilled water was topically applied to twenty healthy test guinea pigs, once each week for a three-week induction period. Twenty-seven days after the first induction dose, a challenge dose of the test substance at its highest non-irritating concentration (HNIC, determined in the preliminary irritation screen to be a 70% w/w mixture in distilled water) was applied to a naive site on each guinea pig. A naive control group (ten animals) was maintained under the same environmental conditions and treated with the test substance at challenge only. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema. Based on the results of this study, the test substance is

not considered to be a contact sensitizer.

The positive response observed in the historical positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Distilled water/ alpha-Hexylcinnamaldehyde Technical

3. Test animals:

Species:	Guinea pig
Strain:	Hartley albino
Source:	Elm Hill Breeding Labs, Chelmsford, MA
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

B: STUDY DESIGN AND METHODS

In life dates: 2004-05-03 to 2004-06-03

Animal assignment and treatment:
In order to evaluate the sensitization response at challenge, two indices were used; one for incidence and one for severity (Ritz, H. and Buehler, E., 1980) 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 l/ 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 (Robinson, et al., 1990): At the 24-hour andlor 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

III. CONCLUSION

Based on these findings and on the evaluation system used, Glyphosate Acid Technical is not considered to be a contact sensitizer.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/09	[redacted]	2008	Skin Sensitisation Test for Glyphosate Technical in Guinea Pigs. Buehler Test. [redacted] Data owner: Helm AG Report No.: RF-3996.318.431.07 Date: 2008-09-30 GLP: yes unpublished

Guideline: 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.

Dates of experimental work: 2008-06-12 - 2008-07-12

Executive Summary

The skin sensitisation test with GLYPHOSATE TECHNICAL in guinea pigs (Cavia porcellus) was carried out according to the Buehler Test Method and the Guideline 406 of the Organisation for Economic Co-operation and Development (OECD, 1992), in order to evaluate its skin sensitisation potential. A pilot study was conducted in order to determine the suitable doses of test substance for induction and challenge applications. The induction and challenge doses chosen were 0.5 g of test item (equivalent to 1 ml, of a 50% w/v test solution). One test solution for each application was made using DMSO as vehicle to increase the contact area and to allow transdermal absorption. A sample of each solution was sent for quantitative analysis using High Performance Liquid Chromatography (HPLC) equipment. After each application cotton lint patches were held in contact with the skin for an approximated 6-hour exposure period. Twenty treatment animals were exposed to the test item in inductions and challenge applications. Ten control animals were exposed to the vehicle on inductions and to the test item on challenge application. Skin reactions were evaluated approximately 30 and 54 hours after each application by clinical examination (inductions) and according to the Magnusson & Kligman's grading scale (pilot study and challenge). 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. Therefore, the epidermal application of GLYPHOSATE TECHNICAL using DMSO as vehicle did

not cause skin sensitisation

in guinea pigs, according to the Buehler Test Method.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate

Identification: Glyphosate Techical
Description: White powder
Lot/Batch #: 20070606
Purity: 98.05%

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

2. Vehicle and/ or positive control: DMSO

3. Test animals:

Species:	Guinea Pig
Strain:	Hartley
Source:	ANILAB LTDA., SP, Brazil
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, *ad libitum*
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

B: 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

III. CONCLUSION

The epidermal application of GLYPHOSATE TECBNICAL using DMSO as vehicle did not cause skin sensitisation in guinea pigs, according to the Buehler Test Method.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/10	[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: yes unpublished

Guideline: § 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

Dates of experimental work: 05 September 2005 to 13 October 2005

Executive Summary

Twenty test and ten control animals were used for the main study. Two phases were involved in the main test; an induction of a response by intradermal injection and topical application and a topical challenge of that response. Based on the results of sighting tests, the concentrations of test material for the topical induction and topical challenge phases were selected as follows: Intradermal Induction : 0.195% (v/v) in isotonic sodium chloride solution; Topical Induction : 60% (w/w) in distilled water; Topical Challenge : 60% (w/w) and 30% (v/v) in distilled water. Under the conditions of the test, the test material produced a 0% (0/18) sensitisation rate and was classified as a non-sensitiser to guinea pig skin.
Glyphosate Technical 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.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	white powder
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	No data available

2. Vehicle and/ or positive control: Distilled water

3. Test animals:

Species:	Guinea Pigs
Strain:	albino Dunkin Hartley
Source:	Charles River (F-69592 L'Arbresle)
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

B: 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 B & Kligman A M, (1969) J. Invest. Dermatol. 52: 268 - 276. 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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).

C. BODY WEIGHT

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

D. NECROPSY

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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/11	[redacted]	1996d	Glyphosate Acid: Skin Sensitisation To The Guinea Pig [redacted] Data owner: Syngenta Report No.: CTL/P/4699 Date: 1996-08-23 GLP: yes not published

Guideline: OECD 406 (1992): OPPTS 870.2600 (2003): 96/54/EC B.6 (1996)

Deviations: None

Dates of experimental work: 1996-07-01 to 1996-08-23

Excecutive summary

In a dermal sensitization 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 (1970)***. The study involved the treatment of guinea pigs using two procedures: the potential induction of an immune response and a challenge of that response. In the main study, a 0.1% w/v preparation in deionised water was used for the intradermal injections and a 75% w/v preparation in deionised water was used for the topical application. For challenge concentrations of 75 and 30% (w/v) in deionised water were used.

Challenge of previously-induced guinea pigs with a 75% w/v preparation of glyphosate acid in deionised water elicited a response characteristic of an irritant.

Challenge of previously-induced guinea pigs with a 30% w/v preparation of glyphosate acid in deionised water did not elicit a skin sensitisation response.

A positive control study using hexylcinnamaldehyde demonstrated the sensitivity of the test system.

Glyphosate acid is not a skin sensitiser under the conditions of the test.

I. MATERIALS AND METHODS

A: MATERIALS:

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	Charles River UK Limited, Margate, Kent, UK
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 *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 17 ± 2°C Humidity: 40-70% Air changes: Approximately 25 changes/hour Photoperiod: 12 hours light, 12 hours dark

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 25 April 1995 End: 19 May 1995

Animal assignment and treatment: In a dermal sensitization 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 (1970***). 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 hexylcinnamaaldehyde (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.

II. 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 5.2-36: 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	

III. CONCLUSION

Glyphosate acid is not a skin sensitiser under the conditions of the test.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/12	[redacted]	2007	Glyphosate Technical Material: Skin Sensitisation (Local Lymph Node Assay In The Mouse). [redacted] Data owner: Syngenta Report No.: R61837/1004 Date: 2007-02-09 GLP: yes not published

Guideline:	OECD 429 (2002): OPPTS 870.2600 (2003): 2004/73/EC B.42 (2004)
Deviations:	None
Dates of experimental work:	2007-01-05 to 2007-02-09

Excecutive summary

A sample of glyphosate technical material was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay ***(Kimber and Basketter 1992, Kimber et al, 1994)***. 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. Approximately 25µl of a 10, 25 or 45% w/v preparation of the test substance in dimethyl sulphoxide was applied 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 ^{3}H-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 10ml 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 a known sensitiser (hexylcinnamaldehyde) ***(Dearman et al, 2001)***.

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.

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.

In conclusion, glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

I. MATERIALS AND METHODS

A: MATERIALS:

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	Harlan UK Limited, Shaw's Farm, Blackthorne, Bicester, Oxon, UK.
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.

B: 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 ^{3}H-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 10ml 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 ***(Kimber et al, 1994)***. Consequently, a test substance which does not fulfil the above criterion is designated as unlikely to be a skin sensitiser.

II. 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 5.2-37: 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 5.2-38: Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lynph 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

III. CONCLUSION

Glyphosate technical material is considered not to be a skin sensitiser under the conditions of the test.

Annex point	Author(s)	Year	Study title
IIA, 5.2.6/13	[redacted]	2011	Glyphosate technical: Local lymph node assay in the mouse [redacted] Data owner: Syngenta Report No.: 10/218-037E Date: 2011-04-21 GLP: yes not published

Guideline:	OECD 429 (2010)
Deviations:	None
Dates of experimental work:	2010-10-20 to 2011-04-21

Excecutive summary

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.

The test item solutions were applied on the dorsal surface of ears of experimental animals (25 µL/ear) for 3 consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. On Day 6. Five hours

prior to termination animals were intravenously injected via the tail vein with tritiated methyl thymidine (^{3}HTdR). Cell proliferation in the local lymph nodes was measured by incorporation of ^{3}HTdR and the values obtained were used to calculate stimulation indices (SI).

Groups of 4 mice received 50, 25 or 10% w/v glyphosate technical in propylene glycol (PG), PG alone (negative controls) or 25% α-Hexylcinnamaldehyde in PG (positive controls).

No mortality, systemic toxicity or local irritation was observed during the study. No treatment related effects were observed on animal body weights in any treated groups.

Stimulation index values of the test item were 1.0, 1.0 and 1.2 at treatment concentrations of 50, 25 and 10% (w/v), respectively. A significant lymphoproliferative response (stimulation index value of 12.2) was noted for α-Hexylcinnamaldehyde in this experiment, confirming the validity of the protocol used for this study.

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.

I. MATERIALS AND METHODS

A: MATERIALS:

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	Elevage Janvier, B.P. 4105, Route des Chênes Secs, 53940 Le Genest-St-Isle, France
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 *ad libitum*
Water	Tap water *ad libitum*
Environmental conditions	Temperature: 22±3°C Humidity: 30-70% Air changes: 15-20 air changes per hour Photoperiod: 12 hours light / 12 hours dark

B: 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}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}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 LCNs 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}HTdR incorporation was measured for up to 10 minutes per sample. The β-counter expressed the ^{3}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}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.

II. 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.

III. CONCLUSION

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.

IIA 5.3 Short term toxicity

The 2001 EU evaluation concluded low oral subacute and subchronic toxicity of glyphosate acid.
The lowest NOEL observed was 50 mg/kg bw/day in a subacute rangefinding study. However the subsequent definitive 90-day study did not demonstrated these effects when dosed up to 1000 mg/kg/day and therefore the rangefinder observations were considered spurious. NOAEL values ranged from about 168 to over 1200 mg/kg bw/day in 90-day feeding studies in rats, which reflect differences in dose selection. Some lower values found in dogs were obtained in studies of limited scientific value but these rather equivocal findings were not confirmed in more recent experiments using much higher doses. Changes in clinical chemistry parameters and liver weight changes might indicate liver effects at high doses in rats. Soft stools and diarrhea, together with occasionally reduced body weight gain and food consumption, suggest irritation of the gastrointestinal tract. In some oral rat studies and in one experiment with mice, effects on salivary glands are considered adaptive responses to local oral irritation due to chewing an organic acid mixed into the diet (See IIA 5.10). Repeated dermal exposure of rabbits and rats to glyphosate did not result in any systemic effects. Dermal irritation was observed only at doses as high as 5000 mg/kg bw/day in rabbits or 1000 mg/kg bw/day in rats. Subacute inhalation toxicity of glyphosate

active ingredient in rats is low. Up to the highest tested concentration of 3.8 mg/L air, neither local nor systemic toxicity was noted upon repeated exposure.

IIA 5.3.1 Oral 28-day toxicity

No new sub-acute oral toxicity studies have been performed. Table 5.3-1 summaries the studies performed in rats and dogs previously evaluated in the 2001 EU glyphosate evaluation. Despite some slight changes in haematological, clinical chemistry parameters and soft faeces, there were no adverse effects in rats after exposure to glyphosate. In dogs there were no treatment-related findings observed up to 1000 mg/kg bw/day. In rats, a NOAEL was found to be 50 mg/kg bw day when administered for four weeks in a range finding study, but these effects were not identified in the subsequent 90-day definitive study and thus were considered spurious. The 2004 JMPR review also concluded "In contrast to a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989), the incidence of nephrocalcinosis in the 13-week study ([redacted] 1991) was evenly distributed among dose groups and sexes and did not follow a dose–response relationship, and is therefore clearly not treatment-related. Thus, the previous finding was not confirmed". The glyphosate taskforce proposes that the NOAEL for this study is 250 mg/kg bw/day and the LOAEL is 1000 mg/kg bw/day based on the increased incidence in soft faeces in males.

Table 5.3-1: Summary of sub-acute toxicity studies with glyphosate acid

	Reference (Data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B-5.3.1, Glyphosate Monograph [redacted] 1991f (FSG)	28-day, oral diet Rat, Wistar	0, 200, 2000, 20000 ppm	NOEL: 2000 ppm (≅ 100 mg/kg bw/day)	20000 ppm ((≅ 1959 mg/kg bw/day (calculated value)) ALAT and BUN ↑, Hb ↓ (effects were reversible)
	Annex B-5.3.1, Glyphosate Monograph [redacted] 1989 (CHE)	4-week, oral diet Rat, Sprague-Dawley	0, 50, 250, 1000, 2500 mg/kg bw/day	NOEL: 50 mg/kg bw/day NOAEL: 250 mg/kg bw/day	≥ 250 mg/kg bw/day: Increased incidence of (very mild to slight) nephrocalcinosis in ♀; equivocal increases in serum ALAT and AP (without corollary histopatholgy) 1000 mg/kg bw/day: soft faeces in ♂; increased plasma phosphate ♂
	Annex B-5.3.1, Glyphosate Monograph Goburdhun & [redacted] 1989 (CHE)	21 / 14-day, oral capsule Dog, Beagle	21-day: 100, 300, 1000 mg/kg bw/day (1st, 2nd, 3rd week) 14-day: 1000 mg/kg bw/day	1000 mg/kg bw/day	No remarkable findings

↓ = decreased; ↑ = increased;

IIA 5.3.2 Oral 90-day toxicity (rodents)

The 2001 EU glyphosate evaluation concluded that the lowest NOEL was about 100 mg/kg bw/day in 90-day feeding studies in rats with the first effects occurring in the range 250-300 mg/kg bw/day, however in most studies higher NOAELs were established. Liver effects were observed indicated by clinical chemistry and organ weight changes in rats. Soft stools and diarrhoea, together with occasionally reduced bodyweight gain and food consumption, suggest irritation of the gastrointestinal tract at high dose levels. Decreased urinary pH was noted in some studies at high doses, with blood in the urine reported in one study. In some oral rat studies and in one mouse study, cellular alterations in salivary glands were observed upon histopathological examination. The glyphosate taskforce believes these salivary gland

findings are a non-adverse adaptive response to treatment with a low pH diet (See IIA 5.10). Overall the mouse is less sensitive than the rat with only effects observed on body weight at very high dose levels.
Additional studies included in this submission also demonstrate that glyphosate is of low oral toxicity. The NOAELs ranged between 79-765 mg/kg bw/day and the lowest LOAEL observed in rats was 569 mg/kg bw/day ([redacted] 1995). Consistent with the previously reviewed studies effects were observed on clinical chemistry parameters (often non-specific markes of mild toxicity), bodyweight gain and food consumption at high dose levels. Additionally caecum distension and an increase in caecum weight were observed in both a 13 week rat study ([redacted] 1995) and a 13 week mouse study ([redacted] 1995) . This effect appeared to be dose related at very high dose levels in both species but was not assocated with any corollary histopathological changes and is therefore of uncertain toxicological relevance. In contrast, in another 13 week dietary rat study ([redacted] 1996) mucosal atrophy of the caecum was observed where there was no associated weight change. Another finding in male mice that had been previously reported was cystitis of the urinary bladder in animals dosed at 6295 mg/kg bw/d.
Overall the NOEL/NOAEL levels established in the 90-day dietary studies in rats varied between approximately 80 and 1600 mg/kg bw/d, the wide range of values being an artifact of dose selection. The lowest effect dose was in the range of 550 mg/kg bw day ([redacted] 1995). Mice appeared to be less sensitive, with substantially higher NOAEL values, the lowest of which was 600 mg/kg bw/d.

Table 5.3-2: Summary of short-term toxicity studies with glyphosate acid

	Reference (data owner)*	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B-5.3.2.1, Glyphosate Monograph [redacted] 1989 (CHE 1)	90-day, oral diet Rat, Sprague-Dawley	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Clinical chemistry, cellular alterations in paratoid salivary glands, decreased urinary pH (♂ only)
	Annex B-5.3.2.1.2, Glyphosate Monograph IIA 5.3.2/02 [redacted] 1993 (Alkaloida, ALK)	13-week, oral diet Rat, Sprague-Dawley	0, 2000, 6000, 20000 ppm (≅ 0, 125.2/156.3, 371.9/481.2, 1262/1686.5 mg/kg bw/day ♂/♀)	NOAEL = 6000 ppm (≅ 379.1/481.2 mg/kg bw/day ♂/♀)	1262.1/1686.5 mg/kg bw/day ♂/♀ (calculated values): Diarrhea, blood in urine, organ weight changes
	Annex B-5.3.2.1, Glyphosate Monograph [redacted] 1992 (FSG)	90-day, oral diet (+ 2-week recovery) Rat, Wistar	0, 200, 2000, 20000 ppm	2000 ppm (147/196 mg/kg bw/day (♂/♀))	1000 mg/kg bw/day: Clinical chemistry, reduced body weight gain

	Reference (data owner)*	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B-5.3.2.1, Glyphosate Monograph [redacted] 1989 (Barclay, BCL)	13-week, oral diet (+ 5-week recovery) Rat, CD	0, 2000, 3000, 5000, 7500 ppm	NOEL: 7500 ppm (ca. 375 mg/kg bw/day)	No treatment-related effects
	Annex B-5.3.2.1, Glyphosate Monograph [redacted] 1987 (MON)	90-day, oral diet Rat, Sprague-Dawley	0, 1000, 5000, 20000 ppm	NOEL: 20000 ppm (1267/1623 mg/kg bw/day (♂/♀))	No treatment-related effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.2/01 [redacted], 1996 (1st revision of study) (SYN)	90-day, oral, diet Rat, Alpk:APfSD	0. 1000, 5000, 20000 ppm	NOEL: 5000 ppm (414/447 mg/kg bw/day ♂/♀	1612/1821 mg/kg bw/day (♂/♀): Reduced body weight, food consumption and utilisation reduced in ♂ only, clinical chemistry changes (changes (↑ALP, ALT)
	IIA 5.3.2/03 [redacted] 1996 (NUF)	90-day, oral, diet Rat, Sprague-dawley	0, 1000, 10000, 50000 ppm (≅ 0, 79/90, 730/844, 3706/4188 mg/kg bw/day (♂/♀)	1000 ppm (79/90 mg/kg bw/day ♂/♀)	730/844 mg/kg bw/day (♂/♀): Clinical chemistry changes, mucosal atrophy of the caecum
	IIA 5.3.2/04 [redacted], 1995 (ALS 1)	13-week, oral, diet Rat, Sprague-Dawley	0, 3000, 10000, 30000 ppm (≅ 168/195, 569/637, 1735 / 1892 mg/kg bw/day (♂/♀))	NOAEL: 3000 ppm (168/195 mg/kg bw/day ♂/♀)	10000 ppm (≅ 569/637 mg/kg bw/day (♂/♀)): caecum distention; caecum weight increased without histopathological findings 30000 ppm (≅ 1735 / 1892 mg/kg bw/day (♂/♀)): caecum distention; caecum weight increased without histopathological findings, reduced body weight and lower food efficiency; increased AP activity in ♀

Reference (data owner)*		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.2/05 [redacted] 1995 (ALS 2)	13-week, oral, diet Mouse, ICR	0, 5000, 10000, 50000 ppm (≅ 0, 600/765, 1221/1486, 6295/7435 mg/kg bw/day (♂/♀)	NOAEL: 5000 ppm (600/765 mg/kg bw/day ♂/♀)	10000 ppm (1221 / 1486 mg/kg bw/day ♂/♀): caecum distention ♀, increased absolute and relative caecum weight 50000 ppm 6295 / 7435 mg/kg bw/day ♂/♀):: reduced bodyweight and food consumption, decreased fodd efficiency ♀, haematological changes in ♀, blood chemistry changes, caecum distention and increased absolute and relative caecum weight in both sexes without histopathological changes in the caecum; cystitis of the urinary bladder in ♂
Studies from the 2001 evaluation	Annex B-5.3.2.2, Glyphosate Monograph [redacted] 1991 (CHE 2)	13-week, oral, diet, Mouse, CD-1	0, 200, 1000, 4500 mg/kg bw/day	NOEL: 4500 mg/kg bw/d	No treatment-related effects

*: Number refers to data presented in Figure 5.11-2 in chapter IIA 5.11.
↓ = decreased; ↑ = increased;

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/01	[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 GLP: yes not published

Guideline:	No guideline statement, but in accordance with OECD 408 (1998), OPPTS 870.3100 (1998), 2001/59/EC B.26 (2001)
Deviations:	None
Dates of experimental work:	1996-02-25 to 1996-05

Executive Summary
In a subchronic toxicity study, groups of twelve male and twelve female Alpk:AP (now known as Alpk:APfSD) Wistar-derived rats were fed diets containing 0 (control), 1000, 5000 or 20000 ppm glyphosate acid for 90 consecutive days.

Clinical observations, bodyweights, food consumption and blood biochemistry parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathology examination

Males fed 20000 ppm glyphosate acid showed small reductions in bodyweight gain, food consumption and food utilisation efficiency compared to controls. There were associated minor reductions in plasma urea, total protein and triglycerides. Increased levels of plasma ALT and ALP were seen in both male and female rats at the top dose and are considered to indicate an altered liver metabolism but there was no evidence of histological change in this organ.

Effects in rats fed 5000 ppm glyphosate acid were confined to marginal increases in plasma ALT levels in females and in both plasma ALT and ALP levels in males. The magnitude and isolated nature of these effects leads them to be considered of no biological significance.

5000 ppm glyphosate acid is considered to represent the no-effect level for glyphosate acid in this study.

Conclusion
Glyphosate acid when fed to rats at a level of 20000 ppm resulted in reduced growth (males only) and associated changes in clinical chemistry. The latter also provided limited evidence for an altered liver metabolism which was not associated with any histopathological change.

Toxicologically significant changes were confined to the 20000 ppm glyphosate acid dose level and occurred mainly in male rats. The minor changes in clinical chemistry seen at 5000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the no-effect level for glyphosate acid in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P15
Purity:	97.4%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Alpk:APfSD
Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderly Park, Macclesfield, Cheshire, UK
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 1275cm^2
Environmental conditions:	Temperature: 21 ± 2°C
	Humidity: 36 - 60%
	Air changes: ≥ 15/hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1986-02-25 to 1986-05

Animal assignment and treatment:
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 study consisted of one control and three treatment groups each containing twelve male and twelve female rats.

Table 5.3-3: 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 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 untilisation
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, prostrate, 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 chemisty – 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).

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. 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.

C. BODY WEIGHT

No relevant differences in the mean body weight gain were noted between controls and animals given 1000 or 5000 ppm.

Bodyweight gain was reduced in male rats fed 20000 ppm glyphosate acid from the first week of the study. The bodyweights continued to diverge from control values as the study progressed, and final bodyweights were approximately 8% lower than those of controls (see Table 5.3-4).

Table 5.3-4: Intergroup comparison of bodyweight gain – selected timepoints from start of study

Timepoint	Mean cummulative bodyweight gain (g)							
	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**	272.00**	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)

D. 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 5.3-5: 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 5.3-6: 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)

E. 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.

F. 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 (ALAT) 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 20000ppm 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 5.3-7: 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)

G. URINALYSIS

There were no treatment-related findings.

H. 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. A 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.

III. CONCLUSION

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 amles and females, respectively).

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/02	[redacted]	1993	90 Day Range Feeding Study of Glyphosate in Rats [redacted] Laboratory Report No.: 011-0001 Data owner: Alkaloida - Monsanto Date: 1993 GLP: yes not published

Guideline:	No guideline statement, but in accordance with OECD 408 (1981)
Deviations:	None
Dates of experimental work:	1993-02-17 to 1993-05-21

Executive Summary
In a subchronic toxicity study, groups of ten male and ten female Sprague-Dawley rats were fed diets containing 0 (control), 2000, 6000 or 20000 ppm glyphosate acid for 13 consecutive weeks.

Clinical observations were done daily. Bodyweights and food consumption was assessed in weekly intervals. Haematological, blood biochemistry parameters, as well as urine analysis were conducted prior to start of treatment and at termination. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem, and selected organs were weighed and specified tissues were taken for subsequent histopathology examination

There were no mortalities in any of the dose groups. The only treatment-related clinicalfinding was diarrhoea in all high-dose males and in 9 high-dose females. Mean body weights were comparable between all groups. The mean body weight gains were significantly different between groups at several time points, but the total mean body weight gain was similar in all dose groups. There were no differences in food consumption. There were no treatment-related findings in the haematological and clinical chemistry parameters. Urinalysis showed marginal increases in the mean scores for ketones, blood, protein and RBCs in mid- and high-dose males, which were of no biological relevance. In mid- and high-dose females the number of rats with 1 RBC/hpf was also increased. Together with the appearance of blood in these groups this change seems to be treatment-related. There were no indications for microscopic hematuria found during the histopathological examinations.

Conclusion
Glyphosate acid when fed to rats at a level of 20000 ppm resulted in clinical signs of toxicity and slight increases in blood urine and RBC counts in urine. The minor changes in urinalysis seen at 6000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the NOAEL for glyphosate acid in this study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
Description:	Greyish-white or yellowish-white crystalline powder
Lot/Batch #:	46540992
Purity:	97.5%
Stability of test compound:	1994-10-01

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD®BR VAF/Plus®)
Source:	Charles River Laboratories, Portage, MI, USA
Age:	approx. 5 weeks
Sex:	Male and female
Weight at dosing:	♂ 208.2-249.9 g; ♀ 159.8-202 g
Acclimation period:	14 days
Diet/Food:	Purina certified Laboratory Rodent Chow 5002, *ad libitum,* (except during collection of urine samples)
Water:	Tap water, *ad libitum*
Housing:	Individually in stainless steel cages
Environmental conditions:	Temperature: 18 - 26°C Humidity: –55 ± 15% Air changes: ≥ 10/hour 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1993-02-17 to 1993-05-21

Animal assignment and treatment:
In a 90-day oral toxicity study groups of 10 Sprague-Dawley per sex received daily doses of 0, 2000, 6000 and 20000 ppm in the diet. The test diets were prepared weekly and stored at room temperature. Samples of the control and test substance diets were analysed for stability and homogeneity. Dietary preparations were analysed for achieved concentrations weekly during the first 4 weeks, and then every fourth week thereafter.

Table 5.3-8: Group mean weekly bodyweights and standard deviations (sd)

Test group	Dietary concentration (ppm)	Males	Females
Control	0	10	10
Low	2000	10	10
Mid	6000	10	10
High	20000	10	10

The experimental diets were prepared weekly by adding the appropriate amount of glyphosate acid to the diet.
The homogeneity of glyphosate in the diet was determined by replicate samples from each dose level. Achieved concentrations were determined by analysing samples from each dose level during weeks 1 to 4, and every fourth week thereafter The chemical stability of glyphosate acid in diet at room temperature was determined for all dose levels after preparation. Analysis was done by high performance liquid chromatography (HPLC).

Mortality
Each animal was checked for mortality or signs of morbidity twice daily during the treatment period.

Clinical observations
A check for clinical signs of toxicity was made once daily on all animals.

Body weight
The bodyweight of each animal was recorded on Day 1 before start of treatment and weekly thereafter.

Food consumption and untilisation
Individual food consumption was recorded weekly.

Ophthalmoscopic examination
The eyes of all animals from all dose groups were examined prior to initiation and just prior to termination. The examoination included macroscopic and opthalmoscopic examinations of the anterior portion of the eye, the optic media, and the ocular fundus.

Haematology and clinical chemistry
Prior to initiation and at termination, blood samples from all rats were taken by puncture of the retroorbital sinus after anesthetization. These samples were submitted for haematological and clinical chemistry examination. The following haematological parameters measured were measured: haemoglobin, haematocrit, red blood cell count, MCV, MCHC, MCH, thrombocytes, leucocytes, and a blood smear examination (including differential white cell count)..

For clinical chemistry analysis the following parameters measured: glucose, urea, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, creatinine, calcium, phosphorous, sodium, potassium, chloride, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase.

Urinalysis
Urine samples were collected overnight from all rats prior to start of treatment and at termination. During urine collection, the rats were individually housed in metabolism cages and denied access to food. The following parameters were measured: volume, pH, specific gravity, nitrites, blood, leukocytes proteins, glucose, ketones, bilirubin and urobilinogen.

Sacrifice and pathology
After 13 weeks of consecutive treatment, 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, seminal vesicles, and testes or ovaries. Organ-to-bodyweight and organ-to-brain weights were determined.

Tissue samples were taken from the following organs and preserved in buffered formalin: all gross lesions, adrenals, aorta, bone marrow (sternum), brain, caecum, colon, duodenum, epididymides, eyes, femur (incl. articular surface), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal turbinate, nasal cavity, oesophagus, ovaries, pancreas, pituitary gland, prostrate, rectum, salivary glands (sublingual), spinal cord, sciatic nerve, skeletal muscle, skin, spleen, stomach, testes, thymus, thyroid/parathyroids, trachea, urinary bladder All tissue sampled freom the control and high dose group were examined histopathologically. From the mid- and low- dose only tissues from lungs, liver, kidneys and gross lesions were subjected to histopathological evaluation.

Statistics
Mean and standard deviation were calculated for all quantitative data. Comparisons with controls were done using ANOVA with a post hoc Dunnett´s t Test or Duncan´s multiple range test. A 95% confidence level ($p<0.05$) was used to determine statistically significant differences between control and treated groups.

II. RESULTS AND DISCUSSION

A. MORTALITY

There were no mortalities.

B. CLINICAL OBSERVATIONS

The only obvious treatment-related finding was diarrhoea observed in 10/10 males and 9/10 females in the high-dose group. Diarrhoea was also observed in one male of the low dose group.

Table 5.3-9: Intergroup comparison of clinical findings

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Finding	0	2000	6000	20000	0	2000	6000	20000
Diarrhoea	0/10	1/10	0/10	10/10	0/10	0/10	0/10	9/10

* Statistically significant from controls, p<0.05)
** Statistically significant from controls, p<0.01

C. BODY WEIGHT

Mean body weights were not significantly different between testsubstance and control animals. The mean body weight gains were however significantly different from controls at several intervals (see Table 5.3-10). The total mean body weights over the entire study period were not different.

Table 5.3-10: Intergroup comparison of body weights and bodyweight gain – selected timepoints from start of study

Timepoint	Mean bodyweight or body weight gain (g)					
	Initial body weight	Final body weight	Weigh gain Day 43	Weight gain Day 50	Weight gain Day 85	Total weight gain
Dose (ppm)	Males					
0	224.6	510.3	-3.4	39.4	12.6	285.7
2000	231.0	530.8	9.1	29.3	9.2	299.7
6000	229.0	526.0	17.1*	28.6	11.1	297.0
20000	224.6	512.9	21.1**	19.3**	1.9*	288.4
	Females					
0	178.3	321.9	6.9	16.6	6.1	143.7
2000	179.7	331.3	7.5	14.7	6.2	151.6
6000	176.0	316.1	7.8	15.7	2.4	140.1
20000	176.9	309.0	7.8	4.8*	2.8	132.0

* 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)

D. FOOD CONSUMPTION AND TEST SUBSTANCE INTAKE

There were no treatment-related effects. The mean food consumption in treatment groups was not significantly different from controls..

Calculated mean test compound intakes are presented in the following table.

Table 5.3-11: Overall mean test compound intake (mg/kg bw/day)

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Achieved dose (mg/kg bw/day)	0	125.2	371.9	1262.1	0	156.3	481.2	1686.5

E. OPHTHALMOSCOPIC EXAMINATION

There were no test substance-related ophthalmological findings at the end of the treatment period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related differences noted in any dose group.

Blood clinical chemistry

At termination, low dose males had an significantly decreased mean K value as compared to control animals. Since this finding was restricted to the low dose group, it was not considered to be treatment-related.

High dose males showed a significantly lower ALT value at termination than control males. However, because of the minor degree of change, the absence of a dose-response at the other dose levels this findings was considerd to be of no biological relevance. The increased total bilirubin value in high-dose males at termination is also considered to be of no biological relevance.

Table 5.3-12: Intergroup comparison of selected clinical chemistry parameters pre-dose and at termination

	Dietary concentration of glyphosate acid (ppm)							
	Males				**Females**			
Parameter	**0**	**2000**	**6000**	**20000**	**0**	**2000**	**6000**	**20000**
ALT(U/L)	48	35	41	32*	42	39	35	30
Total bilirubin (mg/dL)	0.3	0.3	0.5	0.6*	0.2	0.3	0.3	0.3
Potassium(mmol/L)	6.9	5.8**	6.2	6.8	5.8	6.2	5.7	6.2
Potassium(mmol/L) (pre-dose)	5.2	5.1	4.8	5.2	5.2	4.6*	4.4**	4.7

* Statistically significant from controls, $p<0.05$)
** Statistically significant from controls, $p<0.01$

G. URINALYSIS

At termination there were marginal increases in the mean scores for ketones, blood, protein and red blood cells (RBCs) in the mid- and high-dose males. It has to be noted that the unusual appearance of ststistical differences among zeros (ketones) or 1 (protein) was the result of rounding (see Table 5.3-13). The number of affected rats, and their respective scores are shown in Table 5.3-14 below. The lower protein value observed in high-dose males was statistically different from control, but still within the normal range. The presence of blood and RBCs is minimally elevated in all treated groups when copmpared to control animals. However, the observation of a few RBCs is common in male rats and this mild degree cannot be attributed unequivocally to the test substance. In mid- and high-dose females the number of rats with 1 RBC/hpf was also increased. Together with the appearance of blood in these groups this change seems to be treatment-related. In addition, there were no indications for microscopic hematuria found during the histopathological examinations. Therefore, at least the changes in urine analysis parameters in the mid-dose group are considered not to be adverse effects.

Table 5.3-13: Urineanalysis at termination – group mean values for selected parameters

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones (mg/dL)	0	0	0*	0	0	0	0	0
Blood (mg/dL)	0	2	1	2**	0	0	1	1
Protein (mg/dL)	1	1	1	1*	0	0	0	0
RBC (cells/hpf)	0	1	1	2**	0	0	1**	1

* Statistically significant from controls, p<0.05)
** Statistically significant from controls, p<0.01

Table 5.3-14: Urineanalysis at termination – affected animals for selected parameters

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Parameter	0	2000	6000	20000	0	2000	6000	20000
Ketones (Score 0)	10/10	10/10	6/10	9/10	10/10	10/10	10/10	10/10
Ketones (Score 1)	4/10	1/10	4/10	1/10	0/10	0/10	0/10	0/10
Blood (score 0)	8/10	3/10	3/10	2/10	8/10	8/10	5/10	7/10
Blood (score 1)	2/10	3/10	3/10	3/10	2/10	2/10	2/10	0/10
Blood (score 2)	0/10	2/10	2/10	1/10	0/10	0/10	2/10	3/10
Blood (score 3)	0/10	0/10	1/10	0/10	0/10	0/10	0/10	0/10
Blood (score 4)	0/10	2/10	1/10	4/10	0/10	0/10	1/10	0/10
Protein (score 1)	5/10	6/10	7/10	3/10	1/10	2/10	3/10	0/10
Protein (score 2)	4/10	1/10	2/10	1/10	0/10	0/10	0/10	0/10
RBC (score 1)	3/10	7/10	8/10	6/10	1/10	2/10	8/10	6/10
RBC (score 2)	-	-	-	1/10	-	-	1/10	-
RBC (score 3)	-	-	-	0/10	-	-	-	-
RBC (score 4)	-	-	-	2/10	-	-	-	-

-: no finding

H. NECROPSY

Organ weights

The mean adrenal weights in high- and low-dose males was significantly decreased, whereas the relative adrenal weights were only signicantly decreased high dose males. In females of the high-dose group relative spleen weights were significantly increased when compared to controls.

Table 5.3-15: Results from absolute and relative organ weight determination

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
	0	2000	6000	20000	0	2000	6000	20000
Mean adrenal weight (g)	0.070	0.059*	0.061	0.052**	0.093	0.080	0.086	0.080
Relative adrenal weight (% bw)	0.014	0.012	0.012	0.011**	0.031	0.026	0.029	0.028
Spleen weight (g)	0.785	0.786	0.808	0.752	0.548	0.598	0.589	0.648
Relative spleen weight (% bw)	0.257	0.250	0.279	0.266	0.180	0.192	0.198	0.223**

* Statistically significant from controls, p<0.05)
** Statistically significant from controls, p<0.01

Gross pathology
A few gross lesions were noted at necropsy in all dose groups. The most prevalent abnormality was the swollen, reddened sublingual salivary glands. This lesion was observed in control males, and in one treated male and female of some test substance groups (see Table 5.3-16). Therefore, this finding is considered not to be related to treatment.

Table 5.3-16: Summary of necropsy findings

	Dietary concentration of glyphosate acid (ppm)							
	Males				Females			
Finding	0	2000	6000	20000	0	2000	6000	20000
Sublingual salivary glands: enlarged and/or reddened	1/10	0/10	1/10	1/10	0/10	1/10	0/10	1/10

Histopathology
There were no histopathological findings related to treatment. The incidence of findings was low and of a type commonly found in rats of this strain and age

III. CONCLUSION

Glyphosate acid when fed to rats at a level of 20000 ppm resulted in clinical signs of toxicity and slight increases in blood urine and RBC counts in urine. The minor changes in urinalysis seen at 6000 ppm glyphosate acid were considered biologically insignificant and this was, therefore, judged to be the NOAEL for glyphosate acid in this study.

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/03	[redacted]	1996	Technical Glyphosate: Ninety Day Sub-Chronic Oral (Dietary) Toxicity Study In The Rat [redacted] SPL Project No.: 434/016 Date: 1996-07-16 GLP: yes unpublished

Guideline:	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.
Dates of experimental work:	1995-08-11 - 1996-01-30

Executive Summary
The test material was administered by dietary admixture to three groups, each of ten male and ten female Sprague Dawley (CD) strain rats, for ninety consecutive days, at dietary concentrations of 1000, 10,000 and 50,000 ppm (equivalent to an estimated mean achieved dose level of male; 79, 730 and 3706 mg/kg/day, female; 90, 844 and 4188 mg/kg/day respectively). A further group of ten males and ten females was exposed to basal laboratory diet to serve as a control.
Clinical signs, bodyweight, food and water consumption were monitored during the study. Haematology, blood chemistry and urinalysis were evaluated for all animals at the end of the study. Ophthalmoscopic examination was also performed. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed.

At 1,000 ppm no treatment-related effects were noted in any of the investigation conducted.
In the mid dose group statistically significant reduction in plasma calcium concentration and an increase in alkaline phosphatase was observed in both sexes. Histopathology revealed a mucosal atrophy of the caecum in this group. No other treatment-related findings were observed in this dose group.
Animals treated with 50,000 ppm showed soft faeces/diarrhoea from Day 4 which continued throughout the study period. In addition, body weight gain, food intake and food efficiency in animals of both sexes in the high-dose group was reduced over the first four weeks of treatment when compared with controls. Bodyweight development, food consumption and efficiency recovered in females and were comparable with the control group by the end of the treatment period. In males bodyweight gain showed only a partial recovery, and an adverse effect on dietary intake was still apparent during the remaining treatment period.
Animals of both sexes treated with 50,000 ppm showed a statistically significant reduction in plasma calcium concentration and creatinine levels, as well as an increase in alkaline phosphatase and inorganic phosphorous in comparison with controls. Reductions in total protein and albumin were observed only in high-dose females. Urinalysis revealed increased levels of haemoglobin when compared with controls. Microscopic examination of sediment revealed unidentified particulate matter in the samples obtained from males treated with 50,000 ppm. At necropsy high dose animals of both sexes showed an enlarged and fluid-filled caecum, as well as statistically increased liver and kidney weights. Microscopic examination of the caecum revealed changes identified as mucosal atrophy for animals of both sexes treated with 50,000.

Conclusion
Dietary administration of the test material, technical Glyphosate, to rats for a period of 90 consecutive days at 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 1,000 ppm (equivalent to 79 mg/kg bw/day for males, and 90 mg/kg bw/day for females).

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	Charles River Limited, Manston, Kent, UK
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

B: 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, prostrate, 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 $p < 0.1$ $^{(*)}$.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. 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.

C. 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 5.3-17). 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 bodyweight gain remaining slightly lower than the control group values during the subsequent weeks of treatment.
Bodyweight development was unaffected by treatment with the test material at the remaining dose levels.

Table 5.3-17: Group mean weekly bodyweights and standard deviations (sd)

Dietary concentration (ppm)		Bodyweight (g) at Day													
		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

D. 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 5.3-18). 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 bodyweight 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 5.3-18: 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)	(-1)	(0)	(3)	(4)	(0)	(9)

( ) - % change compared to control group * - Week 13 comprises six days only

E. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

F. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular effects for either sex noted were detected during the study.

G. HAEMATOLOGY AND CLINICAL CHEMISTRY

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 5.3-19). 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 5.3-19: 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	–	–
		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 ($p < 0.001$)

H. 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 5.3-20). 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 5.3-20: Urinanalytical 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 × 10^6 ery/L
++ - ca. 50 × 10^6 ery/L
+++ - ca. 250 × 10^6 ery/L

I. NECROPSY

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 5.3-21).
There were no further direct effects of treatment.

Table 5.3-21: 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.

III. CONCLUSION

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).

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/04	[redacted]	1995	HR-001 : 13-week Subchronic Oral Toxicity Study in Rats. [redacted] Laboratory Report No.: IET 94-0138 Data owner: Arysta LifeScience Date: 1995-07-20 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984; OECD 408 (1981)
Deviations:	None
Dates of experimental work:	1994-12-05 to 1995-07-20

Executive Summary

A subchronic oral toxicity study of HR-001 was conducted in Sprague-Dawkey (Crj:CD) rats of both sexes. The test substance was administered to the rats (12 animals/group/sex) by incorporating it into the basal diet at dose levels of 0, 3000, 10000, and 30000 ppm for a period of 13 weeks (91 days).

- 30,000 ppm goup: Body weights of males and females were slightly lower than in the control throughout the treatment period and statistically significant decreases were sporadically observed. The averaged food efficiency in males and females during the treatment period was slightly lower than that in the control. Females showed a significant increase in alkaline phosphatise (ALP) activity. Distention of the caecum was observed in 9/12 males and 7/12 females with statistical significance. Both sexes showed significant increases in absolute and relative weighs of the caecum (containing contents). Histologically, there were no abnormalities related to treatment in any tissues including the caecum.

- 10,000 ppm group: At necropsy, 3 males showed distention of the caecum. Organ weight analysis revealed a statistically significant increase (females) or an increasing trend (males) in obth absolute and relative weights of the caecum.

- 3,000 ppm group: There were no abnormalities attributable to the treatment in either sex.

Based on these results, the no-adverse effect level (NOAEL), minimum toxic level, and sure toxic level of HR-001 in Sprague-Dawley (Crj:CD) rats under the conditions of the present study were determined as follows.

	Males	Females
No-adverse-effect level (NOAEL)	3,000 ppm (168.4 mg/kg/day)	3,000 ppm (195.2 mg/kg/day)
Minimum toxic level	10,000 ppm (569 mg/kg/day)	10,000 ppm (637 mg/kg/day)
Sure toxic level	30,000 ppm (1735 mg/kg/day)	30,000 ppm (1892 mg/kg/day)

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Plain diet / none

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crj:CD
Source:	Charles River Japan, Inc.
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

B: 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.

Table 5.3-22: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	3000	12	12
Mid	10000	12	12
High	30000	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about50 g each) of each does level takent from top, middles 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 consumptionby 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 parameters were determined: 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. Fresg urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined 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 parameters were determined: 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, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths were noted in the control and treated groups of either sex.

B. CLINICAL OBSERVATIONS
There were no abnormalities related to the treatment in clinical signs 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.

C. 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.

D. 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 averaged, calculated from food consumption and nominal concentrations of the test substance, through the treatment period, were:

Dose (ppm)	Chemical Intake (mg/kg/day)	
	Male	Female
3 000	168.4	195.2
10 000	569	637
30 000	1735	1892

E. 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.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

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.

G. URINALYSIS

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.

H. NECROPSY

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.

III. CONCLUSION

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).

Annex point	Author(s)	Year	Study title
IIA, 5.3.2/05	[redacted]	1995	HR-001: 13-week Subchronic Oral Toxicity Study in Mice. [redacted] Laboratory Report No.: IET 94-0136 Data owner: Arysta LifeScience Date: 1995-07-24 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985; U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 408 (1981)
Deviations:	None
Dates of experimental work:	1994-12-15 to 1995-07-24

Executive Summary

In order to evaluate the subchronic toxicity of HR-001 in mice, the test substance was administered by incorporating itinto a basal diet to each dose group of 12 males and 12 females of SPF ICR mice (Crj:CD-1) at a dose level of 0, 5000, 10000 or 50000 ppm for a period of 13 weeks.

- 50,000 ppm goup: Males showed a depressed body weight gain associated with lowered food consumption and food efficiency throughout the treatment period. Decreased food efficiency was also observed in females. In haematological examinations, females showed decreases in hematocrit (Ht), haemoglobin concentration (Hb) and erythrocyte count (RBC). Blood chemical exmaniations revealed increases of alkaline phosphatase (ALP) in males and females and inorganic phosphorous (P) in females. At necropsy, males and females revealed increased incidences of distention of the caecum. In organ weight analysis, males and females showed increases of absolute and relative weights of the caecum. Histopathologically, males showed an increase in incidence of cystitis of the urinary bladder.

- 10,000 ppm group: Distention of the caecum was observed in one female at necropsy. In organ weight analysis, increasing tendencies were noted in absolute and relative weights of the cecum.

- 5,000 ppm group: There were no treatment-related changes in either sex in any parameters.

Based on these results, the no-adverse effect level (NOAEL), minimum toxic level, and sure toxic level of HR-001 in ICR (Crj:CD-1) mice under the conditions of the present study were determined as follows.

	Males	Females
No-adverse-effect level (NOAEL)	5,000 ppm (600.2 mg/kg/day)	5,000 ppm (756.0 mg/kg/day)
Minimum toxic level	10,000 ppm (1221 mg/kg/day)	10,000 ppm (1486 mg/kg/day)
Sure toxic level	50,000 ppm (6295 mg/kg/day)	50,000 ppm (7435 mg/kg/day)

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: HR-001
Description: White crystal
Lot/Batch #: T-941209
Purity: 97.56%
Stability of test compound: 26/12/1994

2. Vehicle and/or positive control: Plain diet / none

3. Test animals:

Species: Mouse
Strain: Crj:CD-1
Source: Charles River Japan, Inc.
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

B: STUDY DESIGN AND METHODS

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.

Table 5.3-23: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	12	12
Low	5000	12	12
Mid	10000	12	12
High	50000	12	12

Chemical analysis for homogeneity and concentration of the test substance in the diet were performed on samples (about50 g each) of each does level takent from top, middles 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 consumptionby 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 parameters were determined: 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 (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
At 13 week of treatment, all surviving animals were subjected to urinalysis. Fresg urine samples were collected by pressing the lumbodorsal region of the animals. Specific gravity was detmerined 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 parameters were determined: brain, spinal cord, sciatic nerve, pituitary, thyroids with parathyroids, thymus, adrenals, spleen, bone with marrow, tibio-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, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
There were no treatment-related abnormalities in clinical signs in the control and treated groups during the treatment period.

C. BODY WEIGHT
In the 50 000 ppm group, mean body weights of males were lower than those of the control form 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.

D. 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 5000 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:

Dose level (ppm)	Average chemical intake (mg/kg b.w. / 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 almost all measuring points during the treatment. Average food efficiency of males and females were stayed at 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.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophtalmological abnormalities in the animals of both sexes in the highest dose group and the control group.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

The significant changes observed after 13 weeks in the treated groups are summarized in the table below:

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
Hematocrit (Ht)	female	-	-	↘
Hemoglobin concentration (Hb)	female	-	-	↘
Erythrocyte count (RBC)	female	-	-	↘

↘: decreases of 92% to the control. P< 0.01 (estimated by Dunnett multiple comparison test).

In the 50 000 ppm group, females showed significant decreases in hematocrit (Ht), hemoglobin concentration (Hb) and erythrocyte count (RBC), 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 significant changes observed in the treated groups are summarized in the following table:

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 pyrvic transaminase (GPT)	Female	No change	No change	↘

Parameter	Sex	Dose group (ppm)		
		5 000	10 000	50 000
				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

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 pyrvic 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

G. URINALYSIS

In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

H. NECROPSY

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; 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 ; 0/12 of 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.

III. CONCLUSION

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).

IIA 5.3.3 Oral 90-day toxicity (dog)

In oral sub-chronic toxicity studies in the dog previously evaluated in the 2001 EU glyphosate evaluation, only unspecific signs of toxicity (decrease in body weight gain and food consumption) were observed at high dose levels. In two dietary dog studies performed at the same laboratory, liver effects of equivocal toxicological significance were observed at low doses (8-29 mg/kg bw/d). However, the previous evaluation found that because these findings were not confirmed in more recent studies using much higher dose levels they were not considered to be compound-related. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid and the IPA salt.

This NOAEL is supported by four recently conducted studies ([redacted] 2007, [redacted] 1999, [redacted] 1996 [redacted] 1996) . All of these studies are summarised below. All studies conform to current guidelines and were performed under GLP. Again the results of these studies are consistent with the studies previously submitted and reviewed, a reduction in body weight gain and food consumption was observed.

In the [redacted] (2007) study at 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft feces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) and body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11. Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day. 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. The relevance of these findings are uncertain given that in this study 1000 mg/kg bw/d clearly exceeded the maximum tolerated dose.

Overall the lowest NOAEL observed was 252.6 mg/kg bw/d. The lowest effect level was 1000 mg/kg bw/d.

Table 5.3-24: Summary of short-term toxicity studies with glyphosate acid

	Reference (data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.3/01 [redacted] 2007 (NUF)	13-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Liquid/soft faeces, dehydration, thin appearance, vomiting, pallor, body weight gain ↓ ♂, body weight loss ♀, food consumption ↓, ALT ↑, AP ↓, protein ↓, albumin ↓, adipocytes in sternum ↑, Prostrate and uterine atrophy; slight↑ of absolute and relative adreanal weights of ♂ at 300 mg/kg bw/d (increase was not statistically significant)
	IIA 5.3.3/02 [redacted] 1999 (FSG)	90-day, oral diet, Dog, Beagle	0, 200, 2000, 10000 ppm (0, 5.3, 53.5, 252.6 mg/kg bw/day)	NOAEL: 10000 ppm (252.6 mg/kg bw/day)	No treatment-related effects
	IIA 5.3.3/03 [redacted] 1996 (ALS)	13-week, oral diet Dog, Beagle	0, 1600, 8000, 40000 ppm	NOAEL: 40000 ppm (1015/1014 mg/kg bw/day ♂/♀)	No treatment-related effects
	IIA 5.3.3/04 [redacted] 1996 (SYN)	13-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	Decreased body weight, clinical chemistry changes

Reference (data owner)		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.3.2.3.2 Glyphosate Monograph [redacted] 1985 (Luxan / BCL))*	90-day, oral diet, Dog, Beagle	0, 100, 250, 500 mg/kg bw/day)	NOAEL: 250 mg/kg bw/day	500 mg/kg bw/day: reduced body weight gain, reduced food consumption
	Annex B.5.3.2.3.2 Glyphosate Monograph [redacted] 1981* (ALK/MON)	3-month, oral diet Dog, Beagle	0, 200, 600, 2000 ppm	NOAEL: 600 ppm (15/129 mg/kg bw/day ♂/♀)	2000 ppm: Liver: decreased organ weight, congestion, equivocal histological findings

* The study was considered supplementary in the 2001 EU glyphosate evaluation
↓ = decreased; ↑ = increased;

Table 5.3-25: Summary of short-term toxicity studies with glyphosate IPA salt

Reference (data owner)		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Study from the 2001 evaluation	Annex B-5.3.2.3, Glyphosate Monograph [redacted], 1983	6-month, oral capsule Dog, Beagle	0, 10, 60, 300 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	AP ↑; equivocal impact on body weight gain

↓ = decreased; ↑ = increased;

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/01	[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 GLP: yes not published

Guideline:	OECD 409 JMMAF 12 NohSan No. 8147
Deviations:	None
Dates of experimental work:	2005-06-08 - 2005-09-22

Executive Summary
Groups of four Beagle dogs per sex received the test item, glyphosate technical, by daily administration (capsule) at dose-levels of 0, 30, 300 or 1000 mg/kg bw/day for 11/13 weeks. The duration of the treatment period for the high-dose group was shortened to 11 weeks for ethical reasons following the observation of relevant toxic effects.
The animals were checked daily for mortality and clinical signs. Body weight was recorded weekly. Food consumption was estimated daily. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations, as well as urinalysis, were performed before the beginning of the treatment period, in Week 7 and at the end of the treatment period. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.
In the low- and mid-dose groups no treatment-related signs were noted. There were no haematological, blood biochemical, urinary or histopathological effects. Only an slight increase of absolute and relative adrenal weights of males receiving 300 mg/kg bw/day was observed. However, the increase was not statistically significant.
At 1000 mg/kg bw/day the test item administration induced marked clinical signs (liquid/soft faeces, dehydration, thin appearance, vomiting and pallor), caused lower body weight gain (males) or body weight loss (females) and reduced food consumption. This led to the early sacrifice of two moribund animals, and to the early termination of the entire group at week 11.
Laboratory investigations in the surviving animals demonstrated some abnormalities (higher alanine aminotransferase activity in both sexes and lower alkaline phosphatase activity, as well as lower protein and albumin levels in females) and urinary changes (decrease in specific gravity in both sexes and increase in urinary volume and markedly less colour of urine in females).
Treatment-related histopathological changes in surviving animals consisted of increased number of adipocytes in the sternum in both sexes, as well as prostate atrophy and uterine atrophy at 1000 mg/kg bw/day, which clearly exceeds the MTD. 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. Further major microscopic changes in moribund sacrificed animals were found in the kidneys (bilateral vacuolation of cortical tubules, sometimes with pigment deposits), liver (diffuse macrovesicular vacuolation, acute inflammation and/or pigment deposits), oesophagus, lung, uterus (atrophy) and/or bone marrow (increased number of adipocytes). These findings were associated with numerous changes in laboratory parameters (hemoconcentration, increased urea and creatinine levels, decreased urea, protein, albumin and bilirubin levels, decreased liver enzyme activities).

Conclusion
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Empty gelatine capsules, size 12 (Torpac, NY, US)

3. 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

B: STUDY DESIGN AND METHODS

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 were 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, 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 Peyers patches), jejunum, kidneys, larynx, liver, lungs (with bronchi), lymph nodes (mandibular and mesenteric), mammary gland, muscle (skeletal), optic nerve, ovaries, oviducts, pancreas, pituitary gland, prostrate, 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), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
Two unscheduled sacrifices (one male and one female) were noted in animals given 1,000 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.

B. 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.

C. 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 5.3-26).

Table 5.3-26: 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

D. FOOD CONSUMPTION

The food consumption was not affected by the test 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.

E. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the end of the treatment period.

F. 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 dehydratation 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 5.3-27):

- 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 5.3-27: 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	5.3	–	–	–
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

G. URINALYSIS

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.

H. NECROPSY

Organ weights

Treatment-related, statistically significant effects 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/02	[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 GLP: yes not published

Guideline:	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, traches and mammary gland.
Dates of experimental work:	1998-03-18 - 1998-06-26

Executive Summary

Three treated groups of four male and four female Beagle dogs received the test item, Glyphosate Technical, at dietary dose-levels of 0, 200, 2,000, or 10,000 ppm (corresponding to 0, 5.3, 53.5 and 252.6 mg/kg bw/day) for 90 days.

The animals were checked daily for mortality and clinical signs. Veterinary examination was carried out before grouping, at start of treatment, monthly throughout the study and at termination. Body weight was recorded weekly. Food consumption was determined weekly. Ophthalmological examinations were carried out before the beginning and at the end of the treatment period. Haematological and blood biochemical investigations were performed before the beginning of the treatment period, after 45 days of exposure and at termination. Urine was analysed at termination. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Designated organs were weighed and specified tissues preserved. A microscopic examination was performed on selected tissues from all the animals.

No signs of toxicity or ophthalmoscopical findings were observed in any dose group. Food consumption was significantly reduced in the high dose group initially (week 2) while body weights remained unaffected.

Hematological parameters appeared in general unaffected (clotting time was increased after 45 days of exposure in both sexes, but no effects on this parameter were visible at termination; other parameters attaining statistical point significance fell within historical control). Slight increases on total bilirubin and gamma-glutamyl-transferase were observed in the high dose group. No effects on urine parameters, organ weights or organ histopathology were observed.

Conclusion

In absence of any histopathological correlate, the inconstistent effects described in hematology and clinical chemistry are considered incidental. The No Observed Adverse Effect Level (NOAEL) is considered to be 10,000 ppm, corresponding to 252.6 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control:

Plain diet

3. Test animals:

Species:	Dogs
Strain:	Beagle
Source:	Rallis Research Centre, Bangalore, India (in-house breed)
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 *ad libitum*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
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

B: STUDY DESIGN AND METHODS

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 and urinalytical 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, Lymp,Eosi,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. Urinalyis 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 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

All animals survived until scheduled necropsy.

B. CLINICAL OBSERVATIONS

No clinical signs of toxicity were observed.

C. BODY WEIGHT

Bodyweights remain essentially unaffected from treatment. A slight initial depression of body weight gain might be concluded (and would be in accordance with the food consumption). Definitely no weight loss was observed.

Table 5.3-28: Group mean weekly bodyweights

	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
2'000	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
10'000	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
2'000	9.8	10.2	10.3	10.2	10.8	10.9	11.0	11.2	11.4	11.3	11.4	11.5	11.6	11.5
10'000	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

C. 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 5.3-29: 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
2'000	305	373	406	347	334	363	358	366	339	321	330	329	339
10'000	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
2'000	212	306	338	309	298	286	290	298	284	278	285	303	283
10'000	212	166*	348	327	303	261	288	294	298	262	268	262	256

The calculated mean daily test substance intake is summarised in Table 5.3-30 below.

Table 5.3-30: 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

D. OPHTHALMOSCOPIC EXAMINATION

There were no ophthalmological findings at the beginning and at the end of the treatment period.

E. 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 5.3-31: Summary of results for clotting time and GGT-actvity

	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

	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 seemed affected; however, in absence of a histopathological correlate on the liver, the effect was not considered adverse.

Table 5.3-32: 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*

F. URINALYSIS

All parameters were in the normal range and comparable between control and treated animals.

G. NECROPSY

Organ weights

No treatment-related effects were observed.

Necropsy

No treatment-related effects were observed.

Histopathology

There were a few incidental findings with equal distribution across control and treated groups – no relation to treatment was observed.

III. CONCLUSION

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).

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/03	[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 GLP: yes not published

Guideline: Japan MAFF Guidelines 59 NohSan No.4200, 1985, U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)

Deviations: None

Dates of experimental work: 1994-12-15 to 1995-07-24

Executive Summary

An oral subchronic toxicity study of HR-001 was conducted in beagle dogs of both sexes. Groups of 4 males and 4 females were given the test substance by incorporating it into a basal diet at a level of 0, 1600, 8000 or 40000 ppm for a period of 13 weeks. Animals were checked daily for general conditions and death, and individual food consumption was also measured daily. Body weights were recorded weekly. All animals were subjected to ophthalmology, urinalysis, haematology, and blood biochemistry periodically. At termination of treatment, the animals were euthanized and subjected to necropsy and organ weight analysis. Histopathological examination was performed on all animals.

- 40,000 ppm goup: Three of the four females showed decrease in urine pH. However, as it was known that test substance was degraded into free acid including acidified urine, the toxicological significance was not indicated in the change.

- 8,000 and 1,600 ppm groups: There were no-treatment related abnormalities in any parameters in either sex.

No significantly adverse effects were observed in beagle dogs of both sexes following the dietary treatment with HR-001 at a concentration as high as 40,000 ppm for 13 weeks. It was determined that the no-observable-effect level of HR-001 was 40,000 ppm (equivalent to 1015 and 1014 mg/kg/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. **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
2. **Vehicle and/ or positive control:** Plain diet / none
3. **Test animals:**

Species:	Dog
Strain:	Beagle
Source:	Narc, Co. (Japan)
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, *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

B: STUDY DESIGN AND METHODS

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.

Table 5.3-33: Study design

Test group	Dietary concentration (ppm)	Males	Females
Control	0	4	4
Low	1600	4	4
Mid	8000	4	4
High	40000	4	4

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:

$$Food\ consumption = [Feeding\ amount\ (250g\ diet + 250g\ water) - \ food\ residue] + 2$$

Chemical intake (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, gallblader, 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, hematologicla parameters, blood chemical parameters, and organ weights).
Data on clinical sign, mortality, ophthalmology, necropsy, and histopathology were evaluated by Fisher's exact probability.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no animals found dead or killed *in extremis* in any groups during the treatment period.

B. CLINICAL OBSERVATIONS
Statistically significant differences in incidence of clinical signs were not observed between the control and treated goups in either sex.

C. BODY WEIGHT
Statistically significant differences in body weights were not observed between the control and treated groups in either sex throughout the treatment.

D. 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/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 5.3-34: Summary of compound intake

Dose level (ppm)	Overall group mean chemical intake (mg/kg/day)	
	Male	Female
1 600	39.7	39.8
8 000	198	201
40 000	1015	1014

E. OPHTHALMOSCOPIC EXAMINATION
No ocular changes were detected in any dose groups of both sexes.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
Statistically significant changes in haematology parameters were observed in the treated groups as shown in the following table:

Table 5.3-35: 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	↘ (148)	-	-	-	-	-	-	-	-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated groups of both sexes, no dose dependency was conceived in the changes. A significant decrease in mean corpuscular hemoglobin concentration (MCHC) observed in females of the 40 000 ppm group was considered to be incidental, because the change was also noted for the pre-treatment measurement and was not accompanied with significant abnormalities of erythrocyte count (RBC), Hematocrit (Ht), and hemoglobin (Hb).

Blood clinical chemistry

Statistically significant changes in blood biochemistry parameters were observed in the treated groups and are shown in the following table:

Table 5.3-36: Results of clinical chemistry examinations

		Dose level (ppm)								
		1600			8000			40000		
Week of treatment		0	7	13	0	7	13	0	7	13
Glucose (Gluc)	Male	↓ (92)	-	-	-	-	-	↘ (93)	-	-
Chloride (Cl)	Male	-	-	↗ (102)	-	-	-	-	-	↗ (102)
Albumin (Alb)	Female	-	-	↗ (107)	-	-	-		-	-

Statistically evaluated by Dunnett's multiples comparison method
Value in parenthesis means percentage of group mean value against control mean value.

Although there were statistically significant differences in some parameters in the treated group of both sexes, no dose dependency was conceived in the changes. Although significant increases in chloride (Cl) were observed in males of the 1 600 and 40 000 ppm groups at week 13, the changes were considered to be incidental because of no dose dependency and their small degrees of alteration.

G. URINALYSIS

In the 40 000 ppm group, 3 of 4 females showed decrease in urine pH at week 13, although there were no statistically significant differences between the control and treated groups of both sexes in any parameters of urinalysis.

There were no significant changes in urinalysis in males and females treated at 16 000 ppm or less.

H. NECROPSY

Organ weights

There were no gross findings with statistically significant differences in incidence and relationship to the treatment in the treated groups of either sex. Although a statistically significant increase was noted for the relative weight of the adrenals in females of the 1 600 ppm group, the change was considered to be incidental due to the lack of dose-dependency.

Gross pathology

Histopathology

There were no histopathological changes related to the treatment in the treated groups of either sex. A female in the 40 000 ppm group showed cutaneous histiocytoma which is a non-specific lesion in young dogs.

III. CONCLUSION

Under the experimental conditions of the study, the No Observed Effect Level (NOEL) is considered to be 40,000 ppm (equivalent to 1015 and 1014 mg/kg bw/day for males and females, respectively).

Annex point	Author(s)	Year	Study title
IIA, 5.3.3/04	[redacted]	1996	First Revision To Glyphosate Acid: 90 Day Oral Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: CTL/P/1802 Date: 1996-11-14 GLP: yes not published

Guideline:	OECD 409 (1998): OPPTS 870.3150 (1998): 2001/59/EC B.27 (2001)
Deviations:	None
Dates of experimental work:	1986-08-04 to 1996-11-14

Excecutive summary

In a subchronic toxicity study glyphosate acid was administered to groups of four male and four female beagle dogs at dose levels of 0 (control), 2000, 10000 or 50000 ppm glyphosate acid in the diet for a period of at least 90 days. The clinical condition and bodyweights 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 microscopically.

Glyphosate acid was palatable to dogs in dietary concentrations up to and including the limit dose of 50000 ppm in the diet.

Toxic effects were confined to dogs given 50000 ppm glyphosate acid, these being small reductions of bodyweight gain. Males also had slightly reduced plasma protein and calcium concentrations.

Liver and kidney weights of males given 10000 and 50000 ppm glyphosate acid were increased. Plasma alkaline phosphatase activity of females given 50000 ppm glyphosate acid was slightly increased. These

effects were not accompanied by a histopathological lesion and are considered to be of no toxicological significance.

There were no haematological, clinical or pathological changes associated with glyphosate acid treatment. The toxicological no effect level of glyphosate acid given in the diet to dogs for 90 days was 10000 ppm, with only minimal effects at 50000 ppm.

An absolute no effect level was 2000 ppm glyphosate acid.

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.

I. MATERIALS AND METHODS

A: MATERIALS:

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 and/or positive control: Glyphosate acid was administered in diet.

Test Animals:

Species	Dog
Strain	Beagle
Age/weight at dosing	22 - 26 weeks
Source	Breeding Unit, Zeneca, Alderley Park, Macclesfield, Cheshire, UK.
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

B: STUDY DESIGN AND METHODS:

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 bodyweights 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.

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.

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.

Bodyweight: 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 and clinical chemistry: Jugular vein blood samples were taken before feeding from all dogs in weeks -1, 4, 8 and 13 and the following parameters measured:

Haemoglobin
Haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
kaolin-cephalin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
blood cell morphology
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
glucose
albumin
total protein
cholesterol
triglycerides
creatine kinase activity
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
calcium
sodium
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
specific gravity
pH
bilirubin
glucose
ketones
protein
blood

Investigations *post mortem:*
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
brain
epididymides
kidneys
ovaries
liver
testes
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
adrenal gland
aorta
brain
bone and bone marrow (rib)
caecum
colon
duodenum
gall bladder
epididymis
eyes
femur (including stifle joint)
heart
ileum
oesophagus
ovary
pancreas
pituitary gland
prostate gland
rectum
salivary gland
spinal cord (lumbar)
skin
spleen
sternum
stomach
testis
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: Bodyweight gains from the start of the study to each week and final bodyweights 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 bodyweight, 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.

II. RESULTS AND DISCUSSION

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.

Bodyweight and weight gain: Bodyweight 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 5.3-37: Intergroup comparison of bodyweight gain (g) (selected timepoints)

	Dietary Concentration ofglyphosate 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 5.3-38: Mean Dose Received (mg/kg/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 50000ppm 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 5.3-39: 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.

Sacrifice and pathology:
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 5.3-40: Intergroup comparison of liver weight (g) in male dogs (adjusted for bodyweight)

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 haemorrhages were found in several animals and were consistent with a subclinical bacterial infection of the lower urinary tract.

III. CONCLUSION

Minimal toxicity was seen when glyphosate acid was administered in the diet for 90 days at the limit dose of 50000 ppm. The toxicological no effect level for glyphosate acid from this study was 10000 ppm in the diet, equivalent to a dose of more than 300 mg glyphosate acid/kg/day.

IIA 5.3.4 Oral 1 year toxicity (dog)

Oral one year toxicity in the dog was previously evaluated in the 2001 EU glyphosate review. The [redacted] (1991) and and [redacted] (1985) studies have been previously evaluated and like the 90-day studies only non-specific signs of toxicity (slight effect body weight and an increase in clinical signs of soft, liquid stools) were observed at limit dose. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid.

Three additional one year dog studies have been conducted that were not previously reviewed in the 2001 EU evaluation. All 3 studies are considered as confirmatory data and are summarised below. Again non-specific effects of toxicity were observed at doses at, or close to limit dose. These effects were characterised as reduction in body weight gain, reduction in urinary pH and minor effects on clinical pathology parameters. An increase in pneumonia in all females dosed at 1259 mg/kg bw/day was observed in the [redacted] (1997) study. In the other female groups, this lesion was observed in only one out of four dogs each. However, the extent of this lesion was very focal and the degree of intensity was slight in all cases. Statistically, no significant differences between control and dose groups were found in the incidence of this lesion.. In this study no treatment-related effects were observed. The lowest dose level where treatment related effects were observed was 926 mg/kg bw/day in the [redacted] (1996) study. The most relevant one year oral dog NOAEL for glyphosate technical is 500 mg/kg bw/day.

Table 5.3-41: Summary of 1 year toxicity studies with glyphosate acid in dogs

Reference (data owner)		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.3.2.3 Glyphosate Monograph [redacted] 1985 (MON)	12-month, oral capsule Dog, Beagle	0, 20, 100, 500 mg/kg bw/day	NOEL: 500 mg/kg bw/day	No treatment-related effects
	Annex B.5.3.2.3 Glyphosate Monograph [redacted] 1991 (CHE)	52-week, oral capsule Dog, Beagle	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: soft, liquid stools (attributable to capsule administration); equivocal impact on body weight gain

	Reference (data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.3.4/01 [redacted] 2008 (NUF)	52-week, oral capsule Dog, Beagle	0, 30, 125, 500 mg/kg bw/day	NOAEL: 500 mg/kg bw/day	No treatment-related effects
	IIA 5.3.4/02 [redacted] 1997 (ALS)	12-month, oral diet Dog, Beagle	0, 1600, 8000, 50000 ppm (≅ 34.1/37.1, 182/184, 1203/1259 mg/kg bw/day (♂/♀)):	8000 ppm (182/184 mg/kg bw/day ♂/♀)	50000 ppm (≅ 1203/1259 mg/kg bw/day (♂/♀)):: loose stool, retarded body weight gain, reduced body weight at termination without stat. significance, urinary pH ↓, slight anemic changes in ♀, slight focal pneumonia / focal granulomatous pneumonia in the lung of all ♀ (extent of the lesion was very focal and slight in intensity), statistically, no significant differences, blood chemistry changes (Cl ↑, albumin ↓, P ↓ ♀)
	IIA 5.3.4/03 [redacted] 1996 (SYN)	1-year, oral, diet Dog,	0, 3000, 15000, 30000 ppm	NOELs ♀: 15000 ppm (447 mg/kg bw/day) ♂: 30000 ppm (906 mg/kg bw/day)	30000 ppm (926 mg/kg bw/day: ♀ only: slight body weight reduction

↓ = decreased; ↑ = increased;

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/01	[redacted]	2008	Glyphosate technical: 52-week Toxicity Study by Oral Route (Capsule)in Beagle Dogs [redacted] Data owner: Nufarm Study No.: 29647 TCC Date: 2007-07-23 GLP: yes not published
Guideline:			OECD 452 (1981); JMAFF 2-1-14 (2001)
Deviations:			None
Dates of experimental work:			2005-10-03 - 2006-10-17

Executive Summary
The chronic toxicity potential of glyphosate technical was assessed in a 1-year oral toxicity study in male and female beagle dogs. Groups of four dogs per sex received daily doses (capsules) of 0, 30, 125, and 500 mg/kg bw/day for 52 consecutive weeks. (dose level selection was based on the results of a 13 week study run in the same laboratory). Observations covered mortality, clinical signs, body weight, food consumption, ophthalmological examinations, haematology, clinical chemistry, urine analysis, organ weights, necropsy and histopathological examination.
No unscheduled deaths or premature sacrifices occurred during the study. There were no treatment-related effects on clinical signs, eyes, body weight, body weight gain, food consumption, haematology, clinical chemistry and urine analysis parameters in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects.
In conclusion, the NO(A)EL for oral toxicity of glyphosate technical was 500 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	Glyphosate tech
Description:	White crystalline powder
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	Expiry date: 2008-03-25

2. Vehicle and/ or positive control: Gelatine capsules size 12 (Torpac, New York, USA)

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	Marshall Farms, North Rose, New York, USA
Age:	Approx. 6 month
Sex:	Males and females
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, *ad libitum*
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

B: STUDY DESIGN AND METHODS

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 ♂, 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.

Urinanalysis
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 weight: 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), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortalities or premature sacrifices occurred during the treatment period.

B. 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 localized 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.

C. 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 5.3-42). 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 5.3-42: Mean body weight and body weight changes (kg)

Dose level (mg/kg bw/day)	Males				Females			
	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.

D. FOOD CONSUMPTION

There was no treatment-related effect on food consumption noted during the study.
The reduce 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.

E. OPHTHALMOLOGY

There were no ophthalmological findings observed at the end of the study period.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

There were no treatment-related effects noted in the haematological parameters.
The significant differences observed for the activated partial thromboplastin time (↓), MCHC (↓) and eosinophil counts (↓) 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 the treated animals when compared to control dogs were only slight and not dose-related.

G. URINEANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

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 brain weight (see Table 5.3-43) observed in males at 125 mg/kg bw/day was dose-independent. In addition, there were no macroscopic or histopathological findings noted in this organ. Thus, this finding is considered incidental.

Table 5.3-43: 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/02	[redacted]	1997	HR-001: 12-Month Oral Chronic Toxicity Study in Dogs. [redacted] Data owner: Arysta LifeScience Study No.: IET 94-0157 Date: 1997-03-20 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 409 (1981)
Deviations:	None
Dates of experimental work:	1996-03-05 to 1997-04-03

Executive Summary
An oral chronic toxicity study of HR-001 was conducted in eagle dogs of both sexes. Groups of 4 males and 4 females each were given the test substance by incorporating it into basal diet at a level of 0, 2000, 10000 and 30000 ppm for a period of 12 months. Animals were checked daily for general conditions. Body weights and food consumption were measured periodically. All animals were subjected to urinalysis at weeks 25 and 51 and to hematology and blood chemistry at weeks 26 and 52. Ophthalmological examinations were performed at week 52. At termination of treatment, animals were euthanized and subjected to organ weight analysis and necropsy. Histopathological examinations were performed on representative organs/tissues from all animal used.
Findings related to the treatment were demonstrated in clinical observation, body weight, urinalysis, hematology and blood chemistry.

- 50,000 ppm group: Loose stool was observed was observed in 3 of 4 males and 4 of 4 females. These animals frequently sowed the clinical sign through the treatment period, whereas in the control group, only one animal in each sex showed the sign at a restricted period during treatment. Body weight gain was retarded gradually with progression of the treatment in both sexes, when compared to the controls. Consequently, the difference in mean body weight between the 50000 ppm and control groups became great with time, although statistical signific ance was not observed. Hematologically, slight anemic changes were noted for females at weeks 26 and 52. Females also showed a significantly increased plasma level of chloride at week 26 and significantly decreased plasma levels of albumin and inorganic phosphorous at week 52. Significantly lowered urine pH values were continuously observed in males and females. However, this finding was not recognized as a toxic change since it is known that the test substance is secreted with little metabolisation into urine, degraded to a free acid in urine, and consequently, make the urine acidic.

- 8,000 and 1,600 ppm groups: There were no treatment-related abnormalities in either sex.

Based on the results, the no-observable-effect level of HR-001 is 8000 ppm (equivalent to 182 and 184 mg/kg/day for males and females, respectively).

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Dog
Strain:	Beagle
Source:	Narc, Co. (Japan)
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

B: STUDY DESIGN AND METHODS

In life dates: 1996-03-05 to 1997-04-03

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:

$$Food\ consumption = [Feeding\ amount\ (250g\ diet + 250g\ water) - \ food\ residue] \div 2$$

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 hemoglobnin, 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).

Urinanalysis
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 weight: 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 gallblader , 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), summarising the most common statistical procedures used for analysis of data in toxicology studies, together with their most likely outcomes.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no deaths in any dose groups of either sex.

B. 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.

C. BODY WEIGHT
In the 50 000 ppm group 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 50 000 ppm.

D. 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 coparable 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 5.3-44: 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

E. OPHTHALMOLOGY

No remarkable ocular changes were detected in animals in any dose groups at week 52.

F. HAEMATOLOGY AND CLINICAL CHEMISTRY

Haematology

Statistically significant changes in haematology that were observed in treated groups are presented in the following table:

Table 5.3-45: 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.
Female 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 5.3-46: 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 50 000 ppm group showed a significant increase in chloride (Cl) at week 26 and significant decreases in albumin (Alb), calcium (Ca), a,d 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 8 000 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.

G. URINEANALYSIS

There were no findings among the quantitative or semi-quantitative and qualitative parameters during the treatment period.

H. NECROPSY

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 very focal and the degree of intensity 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.

III. CONCLUSION

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).

Annex point	Author(s)	Year	Study title
IIA, 5.3.4/03	[redacted]	1996	Glyphosate Acid: 1 Year Dietary Toxicity Study in Dogs [redacted] Data owner: Syngenta Report No.: CTL/P/5079 Date: 1996-09-24 GLP: yes not published

Guideline:	OECD 452 (1981): OPPTS 870.4100 (1998): 87/302/EEC B.30 (1988)
Deviations:	None
Dates of experimental work:	1995-02-28 to 1996-09-24

Excecutive summary

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.

Clinical observations and veterinary examinations (including ophthalmoscopy) were made and bodyweights, food consumption and clinical pathology parameters were measured and at the end of the scheduled period, the animals were killed and subjected to a full examination *post mortem*. Selected organs were weighed and specified tissues were taken for subsequent histopathology examination.

Mild toxicity was evident at 30000 ppm glyphosate acid, as a slight reduction in bodyweight in females throughout the latter half of the study. This reduction was generally independent of any reduction in food consumption and does not, therefore, reflect a palatability effect. There were no other toxicologically significant effects and the pathological no-effect level was 30000 ppm glyphosate acid.

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 30000ppm glyphosate acid.

The no-observed effect level (NOEL) 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 (NOEL) for toxicity over 1 year, for males is 30000 ppm glyphosate acid (equivalent to an overall mean dose of 906 mg/kg/day).

I. MATERIALS AND METHODS

A: MATERIALS:

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 and/or positive control: The test substance was administered in the diet.

Test Animals:

Species	Dog
Strain	Beagle
Age/weight at dosing	20 – 29 weeks
Source	Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK.
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) *ad libitum*
Water	Mains water *ad libitum*
Environmental conditions	Temperature: 19 ± 2°C Humidity: 40-70% Air changes: Approximately 15 changes / hour Photoperiod: 12 hours light / 12 hours dark

B: STUDY DESIGN AND METHODS:

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 bodyweight 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 5.3-47: 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).

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 where 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.

Statistics: All data were evaluated using analysis of variance and / or covariance for each specified parameter using the GLM procedure in SAS (1989).

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.

Bodyweight: 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 and clinical chemistry: 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
total bilirubin
creatine kinase activity
sodium
potassium
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
colour (if abnormal)
specific gravity
pH
glucose
ketones
protein
bilirubin
blood

In addition, each urine sample was centrifuged and the sediment stained and examined microscopically to identify the components.

Investigations *post mortem:*
Macroscopic examination: All animals were 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
brain
epididymides
thyroid glands
kidney
liver
testes

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
adrenal gland
aorta
brain (cerebrum, cerebellum and brainstem)
bone marrow (sternum)
caecum
colon
duodenum
epididymis
eyes (retina, optic nerve)
femur (including stifle joint, stored not examined)
Gall bladder
heart
ileum
jejunum
kidney
larynx
oesophagus
ovary
pancreas
parathyroid gland
pituitary gland
prostate gland
rectum
salivary gland
spinal cord (cervical, thoracic, lumbar)
skin
spleen
sternum
stomach
testis
thymus
thyroid gland
trachea

liver
lung
lymph node - prescapular
lymph node - mesenteric
mammary gland (females only)
urinary bladder
uterus (with cervix)
voluntary muscle

Microscopic examination: All processed tissues were examined by light microscopy.

II. RESULTS AND DISCUSSION

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 5.3-48: Intergroup comparison of bodyweights (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	11.76**

* 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 and compound intake: 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 5.3-49: Mean Dose Received (mg/kg/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.

Sacrifice and pathology:
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.

III. CONCLUSION

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).

IIA 5.3.5 28-day inhalation toxicity (rodents)

One study on sub-acute inhalative toxicity (14 days) in rodents ([redacted] 1985: non-GLP (pre-GLP) study) was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL was 3.8 mg/L. Since glyphosate is non-volatile (VP = 1.31 x 10^{-5} Pa (25°C)) the conduct of 28-day inhalation studies are not needed according to EU requirements. Therefore, no further inhalation studies were conducted.

Table 5.3-50: Summary of short-term toxicity studies with glyphosate acid rats

Reference (data owner)		Type of study Species	Dose levels [mg/L]	NOEL / NOAEL [mg/L]	Targets / Main effects
Study from the 2001 evaluation	Annex B.5.3.3.3.2 Glyphosate Monograph [redacted] 1985 (EXC)*	14-day, inhalation (6h/day) Rat, Wistar	0, 0.28, 0.93, 3.8	NOEL: 3.8	No treatment-related effects

* Study was considered supplementary data in the 2001 EU glyphosate evaluation

IIA 5.3.6 90-day inhalation toxicity (rodents)

90-day inhalation studies are only required for volatile substances (i.e. substances with an vapour pressure (VP) > 10^{-2} Pa). Since glyphoate is non-volatile (VP = 1.31 x 10^{-5} Pa (25°C)), no 90-day inhalation toxicity study was conducted.

IIA 5.3.7 Percutaneous 28-day toxicity (rodents)

The short-term percutaneous toxicity of glyphosate has been investigated in the rat and rabbit. In both Sprague-Dawley (SD) (Heath *et al.*, 1993) and Wistar derived (Pinto, 1996) rats, no signs of systemic toxicity were noted following dosing for 21 days at 1000 mg/kg bw/day, the limit dose for this study type. Three studies were conducted in New Zealand White rabbits (Bhide, Johnson and Tornai) and doses ranged from 1000 mg/kg bw/day to 5000 mg/kg bw/day. No signs of treatment related systemic toxicity were noted in any study, the highest NOAEL being 5000 mg/kg bw/day.

An *in vitro* dermal absorption study in the rabbit (Hadfield 2012, see IIA 5.3.7/02) was conducted to estimate how much of a dose of a 5000 mg/kg dermal dose would have been systemically available. A total of 2.66% of the applied dose was concluded to be systemically available and this percentage was used to calculate a systemic equivalent dose for the NOAEL established. An applied dose of 5000 mg/kg bw/day was concluded to equate to a systemic dose of 133 mg/kg bw/day. This systemic dose in rabbits is higher than that used to calculate the AOEL from orally dosed rabbits. This implies that mortality observed in orally dosed rabbits was not due to systemic toxicity, but rather a consequence of the route of administration.

In both the rat and the rabbit no signs of systemic toxicity were evident following repeated application of glyphosate to the skin. The NOAEL for short term percutaneous toxicity was 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit as previously concluded in the 2001 EU glyphosate evaluation.

Table 5.3-51: Summary of percutaneous 28-day toxicity studies with glyphosate acid

Reference (data owner)		Type of study Species	Dose levels (mg/kg bw/day)	NOAEL (mg/kg bw/day)	Targets / Main effects
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph [redacted] 1993 (CHE)	21-day, dermal Rat, Sprague-Dawley	0, 1000	1000	Weak dermal irritation at 1000 mg/kg bw/day; no systemic effects
Study not reviewed in the 2001 evaluation	IIA 5.3.7/04 Pinto, 1996 (SYN)	21-day, dermal Rat, Alpk: AP_fSD	0, 250, 500, 1000	1000	No treatment-related effects
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph [redacted] 1985 (EXC)	21-day, dermal Rabbit, NZW	0, 500, 1000, 2000	2000	No treatment-related effects
	Annex B.5.3.3.1 Glyphosate Monograph IIA, 5.3.7/01 [redacted] 1982 (MON)	21-day, dermal Rabbit, NZW	0, 100, 1000, 5000	5000	Dermal irritation at 5000 mg/kg bw/day; no systemic effects
Study not reviewed in the 2001 evaluation	IIA, 5.3.7/02 [redacted] (2012a)	In vitro rabbit dermal absroption	Equivalent of 5000	133 systemic NOAEL in rabbits	2.42% absorbed 0.243% remaining in dermis 2.66% systemic = 133 mg/kg/day
Study from the 2001 evaluation	Annex B.5.3.3.1 Glyphosate Monograph IIA, 5.3.7/03 [redacted] 1994a (ALK)	28-day, dermal Rabbit, NZW	0, 500, 1000, 2000	2000	No effects

Tier II summaries are only presented for representative studies.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/01	[redacted]	1982	21-Day dermal toxicity study in rabbits [redacted] Data owner: Monsanto Monsanto Report No. IR-81-195 Date: 1982-03-10 GLP: no (pre-GLP study) unpublished

Guideline:	Non-stated Study is in general accordance with OECD guideline 410 (1981)
Deviations:	The application area in the high-dose group was about 1.5 – 2 times higher than the recommended 10 % of the body surface area.
Dates of experimental work:	Not reported

Executive Summary
The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits on intact and on abraded skin. Doses of 0, 100, 1000 or 5000 mg/kg bw/day were applied five days per week for three consecutive weeks. For application the solid test substance was moistened with an appropriate amount of water, and spread evenly over the application site. It has to be noted that the surface areas covered (i.e. 1 - 2 %, 5 – 10 % and 15 – 20% for the low, mid- and high-dose group, respectively) were below and above the area of 10 % recommended by actual guidelines. Due to the higher exposed surface area in the high dose group, it has to be considered that more test substance can be absorbed through the skin and could be therefore systemically available.
There were no mortalities and no treatment-related signs of systemic toxicity. There were also no signs of dermal irritation observed in the control, low- and mid-dose group. At 5000 mg/kg bw/day slight dermal irritation consisting of barely perceptible to slight erythema and oedema was noted. However, this effect is considered not to be of biological significance and no signs of irritation were seen in the histopathological examination.
There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.

Conclusion
Repeated dermal administration of glyphosate technical to rabbits for a period of 21 consecutive days at doses of up to 5000 mg/kg bw/day resulted only in slight dermal irritation at 5000 mg/kg bw/day. No such effects were observed in the 0, 100 and 1000 mg/kg bw/day treatment group. There were no treatment-related systemic signs of toxicity. Thus, the "No Observed Adverse Effect Level" is considered to be 5000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	NBP 1992026
Purity:	98.4%

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

2. Vehicle and/ or positive control: None

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	Davidson´s Mill Farm, New Jersey, USA
Age:	Young, adult
Sex:	male and female
Weight at dosing:	♂ 2359 - 2883 g; ♀ 2344 - 2955 g
Acclimation period:	14-16 days
Diet/Food:	Purina Certified Rabbit Chow #5322 (Ralston Purina Company, Missouri, USA), *ad libitum*
Water:	tap water, *ad libitum*
Housing:	Individually in wire mesh cages.
Environmental conditions:	Temperature: Exact range not reported Humidity: Exact range not reported Air changes: Exact value not reported 12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1981-07-28 to 1981-08-19

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Ten rabbits per sex per dose received daily dermal applications of 0, 100, 1000 or 5000 mg/kg bw. The dose groups were further divided in halves. One half received treatment on intact skin, the other half on abraded skin. Abrasion was done twice per week immediately prior to test substance application by producing shallow incisions (not deep enough to cause bleeding) with the blunt end of a scalpel blade.
The day prior to the first application about 30 % of the skin of the back of the animals was clipped free of hair. During the study rabbits were shaved as needed.
For each application the test substance was moistened with an appropriate amount of physiological saline. Each dose was spread evenly over the maximum body surface area possible (see Table 5.3-52), covered with a semi-occlusive dressing. After an exposure period of six hours the dressings were removed and the application site was cleaned with tepid tap water and dried with paper towels. Applications were performed once daily , five days per week for three consecutive weeks. Individual doses were adjusted weekly based on the body weight determined at the beginning of each study week.

Table 5.3-52: Application details for repeated dermal applications

Dose group (mg/kg bw)	Number of animals				Volume of physiological saline used for moistening	Total percent of the body surface covered by test substance
	Intact skin		Abraded skin			
	♂	♀	♂	♀	(mL)	(%)
0	5	5	5	5	--	--
100	5	5	5	5	0.2	1 – 2
1000	5	5	5	5	1.5 – 2.0	5 – 10
5000	5	5	5	5	8.0 – 9.0	15 – 20

It has to be noted that the application area according to current guidelines (OECD and EC) should be about 10% of the body surface. Thus, the body surface covered with test material in the 100 mg/kg bw/day dose group is lower than recommended, whereas the treatment-area in the high dose group is about 1.5 – 2 times higher than recommended. Due to the higher exposed surface area in the high-dose group a higher amount of test substance can be absorbed and would be therefore potentially be systemically available.

Clinical observations
A check for mortality was made twice daily. Observations for clinical signs of toxicity and behavioural changes were made once daily on all animals. The applications sites were assessed for signs of irritation.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed daily for each individual animal by visual inspection.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on 5 rabbits per sex and dose group with intact and abraded skin on Day 21 after an overnight fast.
The following parameters were measured: Haematocrit, haemoglobin, erythrocyte count, reticulocyte count, platelet count, total leukocyte count, differential leukocyte count, MCV, MCH, MCHC, alkaline phosphatase, aspartate amino transferase (AST), alanine aminotransferase (ALT), γ-glutamyl-transferase, creatine kinase, creatinine, blood urea nitrogen, total protein, glucose, albumin, globulin (calculated), total bilirubin, creatinine, lactate dehydrogenase, total cholesterol, inorganic phosphorus, calcium, sodium, potassium, and chloride.

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, gonads, heart, kidneys, liver, pituitary and thyroid (with parathyroid).
Tissue samples were taken from the following organs and preserved in buffered formalin: treated and untreated skin (3 samples/each), adrenals, bone & bone marrow (sternum), brain (at three levels), colon, duodenum, eyes with Harderian gland, gross lesions, heart, ileum, jejunum, kidneys, liver, lungs with main stem bronchi, mammary gland, lymph nodes (mediastinal, mesenteric and regional when applicable), muscle (skeletal), oesophagus, ovaries, pancreas, pituitary, prostrate, salivary glands, sciatic nerve, seminal vesicles, spinal cord (cervical), spinal cord and vertebrae (lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus and vagina.
Histopathological examinations were performed on the following tissues: treated and untreated skin, liver, kidney, gonads and any gross lesions.

Statistics
Terminal body weights, haematological and clinical chemistry parameters, absolute and relative organ weights were analysed by one-way analysis of variance, Bartlett´s test for homogeneity of variance and appropriate t-test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
A number of incidental findings were observed in some animals in all dose groups. The most frequent signs were soft stool, diarrhoea, mucoid diarrhoea, and ocular and nasal discharge.

No signs of dermal irritation were observed in the control, low- and mid-dose group. In the high-dose group at 5000 mg/kg bw/day doubtful or barely perceptible to very slight erythema and doubtful or barely perceptible oedema were noted. There were no differences between the animals with intact and abraded skin.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups (with abraded and intact skin).

D. FOOD CONSUMPTION
There were no major differences in food consumption between the control and the treated groups.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
No treatment-related effects were detected in the haematological parameters measured.
There were some statistical significance differences in some parameters. However, these were incidental and considered to be biologically insignificant (see Table 5.3-53)

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed were considered not to be biologically significant (see Table 5.3-53)

Table 5.3-53: Group mean haematological and blood chemical values and standard deviations (sd)

Dose level (mg/kg bw/day)		Hb (g/dL)	Haematocrit (%)	Sodium (meq/L)	Glucose (mg/dL)	LDH (IU/L)
		Males				
0	mean	11.3	34.1	143	121	–
	sd	0.68	1.78	1.3	21.6	–
100	mean	12.5*	37.3*	144	134	–
	sd	0.63	1.92	2.1	14.1	–
1000	mean	11.4	34.2	145	149	–
	sd	0.46	1.41	5.1	34.7	–
5000	mean	11.7	35.5	146*	125	–
	sd	0.61	1.13	2.5	6.1	–
		Females				
0	mean	–	–	–	102	189
	sd	–	–	–	16.2	125.9
100	mean	–	–	–	137**	149
	sd	–	–	–	18.7	109.1
1000	mean	–	–	–	123	258
	sd	–	–	–	17.1	204.4
5000	mean	–	–	–	129*	28*
	sd	–	–	–	4.7	6.2

LDH lactate dehydrogenase
– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)

I. NECROPSY

Organ weights

Except for the statistically increased relative kidney weight observed in females at 5000 mg/kg bw/day (see Table 5.3-54), there were no treatment-related effects on absolute and relative organ weights noted. Since no histopathological changes were observed in the kidneys of high-dose females, the increase in relative kidney weights is considered to be of no toxicological relevance.

Table 5.3-54: Group mean absolute and relative kidney weights and standard variations (sd)

Dose level (mg/kg bw/day)		Absolute organ weight (g) Kidney		Relative organ weight (%) Kidney	
		♂	♀	♂	♀
0	mean	17.00	16.41	0.58	0.55
	sd	2.281	2.037	0.078	0.040
100	mean	16.38	15.26	0.57	0.54
	sd	1.371	2.260	0.067	0.069
1000	mean	18.15	16.16	0.59	0.54
	sd	2.653	2.449	0.067	0.048
5000	mean	16.77	18.14	0.60	0.63*
	sd	2.016	1.757	0.097	0.072

* - significantly different from control group ($p < 0.05$)

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There were no treatment-related lesions observed in any dose group.
Microscopic evaluation of treated skin samples demonstrated only mild inflammatory cell infiltration and trace necrosis in the 1000 mg/kg bw/day group. However, in untreated skin samples of three rabbits from the 1000 mg/kg bw/day group from one rabbit of the high-dose group there was also mild necrosis, indicating that this lesion was incidental and unrelated to treatment. The lesions in treated and untreated

skins of the control and test substance groups were similar indicating that the effects were not related to glyphosate treatment.
Trace/mild seminiferous tubule degeneration observed in the testis were not dose related in either incidence or severity and was considered unrelated to treatment. Other lesions observed in kidney, liver, lung, ovary, lymph node and skin (non-application site) were considered incidental or spontaneous (see Table 5.3-55 and Table 5.3-56). In general there were no major differences between the treatment groups of intact and abraded skin.

Table 5.3-55: Histopathological findings in rabbits treated dermally on intact skin*

Dose level	0 (mg/kg bw/day)		100 (mg/kg bw/day)		1000 (mg/kg bw/day)		5000 (mg/kg bw/day)	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Cytoplasmic vacuolation (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Interstitial lymphocytic infiltrates (mild)	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Interstitial inflammation (mild)	0/5	1/5	1/5	0/5	0/5	0/5	2/5	0/5
Infarct (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Mineralisation (trace)	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	1/5	0/5	1/5	1/5
Liver								
Granulome (moderate)	0/5	1/5	1/5	1/5	1/5	0/5	0/5	0/5
Mononuclear cell infiltration (trace)	1/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5
Mononuclear cell infiltration (mild)	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5
Mononuclear cell infiltration (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Necrosis (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Lung								
Abscess (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Lymphocytic infiltration (mild)	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Pneumonia (mild)	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5
Pneumonia (moderate)	1/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Congestion (mild)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Congestion (moderate)	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5
Oedema (moderate)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Testis								
Seminiferus tubule degeneration (trace)	1/5	--	3/5	--	2/5	--	3/5	--
Seminiferus tubule degeneration (mild)	2/5	--	2/5	--	0/5	--	1/5	--
Dilated tubules (moderate)	0/5	--	1/5	--	0/5	--	0/5	--
Ovaries								
Mineralisation (trace)	--	1/5	--	0/5	--	0/5	--	0/5
Salivary gland								
Abscess (moderate)	--	1/5	--	0/5	--	0/5	--	0/5
Skin (non-application site)								
Dermatitis (moderate)	0/5	--	0/5	--	1/5	--	0/5	--
Skin, treated								
Inflammation (trace)	1/5	3/5	2/5	3/5	2/5	5/5	3/5	2/5
Inflammation (mild)	2/5	1/5	2/5	1/5	1/5	0/5	0/5	3/5
Necrosis (trace)	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5
Skin, untreated								
Inflammation (trace)	4/5	1/5	4/5	2/5	3/5	2/5	3/5	2/5
Inflammation (mild)	1/5	3/5	0/5	3/5	1/5	2/5	0/5	3/5
Necrosis (mild)	0/5	0/5	0/5	0/5	3/5	0/5	1/5	0/5

* Number of animals affected / total number of animals; -- not applicable

Table 5.3-56: Histopathological findings in rabbits treated dermally on abraded skin*

Dose level	0 (mg/kg bw/day)		100 (mg/kg bw/day)		1000 (mg/kg bw/day)		5000 (mg/kg bw/day)	
Effect/Lesion	♂	♀	♂	♀	♂	♀	♂	♀
Kidney								
Interstitial inflammation (trace)	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5
Interstitial inflammation (mild)	1/5	0/5	1/5	1/5	1/5	0/5	0/5	1/5
Interstitial inflammation (moderate)	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5
Infarct (moderate)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Mineralisation (mild)	0/5	0/5	0/5	0/5	2/5	1/5	0/5	0/5
Liver								
Mononuclear cell infiltration (trace)	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5
Mononuclear cell infiltration (mild)	1/5	0/5	1/5	2/5	4/5	1/5	1/5	1/5
Necrosis (mild)	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
Lung								
Pneumonia (moderate)	0/5	--	1/5	--	1/5	--	0/5	--
Lymph node, prefemoral								
Lymphadenitis (mild)	--	0/5	--	0/5	--	0/5	--	1/5
Testis								
Seminiferus tubule degeneration (trace)	2/5	--	1/5	--	2/5	--	2/5	--
Seminiferus tubule degeneration (mild)	2/5	--	3/5	--	2/5	--	2/5	--
Ovaries								
Mineralisation (trace)	--	0/5	--	0/5	--	1/5	--	0/5
Mineralisation (mild)	--	0/5	--	0/5	--	1/5	--	0/5
Atretic follicles (mild)	--	0/5	--	1/5	--	0/5	--	0/5
Skin, treated								
Inflammation (trace)	2/5	2/5	3/5	2/5	4/5	2/5	2/5	1/5
Inflammation (mild)	0/5	3/5	2/5	1/5	0/5	3/5	3/5	4/5
Skin, untreated								
Inflammation (trace)	3/5	3/5	2/5	4/5	2/5	2/5	1/5	3/5
Inflammation (mild)	1/5	1/5	3/5	1/5	3/5	2/5	4/5	2/5

* Number of animals affected / total number of animals

III. CONCLUSION

Repeated dermal application of glyphosate technical to rabbits for a period of 21 days at dose levels of up to 5000 mg/kg bw/day resulted in no systemic treatment-related changes. Only a slight degree of dermal irritation was noted at 5000 mg/kg bw/day. The "No Observed Adverse Effect Level" was, therefore, considered to be 5000 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/02	[redacted]	2012a	Glyphosate acid - In Vitro Absorption through Abraded Rabbit Skin using [^{14}C]-glyphosate [redacted] Study No.: JV2182, Report No.: JV2182-REG Date: 2012-04-x18 GLP: yes Unpublished

Guideline: OECD 428

Deviations:	None
Dates of experimental work:	2011-12-12 to 2011-12-22

Executive Summary
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 5.3.7/01, Johnson, 1982). 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 5.3.7/01).

^{14}C-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/cm^2 (corresponding to 48.3 mg glyphosate acid/cm^2) 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).

Conclusion
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 %.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

a) Non radio-labelled test substance:

Identification:	MON 77973 (glyphosate acid)
Description:	White wet cake
Lot/Batch #:	GLP-1103-21149-T
Chemical purity:	85.14 % as glyphosate acid (puritiy: 95.93 %)
Stability of test compound:	Expiry date: 2012-03-09

b) Analytical reference standard:

Identification:	Glyphosate acid
Lot/Batch #:	GLP-0810-1915-A
Chemical purity:	Not reported

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid) [phosphonomethylene-^{14}C]-Glyphosate
Lot/Batch #:	4675JJN002-1
Radiochemical purity:	96.7 % (confirmed by analysis)
Specific activity:	48 mCi/mmol; 1776 MBq/mmol; 2523µCi/mL; 9.35 MBq/mL

2. Test skin source:

Species:	Rabbit
Strain:	New Zealand White Albino
Source:	Harlan
Age:	At least 12 weeks
Type:	Complete pelt

B: 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 5.3.7/01, Johnson, 1982). 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 kΩ 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 5.3.7/01, Johnson, 1982). 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 kΩ 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 µL 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/cm^2 exposed skin area (202.8 mg/cell), corresponding to 48.3 mg glyphoate/cm^2. 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.
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 65ºC 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 µg/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/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.

II. RESULTS AND DISCUSSION

A. ANALYSES OF THE ^{14}C-GLYPHOSATE STOCKSOLUTION

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.

B. 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.

C. MEMBRANE INTEGRITY CHECK

Based on the ER measurements eight cells with abraded skin samples were selected for the absorption study.

D. 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 5.3-57 below.

Table 5.3-57: Summary of results for dermal absorption of ^{14}C-glyphosate

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)			
	µg/cm^2	SD	% of applied dose	SD
Surface compartment				
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
Receptor compartment				
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 [µg/cm^2/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 µg/cm^2/h, which increased to 166 µg/cm^2/h between 1-4 hours. The mean absorption rate subsequently slowed to 72.3 µg/cm²/h between 4-10 hours and declined further to 13.3 µg/cm²/h for the remainder of the absorption period (10-24 hours). The overall absorption rate (0-24 hours) was 53.1 µg/cm^2/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 µg/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

III. CONCLUSION

The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through abraded rabbit skin was generally slow. The vast majority of glyphosate was removed from the skin by the washing procedures after 6 hours exposure.
The total absorbed amount after 24-hour was 2.42 %. The corresponding total potentially absorbable amount, represented by the mean absorbed dose together with the amount in the remaining dermis after 24 hours was 2.66 %.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/03	[redacted]	1994a	Glyphosate technical (Alkaloida, Tiszavasvári): Repeated dose twenty-eight-Day dermal toxicity study in rabbits [redacted] Data Owner: Alkaloida Report No.: MÜF 214/94 (Test Code: GLY-94-410/N Date: 1994-08-30 GLP: yes unpublished

Guideline:	Non-stated Study is in general accordance with OECD guideline 410 (1981)
Deviations:	None
Dates of experimental work:	1994-05-23 to 1994-06-20

Executive Summary
The toxicity potential of glyphosate technical was assessed after repeated dermal application to groups of male and female New Zealand white rabbits. Doses of 0, 500, 1000 or 2000 mg/kg bw/day were applied five days per week for 28 consecutive days. For application the solid test substance was mixed with water resulting in a 50 % (w/v) solution, and spread evenly over the application site.
There were no mortalities and no treatment-related signs of systemic toxicity. Very slight erythema was noted in one high-dose male and one low-dose female. However, this effect is not considered biological significant and were not seen in the histopathological examination.
There were no treatment-related effects on body weight, food consumption, haematological and clinical chemistry parameters observed in any of the dose groups. The macroscopic and histopathological findings observed at necropsy were considered incidental and unrelated to the test substance.
Conclusion
Repeated dermal administration of glyphosate technical to rabbits for a period of 28 consecutive days at doses of up to 2000 mg/kg bw/day resulted only in slight dermal irritation in one high-dose male and one low-dose female. There were no treatment-related systemic signs of toxicity. Thus, the "No Observed Adverse Effect Level" is considered to be 2000 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	39730494
Purity:	99.6%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand
Source:	KATKI Co. Ltd., Gödöllö, Hungary
Age:	Young, adult
Sex:	male and female
Weight at dosing:	♂ 2200 - 2800 g; ♀ 2100 - 2500 g
Acclimation period:	7 days
Diet/Food:	Altromin Rabbit Chow, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Individually in wire mesh cages.
Environmental conditions:	Temperature: 18 ± 2 °C
	Humidity: Exact range not reported
	Air changes: 10/hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1994-05-23 to 1994-06-20

Animal assignment and treatment:
The potential dermal toxicity of glyphosate technical after repeated exposure was assessed using young adult New Zealand albino rabbits (males and females). Five rabbits per sex per dose received daily dermal applications of 0, 500, 1000 or 2000 mg/kg bw, five days per week for a total of 15 applications.
Two days prior to the first application about 15 % of the skin of the dorsal back of the animals was clipped free of hair. The clipping was repeated weekly thereafter.
For each application the test substance was mixed with water to give a final concentration of 50 % (w/v) of glyphosate. Each dose was spread evenly over about 10 % of the body surface area and covered with a semi-occlusive dressing. After an exposure period of six hours the dressings were removed and the application site was cleaned with hand soap, water and clean, absorbent paper pads. Applications were performed once daily, five days per week for a total of 28 days..

Clinical observations
A check for mortality, clinical signs of toxicity, and general appearance and behaviour, as well as a quantitative assessment of food and water intake was made twice daily. The applications sites were assessed for signs of irritation once daily.

Body weight
Individual body weights were recorded at weekly intervals during the pre-test and study periods and before sacrifice.

Food consumption
Food consumption was assessed at weekly intervals during the pre-test and study periods and before sacrifice.

Haematology and clinical chemistry
Haematological and blood chemical investigations were performed on all rabbits at termination.
The following parameters were measured: haematocrit, haemoglobin, erythrocyte count, platelet count, total leukocyte count, differential leukocyte count, MCV, MCH, MCHC, coefficient of variation of erythrocyte volume (RDW), platetelet volume distribution (PDW), mean platelet volume (MPV), thrombocrit (volume % of platelets), aspartate amino transferase (AST), alanine aminotransferase (ALT),

blood urea nitrogen, total protein, glucose, albumin, total bilirubin, creatinine, inorganic phosphorus, calcium, sodium, potassium, and chloride.

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, heart, kidneys, liver, lung, spleen, stomach, thymus, and testes. The organ-to-brain weight ratios were calculated.
Tissue samples were taken from the following organs and preserved in buffered formalin: treated and untreated skin, adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, stomach gl., testes, and thyroid.
Histopathological examinations were performed on all collected tissues from the control and high-dose animals, as well as from abnormal tissues of animals from the low- and mid-dose groups.

Statistics
Body weights, haematological and clinical chemistry parameters, absolute and relative organ weights and histopathology data of treated animals were compared with control animals. Body weight, food consumption and haematology and clinical chemistry parameters were analysed by t-test. Histopathology data were analysed by Fisher´s exact test.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred during the study.

B. CLINICAL OBSERVATIONS
There were no signs of systemic toxicity noted in any animal of any dose group.

Signs of dermal irritation consistent of very slight erythema was observed in one high-dose male and one low dose female. The erythema lasted from day 7 to 20 for the male, and for 5 days for the female.

C. BODY WEIGHT
There were no statistical significant differences observed in body weights or body weight gains between the control and treated groups.

D. FOOD CONSUMPTION
There were statistically significant differences in food consumption between the control and the treated groups. Observed differences were unrelated to treatment.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY
Haematology
No treatment-related effects were detected in the haematological parameters measured.
In females of the mid-and high-dose group MPV and PDW values were significantly lower when compared to controls. However, the values were within the historical control range for female NZW rabbits of this age. Thus, these changes were not considered treatment-related (see Table 5.3-58).

Blood chemistry
There were no treatment-related effects. The incidental significant changes observed urea level in high dose males were within the historical control range of the testing facility (see Table 5.3-58)

Table 5.3-58: Group mean haematological and blood chemical values and standard deviations (sd)

Dose level (mg/kg bw/day)		MPV (fl)	PDW (fl)	Urea (mmol/L)
		Males		
0	mean	7.48	5.80	7.80
	sd	0.28	1.10	0.66
500	mean	7.94	6.80	8.72
	sd	0.62	1.57	1.68
1000	mean	7.64	6.20	7.64
	sd	0.42	1.04	0.50
2000	mean	7.48	5.60	9.82**
	sd	0.04	0.42	1.02
		Females		
0	mean	8.96	9.70	10.24
	sd	0.85	1.86	1.09
100	mean	8.15	7.50	9.70
	sd	0.99	2.71	0.53
1000	mean	7.62*	6.20*	10.58
	sd	0.13	0.57	0.39
2000	mean	7.46*	5.10***	9.96
	sd	0.27	0.65	1.31

MPV mean platelet volume; PDW platetelet volume distribution (coefficient of variance of platelets volume)
– no significant changes
* significantly different from control group ($p < 0.05$)
** significantly different from control group ($p < 0.01$)
*** significantly different from control group ($p < 0.001$)

I. NECROPSY

Organ weights

There were no treatment-related effects on mean absolute and relative organ weights noted.

Gross pathology

There were no treatment-related macroscopic abnormalities observed in the treated skin or any other tissues in any group.

Histopathology

There were no treatment-related lesions observed in any dose group.

III. CONCLUSION

Repeated dermal application of glyphosate technical to rabbits for a period of 28 days at dose levels of up to 2000 mg/kg bw/day resulted in no systemic treatment-related changes. Only a slight degree of dermal irritation was noted at one animal each of the low- and high-dose group. The "No Observed Adverse Effect Level" was, therefore, considered to be 2000 mg/kg bw/day.

Annex point	Author(s)	Year	Study title
IIA, 5.3.7/04	[redacted]	1996	Glyphosate Acid: 21 Day Dermal Toxicity Study In Rats [redacted] Data owner: Syngenta Report No.: CTL/P/4985 Date: 1996-06-24 GLP: yes not published

Guideline: OECD 410 (1981): OPPTS 870.3200 (1998): 87/302/EEC B.28 (1988)

Deviations: None

Dates of experimental work: 1996-01-09 to 1996-06-24

Excecutive summary

In a subchronic toxicity study groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats received 6 hour dermal applications of 0 (control), 250, 500, or 1000 mg glyphosate acid/kg/day. Glyphosate acid was prepared as a paste using deionised water as the control substance and vehicle. A total of 15 applications were made over a 21 day period (5 applications per week).

Clinical observations were made and bodyweights and food consumption were measured, and at the end of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no clinical signs of systemic toxicity at any dose level and no adverse compound related effects on bodyweight, food consumption, haematology, clinical chemistry or organ weights. There was no evidence of toxicity at histopathological examination.

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.

I. MATERIALS AND METHODS

A: MATERIALS:

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	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
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

B: 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 5.3-59: Study design

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.

Bodyweight: The bodyweight 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
haematocrit
red blood cell count
mean cell volume
mean cell haemoglobin
red cell distribution width
activated partial thromboplastin time
mean cell haemoglobin concentration
platelet count
total white cell count
differential white cell count
blood cell morphology
prothrombin time

Clinical chemistry: Blood was collected at termination, by cardiac puncture and the following parameters were examined:

urea
creatinine
glucose
albumin
total protein
cholesterol
triglycerides
total bilirubin
creatine kinase activity
alkaline phosphatase activity
aspartate aminotransferase activity
alanine aminotransferase activity
gamma-glutamyl transferase activity
calcium
phosphorus (as phosphate)
sodium
potassium
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
kidneys
liver
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 and not examined microscopically

Microscopic examination: All selected tissues processed from the control and 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. Bodyweights, on initial (day 1) bodyweight, organ weights on final bodyweight were analysed using analysis of covariance. All data were analysed using ***SAS (1989***).

II. 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 5.3-60: Intergroup comparison of selected haematology parameters

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	**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 5.3-61 Intergroup comparison of selected clinical chemistry parameters

	Dose level of glyphosate acid (mg/kg/day)							
	Males				Females			
Parameter	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 trigycerides	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.

III. CONCLUSION

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.

IIA 5.3.8 Percutaneous 90-day toxicity (rodents)

Due to the low dermal absorption of glyphosate (see II 5.9.9 and IIA 5.3.7/02), as well as the low toxicity observed in percutaneous 28-day studies (see IIA 5.3.7), 90-day percutaneous toxicity studies are not considered necessary, and therefore not provided.

IIA 5.4 Genotoxicity

In the 2001 EU evaluation glyphosate was examined for mutagenicity and clastogenicity in a wide range of test systems covering all relevant endpoints *in vitro* (see IIA 5.4.1 to IIA 5.4.3). Additional studies have been conducted on glyphosate since the last EU review, however, all these studies were negative and are considered confirmatory data. Glyphosate has clearly been proved to have no genotoxcity potential in a wide range of regulatory studies *in vitro*.

IIA 5.4.1 In vitro genotoxicity testing – Bacterial assay for gene mutation

Table 5.4-1: Summary of *in vitro* genotoxicty testing with glyphosate acid

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
***In vitro* gene mutation tests in bacteria**					
Studies from the 2001 evaluation	Annex B.4.1.1.1 Glyphosate Monograph Thompson, 1995 (Herbex)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538	8.0 – 5000 µg/plate (95%); +/- S9	negative
	Annex B.4.1.1.1 Glyphosate Monograph Fassio, 1995	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	50 – 5000 µg/plate (purity not reported); +/- S9	negative
	Annex B.4.1.1.1 Glyphosate Monograph Jensen, 1991a (CHE)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537	- S9: 160 – 2500 µg/plate + S9: 310 – 5000 (98.6%)	negative
	Annex B.4.1.1.1 Glyphosate Monograph Shirasu et al., 1978 (published MON study by Li&Long, 1988)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537, 1538 and *E. coli* WP2 hcr	10 – 5000 µg/plate (98.4%); +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/01 Mie, 1995a (ALS)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	156-5000 µg/plate (95.68%) +/- S9	negative
	IIA 5.4.1/02 Sokolowski, 2007a (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (95.1%) +/- S9	negative
	IIA 5.4.1/03 Sokolowski, 2007b (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (97.%) +/- S9	negative
	IIA 5.4.1/04 Sokolowski, 2007c (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate–incorporation) 33 – 5000 µg/plate (pre-incubation test) (95%) +/- S9	negative
	IIA 5.4.1/05 Ribeiro, 2007 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	648 – 5000 µg/plate (98.01%) +/- S9	negative

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/06 Miyaji, 2008 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	1 – 1000 µg/plate (98.01%) +/- S9	negative
	IIA 5.4.1/07 Flügge, 2009a (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test) (98.8%) +/- S9	negative
	IIA 5.4.1/08 Flügge, 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 3160 µg/plate (plate incorporation and pre-incubation test) (96.4%) +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/09 Sokolowski, 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	3 – 5000 µg/plate (plate incorporation and pre-incubation test) (97.16%) +/- S9	negative
	IIA 5.4.1/10 Wallner, 2010 (HAG)	Ames test	*S. typhimurium* TA 98, 100, 102, 1535, 1537	31.6 – 5000 µg/plate (plate incorporation and pre-incubation test) (98.2%) +/- S9	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.1/11 Thompson, 1996 (NUF)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP *uvrA*	0 – 5000 µg/plate (plate–incorporation) (95.3%) +/- S9	negative
	IIA 5.4.1/12 Callandar, 1996 (SYN)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2P *uvrA* and WP2P	100 – 5000 µg/plate (plate-incorporation) (95.6%)	negative
	IIA 5.4.1/13 Sokolowski, 2009 (SYN)	Ames test	*S. typhimurium* TA 98, 100, 1535, 1537 and *E. coli* WP2 *uvrA* pKM 101and WP2 pKM 101	33 – 5000 µg/plate (plate-incorporation) (96.3%)	negative

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/01	Mie, A.	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 GLP: yes not published

Guideline: U.S. EPA FIFRA Guidelines, Subdivision F

Deviations: None

Dates of experimental work: 1995-02-21 to 1995-03-09

Executive Summary
Reverse mutation tests were performed on HR-001 in *Escherichia coli* WP2 uvrA and four testere strains of *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537). Experiments were carried out with and without metabolic activation system (S9 mix) at dose levels up to 5000 µg/plate. The mean number of revertant colonies did not exceed the factor of 2 above that of the corresponding solvent control in any strain at any dose with or without S9 mix.

Based on the results, HR-001 is non-mutagenic to bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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 – (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 inDMSO; NaN_3: sodium azide dissolved in sterile water
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

3. 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.

4. test organisms: *Escherichia coli* WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

5. test concentrations:

(a) 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.

(b) 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.
Re-tests: Not concerned

B: TEST PERFORMANCE

1. Preliminary cytotoxicity/plate incorporation mutation assay

Results of the range-finding are presented in table below.

Table 5.4-2: 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_2O)	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_2O)	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	17	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.

2. Pre-incubation 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.

3. Statistics

Results were judged without statistical analysis.

4. 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:

1. A two-fold or greater increase above solvent control in the mean number of revertants is observed
2. This increase in the number of revertants is accompanied by a dose-response relationship
3. This increase in the number of revertants is reproducible.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. PRELIMINARY CYTOTOXICITY ASSAY

HR-001 did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

C. MUTATION ASSAYS

Results are shown in table hereafter

Table 5.4-3: summary data – experiment 1

	Dose	Revertants (n° colonies/plate)*				
		Base-change type			Frameshift type	
	(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix	Solvent control (H_2O)	117	12	21	37	3
	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

		Dose	Revertants (n° colonies/plate)*				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
+ S9 mix		Solvent control (H_2O)	78	9	21	35	7
		156	83	6	19	36	9
		313	77	7	19	31	5
		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		compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	- S9 mix	µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	510	524	305	621	786
		compound	2-AA	2-AA	2-AA	2-AA	2-AA
	+ S9 mix	µg/plate	1	2	10	0.5	2
		Revertants/plate	606	392	522	360	75

* values are the mean of three plate

Table 5.4-4: summary data – experiment 2

		Dose	Revertants (n° colonies/plate)*				
			Base-change type			Frameshift type	
		(µg/plate)	TA100	TA1535	WP2uvr A	TA98	TA1537
- S9 mix		Solvent control (H_2O)	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
+ S9 mix		Solvent control (H_2O)	123	8	17	37	7
		156	112	7	15	32	10
		313	125	7	13	29	9
		625	113	8	20	35	9
		1250	107	7	14	28	9
		2500	89	7	19	20	8
		5000	67	4	17	17	4
Positive controls		compound	AF-2	NaN_3	AF-2	AF-2	9-AA
	- S9 mix	µg/plate	0.01	0.5	0.01	0.1	80
		Revertants/plate	595	527	252	742	909
		compound	2-AA	2-AA	2-AA	2-AA	2-AA
	+ S9 mix	µg/plate	1	2	10	0.5	2
		Revertants/plate	768	322	605	327	87

* values are the mean of three plates

III. CONCLUSIONS

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.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/02	Sokolowski, A.	2007a	Salmonella typhimurium and Escherichia coli Reverse mutation assay with Glyphosate technical (NUP-05068) RCC Ltd., Itingen, Switzerland Data owner: Nufarm RCC Study No.: 1061401 Date: 2007-03-16 GLP: yes unpublished

Guideline:	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
Dates of experimental work:	January 15, 2007 to January 25,2007

Executive Summary

This study was performed to investigate the potential of Glyphosate technical (NUP05068) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both 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, occurred in the test groups with and without metabolic activation. Only in experiment II a minor reduction in the number of revertants, occurred in strain TA 1537 in the absence of metabolic activation at 5000 µg/plate. 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 and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP-05068)
Description:	Crystalline powder White
Lot/Batch #:	200609062
Purity:	95.1 %
Stability of test compound:	Not specified

2. 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
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 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 15, 2007 to January 25,2007

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.

II. RESULTS AND CONCLUSION

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.

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

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/03	Sokolowski, A.	2007b	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 GLP: yes unpublished

Guideline:	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
Dates of experimental work:	January 15, 2007 to January 25,2007

Executive Summary

This study was performed to investigate the potential of Glyphosate technical (NUP05070) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.

The assay was performed in two independent experiments both 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, 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 and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP-05070)
Description:	Crystalline powder White
Lot/Batch #:	20060901
Purity:	97.7%
Stability of test compound:	Not specified
2. 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
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 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 15, 2007 to January 25,2007

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.

II. RESULTS AND CONCLUSION

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 bserved 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.

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

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/04	Sokolowski, A.	2007c	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 GLP: yes unpublished

Guideline:	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
Dates of experimental work:	January 16, 2007 to January 25,2007

Executive Summary
This study was performed to investigate the potential of Glyphosate technical (NUP050067) to induce gene mutations in the plate incorporation test (experiment I) and the preincubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both 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, 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-05067) 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 and showed a distinct increase of induced revertant colonies.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical (NUP-05067)
Description:	Crystalline powder White
Lot/Batch #:	0609-1
Purity:	95%
Stability of test compound:	Not specified

2. 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
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 NaCI (MERCK, 0-64293 Darmstadt)
Test concentrations::	0, 33, 100, 333, 667, 1000, or 5000 µg / plate with or without metabolic activation.g

B: STUDY DESIGN AND METHODS

In life dates: January 16, 2007 to January 25,2007

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 89 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 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.

II. RESULTS AND CONCLUSION

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 follOowing 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.

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

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/05	Riberri do Val, R.	2007	Bacterial reverse mutation test (Ames Test) for Glifosato Téchnico Helm TECAM Technologia Ambiental Ltda., Brazil Data owner: HAG Report No.: 3393/2007-2.0AM-B Date: 2007-12-13 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	30/11/2007 – 03/12/2007

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535 and TA1537. The assay was carried out with and without metabolic activation. Concentrations of test item ranged between 648 and 5000 µg/plate. The mutation rates after 72 hours of incubation were lower than 2. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification: Glifosato Téchnico Helm
Description: Solid
Lot/Batch #: 2007091801
Purity: 980.1 g/kg
Stability of test compound: Stable (CIPAC MT 46, 54°C, 14 days)

2. 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

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535; TA1537
Source: Moltox Inc. (Annapolis, MD, USA)

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. Test concentrations:

Preliminary cytotoxicity test: 8, 40, 200, 1000, 5000 µg/plate
Mutation assay: 648, 1080, 1800, 3000, 5000 µg/plate

B: STUDY DESIGN AND METHODS

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 *Salomonella* 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.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
None of the concentrations tested showed cytotoxic effects.

B. MUTATTION ASSAYS
No significant mutation rate was observed up to a concentration of 5000 µg of test item per plate.

C. LIABILITY CHECK
All acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/06	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 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	05/06/2008 – 30/06/2008

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA97a; TA98; TA100; TA102 and TA1535. The assay was carried out with and without metabolic activation. Concentrations of test item ranged between 0.001 and 1.0 mg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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)

2. 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

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA97a; TA98; TA100; TA102; TA1535
Source: Moltox Toxicology, Inc.

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. 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

B: STUDY DESIGN AND METHODS

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

II. RESULTS AND DISCUSSION

A. 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.

B. MUTATTION ASSAYS
No significant mutation rate was observed up to a concentration of 1.0 mg of test item per plate.

C. LIABILITY CHECK
The concentrations of the lowest and highest concentrations were confirmed.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/07	Flügge, C.	2009a	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 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	04/02/2009 – 27/02/2009

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535, TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 31.6 and 3160.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: Dr. Bruce N. Ames

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. 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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. 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.

B. MUTATTION 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.

C. LIABILITY CHECK
The genotypes of the 5 strains used were confirmed regularly.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/08	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 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	15/10/2009 – 23/11/2009

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535, TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between

31.6 and 3160.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA102; TA1535, TA1537
Source: Dr. Bruce N. Ames

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Aroclor 1254)

5. 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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. 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.

B. MUTATTION 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.

C. LIABILITY CHECK
The genotypes of the 5 strains used were confirmed regularly.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/09	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 GLP: yes unpublished

Guideline:	OECD 471
Deviations:	None
Dates of experimental work:	17/03/2010 – 22/03/2010

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA1535 and TA1537 and the *Escherichia coli* strain WP2 uvrA. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 3.0 and 5000.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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

3. Test organisms/cells:

Species: *S. typhimurium*
Strain: TA98; TA100; TA1535, TA1537

Species: *Escherichia coli*
Strain: WP2 uvrA

Source: Trinova Biochem GmbH (35394 Gießen, Germany)

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

5. 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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. 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.

B. MUTATTION 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).

C. LIABILITY CHECK
The acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/10	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 GLP: yes unpublished

Guideline: OECD 471

Deviations: None

Dates of experimental work: 25/03/2010 – 06/04/2010

Executive Summary
The reverse mutation assay was performed to study possible mutagenic effects of technical Glyphosate on the *Salmonella typhimurium* strains TA98; TA100; TA102; TA1535; TA1537. The assay was carried out in two independent experiments with and without metabolic activation. The first one was a plate incorporation test and the second one a preincubation test. Concentrations of test item ranged between 31.6 and 5000.0 µg/plate. The test item did not cause any mutagenic effect with or without metabolic activation.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. 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

3. Test organisms/cells:

Species:	*S. typhimurium*
Strain:	TA98; TA100; TA102; TA1535, TA1537
Source:	MOLTOX, INC, NC 28607, USA

4. Metabolic activation system:

S9 (microsomal fraction of rat liver induced with Phenobarbital/β-Naphthoflavone)

5. 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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
No preliminary cytotoxicity assay was performed.

B. MUTATTION 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.

C. LIABILITY CHECK

The acceptance criteria were met.

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/11	Thompson, P.W.	1996	Technical glyphosate: Reverse mutation assay "Ames test" using Salmonella typhimurium and Escherichia coli Safepharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/014 Date: 1996-02-20 GLP: yes unpublished

Guideline:	OECD 471 (1983) Commission Directive (EC) 92/69/EEC (1992), Method B14 US EPA (TSCA) guidelines
Deviations:	None
Dates of experimental work:	August 19, 1995 to November 13, 1995

Executive Summary

This study was performed to investigate the potential of Technical Glyphosate to induce gene mutations in the Ames plate incorporation method using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test item was tested at the following concentrations:
0, 50, 150, 500, 1500, and 5000 µg/plate with and withput metabolic activation.
The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim 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.
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle/Controls

Vehicle = sterile distilled water

Negative/solvent control:	Vehicle/solvent controls were performed.
Positive control:	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. 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% Dicfo 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 withput metabolic activation. In triplicate for each bacterial strain and for each concentration of test material with an without S9-mix.

B: STUDY DESIGN AND METHODS

In life dates: August 19, 1995 to November 13, 1995

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.

II. RESULTS AND CONCLUSION

The plates incubated with the test item caused no visible reduction in the growth of the bacterial lawn at any dose level up to maximim 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.
In conclusion, the test material was found to be non-mutagenic under the conditions of this test.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/12	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 GLP: yes not published

Guideline:	OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
Dates of experimental work:	1995-10-23 to 1996-02-16

Excecutive summary
In a reverse gene mutation assay in bacteria (***Gatehouse et al, 1990***, based on the method of ***Maron and Ames, 1983***), four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and two strains of *Escherichia coli* (WP2P and WP2 *uvrA*) were exposed to glyphosate acid in the presence and absence of rat liver-derived metabolic activation system (S9-mix).

In two separate experiments, glyphosate acid did not induce any significant, reproducible increases in the observed numbers of revertant colonies in any of the tester strains used, either in the presence or absence of S9-mix.

The sensitivity of the test system, and the metabolic activity of the S9-mix, were clearly demonstrated by the increases in the number of revertant colonies induced by positive control substances.

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.

I. MATERIALS AND METHODS

A: MATERIALS:

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 (final concentration): Dimethylsulphoxide – DMSO (10 µL/plate)

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 Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM), in sterile deionised water and adjusted to a final pH of 7.4.

Test organisms:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		

E. coli strains

X	WP2P (pKM101)	X	WP2P *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, 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.

B: STUDY DESIGN AND METHODS:

In-life dates: Start:28 November 1995 End: 11 December 1995

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 controlshad 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.

II. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.4.1/13	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 Date: 2009-12-18 GLP: yes not published

Guideline:	OECD 471 (1997): OPPTS 870.5100 (1998): 2008/440/EC B.13/B.14 (2008)
Deviations:	None
Dates of experimental work:	2009-09-15 to 2009-12-18

Excecutive summary
This study was performed to investigate the potential of Glyphosate technical (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).

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 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. Positive control chemicals showed appropriate responses in the relevant strains.

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.

Therefore, Glyphosate technical is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

I. MATERIALS AND METHODS

A: MATERIALS:

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 (final concentration):	100µl/plate
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 $MgCl_2$
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:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		

E. coli 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

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 23 September 2009 End: 13 October 2009

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.

II. RESULTS AND DISCUSSION

A. PRELIMINARY CYTOTOXICITY ASSAY
Not performed.

B. MUTATTION 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.

III. CONCLUSION

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.

IIA 5.4.2 In vitro genotoxicity testing – Test for clastogenicity in mammalian cells

Table 5.4-5: Summary of *in vitro* genotoxicty testing with glyphosate acid

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
Study from the 2001 evaluation	Annex B.5.4.1.2 Glyphosate Monograph Van de Waart, 1995 (AGC, MON)	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 (96%)	negative
Studies not reviewed in the 2001 evaluation	IIA 5.4.2/01 Kyomu, 1995 (ALS)	Cytogenicity	CHL cells	- S9: 62.5 – 1000 µg/mL (+ S9: 255 – 2000 µg/mL (95.68%)	negative
	IIA 5.4.2/02 Wright, 1996 (NUF)	Cytogenicity	CHL cells	+/- S9: 0 - 1250 µg/mL (95.3%)	negative
	IIA 5.4.2/03 Fox, 1998 (SYN)	Cytogenicity	Human lymphocytes	- S9: 100 – 1250 µg/mL + S9: 100 – 1250 µg/mL (95.6%)	negative

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.4.2/01	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 GLP: yes not published

Guideline:	U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:	None
Dates of experimental work:	1995-03-13 to 1995-05-09

Executive Summary
The objective of the study was to evaluate the clastogenic potential of HR-001 in cultures Chinese Hamster lung cells. HR-001 was tested in the direct method at concentrations of 125, 250, 500 and 1000 µg/ml (for the 24-hr treatment) and 62.5, 125, 250 and 500 µg/ml (for the 48-hr treatment). The positive control was mitomycin C (MMC, 0.1 µg/ml). HR-001 was also tested with and without metabolic activation (S9) system at concentrations of 250, 500, 1000 and 2000 µg/ml. The positive control was Benzo (a) pyrene (B(a)P, 40 µg/ml).

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. **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 balances salt solution and culture medium

2. **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)

3. 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.

4. Test organisms: CHL cells established from the lung of Chinese hamster

5. Culture medium: The growth medium was Eagle's MEM supplemented with 10% newborn calf serum

6. 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

7. Replicates:

Preliminary cytotoxicity test: Duplicate

Metaphase analysis: Duplicate

B. TEST PERFORMANCE

1. In life dates 1995-03-13 to 1995-05-09

2. Preliminary cytotoxicity test

CHL cells were seeded at a density of 1.0 x 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 48hours. 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.

3. Metaphase analysis

CHL cells were seeded at a density of 2.0 x 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 48hours.

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 plyploid cell having 3 or more copies of haploid number of chromosomes was scored as a numerical chromosome aberration cell.

4. 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.

II. RESULTS AND DISCUSSION

A. 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 5.4-6: Preliminary growth inhibition test

Concentration (µg/ml)	Relative cell growth (%)			
	Direct method 24 h	Direct method 48 h	Metabolic activation method 6-18 h (1st exp.)	Metabolic activation 6-18 h (2nd 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

B. METAPHASE ANALYSIS

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.
It was noticed that in the both methods the color of the culture medium was turned to yellow at 500 µg/ml and 1000 µg/ml, indicating a decline of pH.

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 and in the concurrent control experiment (without S9 mix), 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 in either presence or absence of S9 mix.

It was noticed that in both treatments (with and without S9 mix) pH of the culture medium of the cultures treated at 500, 1000 and 2000 µg/ml went down.

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.

Table 5.4-7: Preliminary: Cytogenetics test (direct method 24-hr and 48-hr treatment)

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	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	-
Sovent 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	0	1.5	0.5	-
			500	100	5.6	0	-	1.5	0.5	0	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	0	1.0	0.5	-
			250	100	3.1	1.0	-	1.5	0.5	0	0	0.5	0	0	2.5	1.0	-
			500	100	3.0	0	-	1.5	1.0	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 5.4-8: Preliminary: Cytogenetics test (direct method 24-hr and 48-hr treatment)

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	Fragmen-tation	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	-
Sovent 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	0.5	-	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	3.8	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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.4.2/02	Wright, N.P.	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro Safepharm Laboratories, Derby, UK Data owner: Nufarm SPL Project No.: 434/015 Date: 1996-03-13 GLP: yes unpublished

Guideline: not specified

Deviations: not specified

Dates of experimental work: 30 August 1995 and 4 January 1996.

Executive Summary

Chinese hamster lung (CHL) cells were treated with the test material at six dose levels, in duplicate, together with negative and positive controls, three dose levels were selected for metaphase analysis. Four treatment regimens were used: 6 hours exposure both with and without the addition of an induced rat liver

homogenate metabolising system at 50% in standard co-factors; 24 hours continuous exposure and 48-hours continuous exposure without metabolic activation. The dose range was selected on the basis of the results of a preliminary toxicity test and a determination of the pH of culture media after the addition of the test material and was 39 to 1250 pglml for the 6-hour treatment both with and without S9 and for the 24 and 48-hour continuous treatments. Technical Glyphosate was observed to reduce the pH to an unacceptable level at 2500 and 5000 pg!ml. The vehicle (solvent) controls gave frequencies of cells with aberrations within the range expected for the CHL cell line. All the positive control treatments except cyclophosphamide without S9 gave highly significant increases in the frequency of cells with aberrations indicating the satisfactory performance of the test and of the activity of the metabolising system. The test material, Technical Glyphosate, did not induce any significant increases in the frequency of cells with aberrations in any of the treatment cases. The test material was shown to be toxic to CHL cells in vitro in the continuous treatment cases, but only when the pH was reduced to an unacceptable level. Technical Glyphosate was shown to be non-c1astogenic to CHL cells in vitro.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	TECHNICAL GLYPHOSATE
Description:	white powder
Lot/Batch #:	H95D 161A
Purity:	95.3% w/w
Stability of test compound:	Not specified
2. Vehicle/Controls	suspended in minimal essential culture media
Negative/solvent control:	
Positive control:	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
Activation:	Lot No. Aro. S9/11/0CT/95 SPL was prepared in-house at Safepharm Laboratories on 11/0CT/95. It was prepared from the livers of male SpragueDawley rats weighing - 200g. These had received a single ip. injection of Aroclor 1254 at 500 mglkg, up to 5 days be
Test organisms::	Hamster CHL line
Culture medium:	Eagle's Minimal Essential medium with Earle's Salts (MEM), supplemented with 10% foetal bovine serum and antibiotics, at 37' C with 5% CO2 in AIR
Test concentrations::	19.5 to 5000 µg/mLg

B: STUDY DESIGN AND METHODS

In life dates: 30 August 1995 and 4 January 1996.

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.

II. RESULTS AND CONCLUSION

- 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 59 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.
In conclusions, 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-c1astogenic to CHL cells in vitro.

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

Annex point	Author(s)	Year	Study title
IIA, 5.4.2/03	Fox, V.	1996	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 GLP: yes not published

Guideline:	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.
Dates of experimental work:	1995-07-25 to 1998-10-29

Excecutive summary

Glyphosate acid was evaluated for its clastogenic potential in an *in vitro* cytogenetic assay using human lymphocytes from two donors treated in the presence and absence of a rat liver derived metabolic activation system (S9-mix). Cultures from both donors were harvested at the standard time of 68 hours after culture initiation and additional cultures from Donor 2 were harvested at the later time of 92 hours after culture initiation.

Cultures treated with glyphosate acid at 100, 750 and 1250 µg/mL were selected for chromosomal aberration analysis along with the appropriate solvent and positive control cultures. A 1250 µg/mL concentration of glyphosate acid reduced the pH of the culture medium by 0.57 pH units. Concentrations higher than this were considered not suitable for chromosomal aberration analysis due to excessive reductions in the pH of the culture medium.

No significant reductions in mean mitotic activity were observed in the presence of S9-mix and small reductions in mean mitotic activity, compared to the respective solvent control values, were observed at the highest concentration selected for chromosomal aberration analysis in the absence of S9-mix.

At the 68 hour sampling time, no statistically or biologically significant increases in the percentage of aberrant cells, compared to the solvent control values, were recorded in cultures from either donor treated in either the presence or absence of S9-mix.

No statistically or biologically significant increases in the percentage of aberrant cells, compared to the solvent control values, were recorded at the 92 hour sampling time in cultures from Donor 2 treated in either the presence or absence of S9-mix.

The sensitivity of the test system, and the metabolic activity of the S9-mix employed, were clearly demonstrated by the increases in the percentage of aberrant cells induced by the positive control agents, mitomycin C and cyclophosphamide.

Glyphosate acid was not clastogenic to cultured human lymphocytes treated *in vitro* in either the presence or absence of S9-mix.

I. MATERIALS AND METHODS

A: MATERIALS:

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 (final concentration): 800 µl/ml
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 Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (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. Bothl 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 *Mycoplasma* 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 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	100 µg/mL 750 µg/mL 1250 µg/mL	1250 µg/mL	1250 µg/mL

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 11 March 1998 End: 26 August 1998

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 have been reported to result in the production of chromosomal aberrations (***Scott et al, 1991***).

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, according to the principles of the criteria recommended by ***Scott et al (1990)***.

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

II. RESULTS

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.

III. CONCLUSION

Glyphosate acid was not clastogenic to cultured human lymphocytes treated *in vitro* in either the presence or absence of S9-mix.

IIA 5.4.3 In vitro genotoxicity testing – Test for gene mutation in mammalian cells

Table 5.4-9: Summary of *in vitro* genotoxicty testing with glyphosate acid

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
***In vitro* tests for gene mutation in mammalian cells**					
Studies from the 2001 evaluation	Annex B.5.4.1.1.2 Glyphosate Monograph Jensen, 1991b (MON/CHE)	Mouse lymphoma test	Mouse lymphoma cells (L5178Y)	- S9: 0.61 – 5.0 mg/L + S9: 0.52 – 4.2 mg/L (98.6%)	negative
	Annex B.5.4.1.1.2 Glyphosate Monograph Li, 1983 (published MON study by Li&Long, 1988)	HGPRT assay	Chinese hamster ovary (CHO) cells	- S9: 5 – 22.5 mg/L + S9: 5 – 252 mg/L (98.7%)	negative
Study not reviewed in the 2001 evaluation	IIA 5.4.3/01 Clay, 1996 (SYN)	Mouse lymphoma test	Mouse lymphoma cells (L5178Y $TK^{+/-}$)	+/- S9: 296 – 1000 µg/mL (95.6%)	negative
***In vitro* tests for DNA damage and repair in mammalian cells**					
Studies from the 2001 evaluation	Annex B.5.4.1.3 Glyphoate Monograph Rossberger, 1994 (FSG)	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.13 – 111.69 mM (>98%)	negative
	Annex B.5.4.1.3 Glyphoate Monograph Williams, 1983 (published MON study by Li&Long, 1988)	UDS assay	Primary rat (F344) hepatocytes	0.13 – 111.69 mM (>98%)	negative

Reference (Owner)		Type of study	Test organism / test system	Dose levels (purity) Metabolic activation	Results
***In vitro* tests for DNA damage and repair in bacteria**					
Study not reviewed in the 2001 evaluation	IIA 5.4.3/02 Mie, 1995b (ALS)	Rec assay	*B. subtilis* strains H17 and M45	+/- S9 : 7.5 – 240 µg/disk (95.68%)	negative

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.4.3/01	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 GLP: yes not published

Guideline:	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..
Dates of experimental work:	1996-01-29 to 1996-05-24

Excecutive summary
In a mammalian cell gene mutation assay L5178Y TK$^{+/-}$ mouse lymphoma cells were treated *in vitro* with various concentrations of test substance, both in the presence and absence of a rat liver derived auxiliary metabolic system (S9-mix). Mutant frequencies were assessed by cell growth in the presence of trifluorothymidine after a 48 hour expression time.

Glyphosate acid was tested in two independent experiments up to a maximum concentration of 1000 µg//mL in the presence and absence of S9-mix as concentrations; in excess of this produced excessive reductions in the pH of the treatment medium. Very little toxicity was seen at the maximum concentration tested. Minimum survival levels, compared to the solvent control cultures, of 90% and 57% were observed in cultures treated with the maximum concentration of glyphosate acid in the presence and absence of S9-mix respectively.

No significant increases in mutant frequency were observed in cultures treated with glyphosate acid in either the presence or absence of S9-mix in either of the independent experiments.

The positive controls induced substantial increases in mutant frequency in all mutation experiments thus demonstrating the activity of the S9-mix and that the assay was performing satisfactorily in being capable of detecting known mutagens.

Glyphosate acid was not mutagenic to L5178Y TK$^{+/-}$ cells in the presence or absence of S9-mix.

I. MATERIALS AND METHODS

A: MATERIALS:

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 (final concentration): 1%
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	Phenobarbitol		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		No
Periodically checked for Mycoplasma contamination?	X	Yes		No
Periodically checked for karyotype stability?		Yes		No
Periodically “cleansed” against high spontaneous background?		Yes		No

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

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 22 November 1995 End: 12 March 1996
Test performance:

Cell treatment: 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, 10^4 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.

II. RESULTS AND DISCUSSION

Preliminary toxicity assay: The maximum concentration of glyphosate acid considered appropriate for testing in the mutation experiments was estimated as 1500 µg/mL in the presence and absence of S9-mix, as a concentration of 2000 µg/mL was found to produce an excessive reduction in the pH of the treatment medium. A maximum concentration of 1000 µg/mL was however selected for evaluation in both mutation experiments as a concentration of 1500 µg/mL was also determined 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.

III. CONCLUSION

Glyphosate acid was not mutagenic to L5178Y TK$^{+/-}$ cells in the presence or absence of S9-mix.

Annex point	Author(s)	Year	Study title
IIA, 5.4.3/02	Mie, A.	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 GLP: yes not published

Guideline:	U.S. EPA FIFRA Guidelines, Subdivision F
Deviations:	None
Dates of experimental work:	1995-02-14 to 1995-02-15

Executive Summary

DNA repair test with Bacillus subtilis strains of H17 and M45 was performed to evaluate the DNA-damaging activity of HR-001 at concentrations of 7.5, 15, 30, 60, 120 and 240 µg/disk with and without S9 metabolic activation. 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 5.3.1.4-1). 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.

Based on the results, HR-001 did not have DNA-damaging activity in the bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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
2. 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)
3. 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.
4. test organisms:	Recombination-wild (rec+) strain H17 Recombination-deficient (recE-) strain M45 of *Bacillus subtilis*
5. test concentrations	6 dose level were tested: 7.5, 15, 30, 60, 120 and 240 µg/diskwith and without S9 metabolic activation

B: TEST PERFORMANCE

1. Test description

DNA-damaging activity was evaluated by a DNA repair test (Rec-Assay), with *Bacillus substilis* 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 (20µl: 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.

2. Statistics

Results were judged without statistical analysis.

3. 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.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. MUTATION 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 5.4-10: Results of DNA-repair

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_2O)		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

III. CONCLUSIONS

Under the conditions used in this experiment, HR-001 did not have DNA-damaging activity in the bacteria.

IIA 5.4.4 In vivo genotoxicity testing (somatic cells) – Metaphase analysis in rodent bone marrow, or micronucleus test in rodents

During the 2001 EU glyphosate evaluation, a number of *in vivo* cytogenicity studies and bone marrow micronucleus tests in rats and mice have been evaluated. The weight of evidence clearly show that glyphosate is not clastogenic *in vivo.* A micronucleus tests performed by [redacted] (1993) using the extremely high and already cytotoxic dose of 5000 mg/kg bw/day may have indicated a weak increase in the incidence of micronuclei in females. This study was previously reviewed in the 2001 EU glyphosate evaluation and it was concluded that the result of this study was unlikely to be relevant because the effect was only seen in females (usually the less sensitive sex in a micronucleus test) and the variation in the % of polychromatic erythrocytes with micronuclei was considerably high among female dose groups compared to controls, whereas the results in the male groups were much more homogeneous. In addition, in a cytogenetic study conducted in the same laboratory under near identical conditions using the same doses and test material did not provide any evidence of chromosome aberrations but did indicate a certain degree of cytoxicity at the highest dose level to bone marrow cells.

The ability of glyphosate to cause chromosomal aberrations was further investigated in the *in vivo* micronucleus test [redacted], 2009b, [redacted] 2007, [redacted] 2008, [redacted], 1999, [redacted] 2006, [redacted], 1996 & [redacted], 2008). All new studies were unequivocally negative except the Durward, 2006 study where a statistical significant increase in PCEs was observed at 600 mg/kg. However, the response observed was very modest and within the historical range for vehicle control animals and was therefore concluded to not be of biological relevance. Overall the new data supports the previous findings. Against the background of this large database and based on the overwhelming weight of evidence, it can be concluded that the active ingredient does not exhibit a genotoxicity risk to humans.

Table 5.4-11: Summary of *in vivo* genotoxicty testing with glyphosate

Reference (Owner)		Type of study	Test organism / test system	Dose levels (purity) Sampling	Results
Studies from the 2001 evaluation	Annex B.5.4.2.1 Glyphosate Monograph [redacted] 1994 (FSG)	Cytogenicity in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day (96.8%) sampling 24 h after second dose	Negative for clastogenicity; mitotic index ↓ at 5000 mg/kg bw
	Annex B.5.4.2.1 Glyphosate Monograph [redacted], 1993c (FSG)	Micronucleus test in bone marrow	Swiss albino mice; daily oral applications for 2 successive days	0, 50, 500, 5000 mg/kg bw/day (96.8%) sampling 24 h after second dose	♂: negative ♀:equivocal
	Annex B.5.4.2.1 Glyphosate Monograph [redacted] 1991 (CHE)	Micronucleus test in bone marrow	NMRI mice, single oral application	0 – 5000 mg/kg bw (98.6%) sampling after 24, 48,72 h	negative
	Annex B.5.4.2.1 Glyphosate Monograph [redacted], 1983 (MON)	Cytogenicity in bone marrow	Sprague-Dawley rats, single i.p. injection	0 – 1000 mg/kg bw (98.7%) sampling after 6, 12,24 h	negative

Reference (Owner)		Type of study	Test organism / test system	Dose levels (purity) Sampling	Results
Studies not reviewed in the 2001 evaluation	IIA 5.4.4/01 [redacted] 2006 (NUF)	Micronucleus test in bone marrow	CD-1 mice ♂; single i.p. dose	0, 150, 300, 600 mg/kg bw (95.7%) sampling after 24 and 48 h	Stat. sign. ↑ in PCE at 600 mg/kg bw (24 h), within historical control overall: negative
	IIA 5.4.4/02 [redacted], 2009b (HAG)	Micronucleus test in bone marrow	CD rat, single oral application	0, 500, 1000, 2000 mg/kg bw/day (98.8%) sampling after 24 and 48 h	♂: negative ♀: negative
	IIA 5.4.4/02 [redacted], 2007 (HAG)	Micronucleus test in bone marrow	Swiss albino mice ♂, daily oral applications for 2 successive days	0, 8, 15, 30 mg/kg bw/day (98.01%) sampling 24 h after second dose	negative
	IIA 5.4.4/02 [redacted] 2008 (HAG)	Micronucleus test in bone marrow	Swiss albino mice ♂+♀, daily i.p. applications for 2 successive days	0, 15.62, 31.25, 62.5 mg/kg bw/day (98%) sampling 24 h after second dose	negative
	IIA 5.4.4/02 [redacted] 1999 (NUF)	Micronucleus test	Swiss albino mice, ♂ + ♀, two i.p. injections (24 h interval)	0, 187.5, 375, 562.5 mg/kg bw (95.49 %) Sampling 24 h after 2nd application	negative
	IIA 5.4.4/02 [redacted], 1996 (SYN)	Micronucleus test in bone marrow	CD-1 mice, 5 ♂+ 5 ♀/dose / sampling point; single oral dose	0, 5000 mg/kg bw (95.6%) sampling after 24 and 48 h	negative
	IIA 5.4.4/02 [redacted], 2008 (SYN)	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. (99.1%)	negative

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/01	[redacted]	2006	Glyphosate Technical: Micronucleus Test In The Mouse [redacted] Data owner: Nufarm Report No.: 2060/014 Date: 2006-02-08 GLP: yes unpublished
Guideline:			OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF

Deviations:	None
Dates of experimental work:	2005-06-07 - 2005-07-20

Executive Summary
The Micronucleus Test was performed to assess the potential of the test material to produce damage to chromosomes or aneuploidy when administered to mice.
Based on the results of a range-finding the main test was performed using only male mice. The micronucleus test was conducted using the intraperitoneal route in groups of seven mice at the maximum tolerated dose (MTD) 600 mg/kg and with 300 and 150 mg/kg bw as the two lower dose levels. Animals were killed 24 or 48 hours later, the bone marrow was extracted, and smear preparations made and stained. Polychromatic (PCE) and normochromatic (NCE) erythrocytes were scored for the presence of micronuclei.
Further groups of mice were given a single intraperitoneal dose of phosphate buffered saline (each of 7 mice) or dosed orally with cyclophosphamide (5 mice), to serve as vehicle and positive controls respectively. Vehicle control animals were killed 24 or 48 hours later, and positive control animals were killed after 24 hours.

A statistically significant decrease in the percentage polychromatic erythrocytes (PCEs) per 1000 erythrocytes was observed in the 24-hour 600 mg/kg bw group when compared to the control group. 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 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 polychromatic erythrocytes in animals dosed 600 mg/kg bw in the 24-hour group when compared to the control group. However, the response was very modest, within the historical range for vehicle control and did not include any individual animal values that would not be acceptable for vehicle control animals. 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. Therefore the response was considered to have no genotoxic significance.
The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

The test material was considered to be non-genotoxic under the conditions of the test.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: PBS

3. Test animals:

Species:	Mouse
Strain:	CD-1

Source:	Charles River Ltd., Margate, Kent, UK
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 Ldt., 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

B: STUDY DESIGN AND METHODS

In life dates: 2005-06-07 to 2005-07-20

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. 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.

C. 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 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 5.4-12 and Table 5.4-13). 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 5.4-12: 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 5.4-13: 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

III. CONCLUSION

The test material glyphosate technical was considered to be non-genotoxic under the conditions of the test.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/02	[redacted]	2009b	Micronucleus test of Glyphosate TC in Bone Marrow Cells of the CD Rat by oral administration [redacted] Data owner: HAG Report No.: LPT 23917 Date: 2009-05-18 GLP: yes unpublished

Guideline:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations:	None
Dates of experimental work:	04/02/2009 – 06/03/2009

Executive Summary
Glyphosate TC was assayed in an in vivo bone marrow micronucleus test in the rat for the detection of damage to the chromosomes or the mitotic apparatus employing three dose levels by oral administration. The dose levels had been selected based on a preliminary oral acute toxicity study employing one animal per dose and sex. Three dose levels of 500, 1000 and 2000 mg test item/kg b.w. were tested. The administration volume was 20 mL/kg. No signs of systemic toxicity were noted and no mortality occurred up to the top dose level of 2000 mg Glyphosate TC/kg b.w. For the main study three ascending doses of 500, 1000 and 2000 mg Glyphosate TC/kg b.w., p.o. were administered. Further groups received the vehicle (0.8% aqueous hydroxypropylmethylcellulose) and one further group the positive reference item cyclophosphamide (27 mg/kg b.w., i.p.). Each group consisted of 5 male and 5 female rats. No signs of systemic toxicity were noted after administration of Glyphosate TC up to the highest reasonable dose level of 2000 mg/kg b.w. until 48 hours after administration (the last sampling time point). Immediately after sacrifice, bone marrow smears were prepared. Two (2) sampling times were employed in this study: 24 hours after administration, samples were prepared from the vehicle, positive reference item and all 3 doses of test item-treated animals; 48 hours after administration, samples were prepared only from the vehicle control and high dose-treated animals. Two thousand (2000) erythrocytes were evaluated per animal. The highest reasonable dose level of 2000 mg Glyphosate TC/kg b.w., p.o. did not result in an increase in the incidence of micronucleated polychromatic erythrocytes (PCE). The incidences of PCEs per 2000 PCEs after 24 hours were 1.2 for male and 0.8 for female animals. After 48 hours, the PCEs were 1.6 for male and 0.8 for female animals (vehicle control: 1.6 (male) and 1.8 (female) after 24 h, 2.0 (male) and 2.2 (female) after 48 h). Cyclophosphamide resulted in a significant increase of 30.2 (male) / 24.0 (female) micronuclei per 2000 PCEs. The ratio of polychromatic to normochromatic erythrocytes (NCE) was not influenced.

In conclusion, under the present test conditions, Glyphosate TC tested up to the highest reasonable dose level of 2000 mg/kg b.w. by oral administration showed no mutagenic properties in the rat bone marrow micronucleus study at the two tested sampling times of 24 hours and 48 hours. In the same system, cyclophosphamide (positive reference item) induced significant damage.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control:

Vehicle: 0.8% hydroxypropylmethylcellulose
Positive Control: Cyclophosphamide

3. Test animals:

Species:	Rat
Strain:	CD
Source:	Charles River Laboratories
Age:	Males: 32 – 33 days
	Females: 33 – 34 days
Sex:	Male and Female
Weight at dosing:	Males: 106 – 132 g
	Females: 88 – 111 g
Diet/Food:	Commercial ssniff® R/M-H V1534, feeding was discontinued approx. 16 hours befor administration
Water:	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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. MORTALITY

No mortality occurred.

B. 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).

C. 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 1) 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 5.4-14.

Table 5.4-14: 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 5.4-15: 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

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/03	[redacted]	2007	Mammalian Erythrocyte Micronucleus Test for Glifosato Téchnico Helm [redacted] Report No.: 3393/2007-3.0MN-B Date: 2007-12-13 GLP: yes unpublished

Guideline:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations:	None
Dates of experimental work:	26/11/2007 – 28/11/2007

Executive Summary
Three groups of Swiss mice were treated by oral administration at 8 mg/kg bw, 15 mg/kg bw and 30 mg/kg bw. Two concurrent control groups, negative and positive received the vehicle (deionized water. 5 mL/kg bw) and cyclophosphamide (75 mg/kg bw), respectively. Bone marrow cells of the animals were blindly evaluated for the presence of micronuelei. as well as for the relation between polychromatic and normochromatic erythrocytes. Comparison between negative and positive controls demonstrated a significant increase in the micronucleus number (χ^2=315.4; p<0.001). The difference between the number of micronucleus in the groups treated with GLIFOSATO TECNICO HELM and the concurrent negative control was not statistically significant at 8 mg/kg bw (χ^2=2.14: p=0.144) and 15 mg/kg bw (χ^2=3.12; p=0.077). At 30 mg/kg bw (χ^2=5.44; p=0.020) the statistically significance was not biologically relevant. Under the conditions of this study, GLIFOSATO TECNICO HELM (batch n° 2007091801) did not induce an increase of micronucleus number in mouse bone marrow erythrocytes.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	GLIFOSATO TECNICO HELM
Description:	Solid,
Lot/Batch #:	2007091801
Purity:	980.1 g/kg
Stability of test compound:	Stable (CIPAC MT 46, 54°C, 14 days)

2. Vehicle and/ or positive control: Vehicle: deonized water
Positive Control: Cyclophosphamide

3. Test animals:

Species:	Swiss mice
Source:	Paulistec (Mairipora - SP)
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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. 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 1). 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 5.4-16: Summary of results

Treatment group / sampling time	Number of PCE with micronuclei /18000 PCE	$\chi 2$
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

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/04	[redacted]	2008	Evaluation of the mutagenic potential of Glyphosate Technical Micronucleus assay in mice [redacted] Data owner: HAG Report No.: RF - 3996.402.395.07 Date: 2008-09-29 GLP: yes unpublished

Guideline:	OECD 474 (1997); Commission Directive 2000/32/EC B.12 (2000), USA EPA, JMAFF
Deviations:	None
Dates of experimental work:	19/05/2008 – 13/08/2008

Executive Summary
A mouse bone marrow micronucleus assay was carried out in order to assess the mutagenic potential of the test substance GLYPHOSATE TECHNICAL. The test substance was diluted in sterile corn oil and administered intraperitoneally twice after an interval of 24 hours at the doses of 15.62, 31.25 and 62.5 mg kg- t (b.w.) corresponding to cytotoxicity analysis. Negative and positive controls were administered with the same schedule of the test substance: two intraperitoneal administrations after an Interval of 24 hours. Negative control group was treated with the dilution vehicle and positive control with cyclophosphamide (25 mg kg", b.w.). After 24 hours of the second application the animals were euthanized, their femurs excised to obtain the bone marrow cells, prepared in smears and stained on slides used for observations.

The results pointed out no increase in the number of micronucleus in polychromatic erythrocytes in animals treated with the test substance when compared to the negative control. As expected, a statistically significant increase in this parameter was observed in animals treated with cyclophosphamide. In the conditions of this study, the results indicated that GLYPHOSATE TECHNICAL produced no evidence of mutagenic activity in mice.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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)

2. Vehicle and/ or positive control: Vehicle: sterile corn oil
Positive Control: Cyclophosphamide

3. 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, Agribrands 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

B: STUDY DESIGN AND METHODS

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No mortality occurred.

B. EVALUATION OF BONE MARROW SLIDES
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 5.4-17).

Table 5.4-17: 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

III. CONCLUSION

The test material glyphosate technical was non-genotoxic.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/05	[redacted]	1999	A micronucleus study in mice for glyphosate técnico Nufarm [redacted] Data owner: Nufarm Study No.: RF-G12.79/99 Date: 1999-12-27 GLP: yes unpublished

Guideline: Not specified. Internal SOP M 069 - Micronucleus Test

Deviations: Not applicable

Dates of experimental work: 28/0ctober/1999

Executive Summary

A mouse bone marrow micronucleus assay was carried out in order to assess the mutagenic potential ofthe GLIFOSATE TECNICO NUFARM by measuring its ability to induce chromosome breakage. The product was diluted in water and administered intraperitoneally twice with a 24 hour interval at levels of 187.5, 375 and 562.5 mgJkg corresponding to 25%,50 % and 75 % ofthe LD50 for mice, respectively. Determination of the LD50 for the product as well as the negative and positive controls, used the two applications protocol with 24 hours interval. Negative control mice were treated with dilution vehicle and positive control mice with 25 mg/kg cyclophosphamide. Samplings were carried out 24 hours after the second application, when femurs were removed and smears of bone marrow cells prepared and stained. No increase in the number of polychromatic or normochromatic erythrocytes containing micronuclei was seen in animals treated with GLIFOSATE TECNICO NUFARM when compared to the vehicle control. A

statisticallysignificant increase in polychromatic and normochromatic erythrocytes containing micronuclei was observed in animals treated with cyclophosphamide.

GLIFOSATE TECNICO NUFARM showed no evidence of mutagenic activity in this study.

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance glyphosate does not require classification for this endpoint.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:**

Identification:	GLIFOSATE TECNICO NUFARM
Description:	White powder
Lot/Batch #:	3578/99
Purity:	95%
Stability of test compound:	No data available

2. **Vehicle and/ or positive control:** Water

3. **Test animals:**

Species:	Mouse
Strain:	Swiss albino
Source:	bred onsite by BIOAGRI-DF
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

B: STUDY DESIGN AND METHODS

In life dates: 28/0ctober/1999

Animal assignment and treatment:
Three levels of the GLIFOSATE TECNICO NUFARM were tested: 187.5, 375 and 562.5 mglkg corresponding to 25 %, 50 % and 75 % of the LDso for mice, with ten animals (five male and five female) per level. The nimals were dosed twice with intraperitoneal injections in volumes of 0.45 mL for 30 glanimal within a 24 hours interval, and sacrificed 24 hours after the second injection. Negative control with water and positive ontrol with cyclophosphamide (1.66 mglmL in physiological solution, corresponding to 25 mglkg), were also applied with the two injections protocol. Mice were killed by

cervical dislocation 24 hours after the econd dosing. From the freshly killed animal both femora were removed in total. The bones were then freed from muscle, the distal epiphyseal portion was tom off by gentle traction and the proximal end of the emur was shortened with scissors until a small opening ofthe 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 PCEslNCEs 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 PCEslNCEs were compared using the Kruskal Wallis test for independent samples (Conover, 1980). 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 :0; 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 ofthe laboratory.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No systemic or local signs of toxicity were observed during the study period.

C. BODY WEIGHT
No significant changes observed.

D. NECROPSY
No macroscopic changes of significance were noted.

E. BONE MARROW EVALUATIONS

Table 5.4-18: Summary of results

Group	Micronuclei in 1000 Polychr. Erythrocyte	Normochr. Erythr.	Polychromatic Erythr.	Normochrom. Erythr.	Polychr./Norm Ratio
Vehicle	0.6	0	879	997.7	0.9151
187.5 mg/Kg	0.3	0.1	779.2	978.1	0.81341
375 mglKg	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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/06	[redacted]	1996	Glyphosate Acid: Mouse Bone Marrow Micronucleus Test [redacted] Data owner: Syngenta Report No.: CTL/P/4954 Date: 1996-03-21 GLP: yes not published

Guideline:	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
Dates of experimental work:	1995-10-04 to 1996-03-21

Excecutive summary

Glyphosate acid has been evaluated for its ability to induce micronucleated polychromatic erythrocytes in the bone marrow of CD-1 mice. A single oral dose was given to groups of 5 male and 5 female mice at a dose level of 5000 mg/kg; this being the limit dose for this assay. Bone marrow samples were taken 24 and 48 hours after dosing.

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were seen in either sex at either of the sampling times investigated.

Comparison of the percentage of polychromatic erythrocytes showed no statistically or biologically significant differences in either sex at either of the sampling times between the vehicle control animals and those treated with glyphosate acid.

The test system positive control, cyclophosphamide, induced statistically significant and biologically meaningful increases in micronucleated polychromatic erythrocytes, compared to the vehicle control values, thus demonstrating the sensitivity of the test system to a known clastogen.

Glyphosate acid, under the conditions of test, was not clastogenic in the mouse micronucleus test.

I. MATERIALS AND METHODS

A: MATERIALS:

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 :	Cyclophosphamide	**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	Charles River Breeding Laboratories, Margate, UK.
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*
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

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 11 December 1995 End: 11 January 1996

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities 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 5.4-19: 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 (cyclophoshamide)	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 Analysus: 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.

II. RESULTS AND DISCUSSION

Preliminary toxicity assay: As no clinical signs or lethalities 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.

III. CONCLUSION

Glyphosate acid, under the conditions of test, was not clastogenic in the mouse micronucleus test.

Annex point	Author(s)	Year	Study title
IIA, 5.4.4/07	[redacted]	2008	Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse [redacted] Data owner: Syngenta Report No.: 1158500 Date: 2008-06-09 GLP: yes not published

Guideline:	OECD 474 (1997): EPA OPPTS 870.5395 (1998): 2000/32/EEC B.12 (2000)
Deviations:	None
Dates of experimental work:	2008-02-11 to 2008-06-09

Excecutive summary
In a NMRI mouse bone marrow micronucleus assay, 6/males/dose were treated orally with Glyphosate Technical at doses of 0, 500, 1000 and 2000 mg/kg bw. Bone marrow cells were harvested at 24h (all doses) and 48 h (only the high dose) post-treatment. The vehicle was 0.5% CMC. All animals were treated once orally (gavage) at an application volume of 20 mL/kg b.w. (except the positive control group, which were treated with 10 mL/kg b.w.).
There were no signs of toxicity during the study. Glyphosate Technical was tested at an adequate dose (maximum recommended dose by the OECD guideline). The positive control induced the appropriate response.
There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.

I. MATERIALS AND METHODS

A: MATERIALS:

Test Material: Glyphosate Technical

Description:	solid, white
Lot/Batch number:	20070545
Purity:	99.1% w/w Glyphosate (estimated error ± 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	Harlan Winkelmann GmbH
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

B: STUDY DESIGN AND METHODS:

In-life dates: Start: 25 February, 2008 End: 13 March, 2008

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethalities or severe toxicity observed over a two-day observation period following a single oral dose.

Micronucleus Test:

Table 5.4-20: 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 CO_2 followed by bleeding.
The animals were sacrificed using CO_2 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.

II. 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.

III. CONCLUSION

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.

IIA 5.4.5 In vivo genotoxicity testing (somatic cells) – Unscheduled DNA synthesis or a mouse spot test

Since all the *in vitro* and *in vivo* studies provided under IIA 5.4.1 to IIA 5.4.4 are negative, further *in vivo* testing in somatic cells is not necessary accoding to the data requirements specified in Council Directive 91/414/EEC or Regulation (EC) No. 1107/2009.

IIA 5.4.6 In vivo studies in germ cells

In the previous 2001 EU glyphosate evaluation genotoxic effects on germ cells were examined in dominant lethal assays in rats and mice. In both species no genotoxcic effect of glyphosate on germinal tissues was found. No new studies were performed since the last review.

Table 5.4-21: Summary of *in vivo* germ cell genotoxicty testing with glyphosate acid

	Reference (Owner)	Type of study	Test organism / test system	Dose levels (purity) metabolic activation	Results
Studies from the 2001 evaluation	Annex B.5.4.2.2 Glyphosate Monograph [redacted] 1992 (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 (96.8 %)	negative
	Annex B.5.4.2.2 Glyphosate Monograph [redacted] et al., 1980 (MON)	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 (98.7 %)	negative

DLT = dominant lethal test

IIA 5.5 Long-term toxicity and carcinogenicity

The long-term toxicity and carcinogenic potential of glyphosate has been assessed in rats and mice. The study results are summarised in Table 5.5-1 and Table 5.5-42. The results of studies previously evaluated in the 2001 EU glyphosate evaluation are included in this table to facilitate an assessment of all the relevant studies. The 2001 EU glyphosate evaluation concluded that in long-term studies in rats and mice there was no evidence of carcinogenicity. It also concluded that in rats, there was no adverse effects on survival or clinical signs. A reduction in body weight gain, increases in alkaline phosphatase and liver weight changes, an increase in incidence of cataracts, inflammation of the gastric mucosa and histopathological changes in the salivary glands were observed sporadically across the studies previously reviewed. In the mouse the previous 2001 review concluded that non-neoplastic treatment related effects were limited to high dose males in the [redacted] (1983) study and comprised of a reduction in body weight gain, hepatocyte hypertrophy and bladder epithelial hyperplasia.
Five additional long term studies have been conducted in the rat and 3 in the mouse that have not been previously reviewed at the EU level. The GTF considers there was no evidence that glyphosate acid is carcinogenic in any of these studies that have not been previously submitted. These studies are summarised in the relevant sections below.

IIA 5.5.1 Long-term (2 years) oral toxicity in the rat

Studies that were not assessed during the 2001 evaluation are summarised below. A 1-year toxicity study ([redacted] 1996, IIA 5.5.1/01) was performed in rats with dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid. Based on body weight and salivary gland effects at 20000 ppm, the NOAEL for toxicity for glyphosate acid was 8000 ppm equivalent to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females.
In another study, by [redacted] (1997, 5.5.2/02), rats received diets providing 0, 3000, 10000 or 30000 ppm glyphosate. The NOAEL for toxicity is 3000 ppm equivalent to 104 and 115 mg/kg bw/day for males and females, respectively, based on histopathological and clinical effects of the caecum together with follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose groups.
From another combined chronic toxicity and carcinogenicity study ([redacted] 2001, 5.5.2/03), which was performed with glyphosate technical in rats receiving diets providing 0, 2000, 6000 or 20000 ppm glyphosate acid, the NOAEL was set at 6000 ppm equivalent to 361 and 437 mg/kg bw/day for males and females, respectively. It was based on liver and kidney effects, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance.
The 2 year dietary rat study conducted by [redacted] 1997) concluded that there were no adverse treatment related effects and the NOAEL was 30000 ppm equivalent to 1290/1740 mg/kg bw/day in males and females respectively.
The most recent rat dietary carcinogenicity study was conducted in 2009 by [redacted] again there were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 1230 mg/kg bw/day.
In the 2001 EU evaluation, salivary glands have been suggested as possible target organ. Histological changes described as "cellular alteration" in the parotid and mandibular salivary glands and a higher organ

weight of these glands were noted at 100 mg/kg bw/day and higher ([redacted] 1993, IIA5.5.2/04). These findings determined the lowest NOAEL in the previous review from the long-term studies. In addition similar changes have been observed in subchronic rat studies (see IIA 5.3). In contrast, there are several chronic studies where no effects on the salivary glands were reported. These differences may be more or less pronounced depending on the rodent strain used or methodological differences.Additional studies were conducted to examine species sensitivity, reversibility of the effects and the hypothesis previously suggested in the WHO/FAO 2004 evaluation of glyphosate; that local irritation of the oral cavity by the organic acid mixed into diet may result in an adaptive salivary gland response (IIA 5.10). Based on the outcome of these examinations the treatment-related pathological findings (increased salivary gland size and flow) can be considered as adaptive responses due to oral irritation from the ingestion of glyphosate acid in the diet. When the salivary glands are viewed in perspective as an adaptive the change the lowest effect level in the long-term rat studies is 354/393 mg/kg bw/day in males and females respectively ([redacted], 1997). Overall the NOEL/NOAEL levels established in the long term studies in rats varied between approximately 31 mg/kg/day (300 ppm in diet, the highest dose tested in this pre-guideline study, considered a supplementary study in the EU monograph) and 1740 mg/kg bw/day.

Table 5.5-1: Summary of long-term toxicity and carcinogenicity studies in rats

Reference (Owner**)		Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Studies not reviewed in the 2001 evaluation	IIA 5.5.1/01 [redacted] 1996 (SYN 1)	1-year, oral diet Rat, Wistar Alpk: AP_fSD	♂ 0, 141, 560, 1409 ♀ 0, 167, 671, 1664 (0, 2000, 8000, 20000 ppm)	560/671 ♂/♀ (1409/1664)	1409/1664: Salivary glands, body weight reduction
Study from the 2001 evaluation	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/01 [redacted] 1996 (FSG 1)	2-year, oral diet Rat, Wistar	0, 7.4, 74, 741 ♂ 0, 6.3, 59.4, 595 ♀ 0, 8.6, 88.5, 886 (0, 100, 1000, 10000 ppm)	595/886 ♂/♀ 741 ♂+♀ (741 ♂+♀)	> 595/886: Only mild effects on clinical chemistry (liver enzymes) without histopathological changes
Studies not reviewed in the 2001 evaluation	IIA 5.5.2/02 [redacted] 1997 (ALS 1)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 104, 354, 1127 ♀ 0, 115, 393, 1247 (0, 3000, 10000, 30000 ppm)	104/115 ♂/♀ (1127/1247)	354/393: Caecum weight increased, distension of caecum, loose stool, follicular hyperkeratosis and/or folliculitis/ follicular abscess, reduced body weight
	IIA 5.5.2/03 [redacted] 2001 (SYN 2)	2-year, oral diet Rat, Wistar Alpk: AP_fSD	♂ 0, 121, 361, 1214 ♀ 0, 145, 437, 1498 (0, 2000, 6000, 20000 ppm)	361/437 ♂/♀ (1214/1498)	1214/1498: Kidney and liver findings. Increased survival due to bw reduction, reduced food consumption, prostatitis, periodontal inflammation

	Reference (Owner**)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.5.1 Glyphosate Monograph IIA 5.5.2/04 [redacted] 1993a (CHE 1)	2-year, oral diet Rat, Sprague-Dawley	0, 10, 100, 300, 1000	300 (1000)	1000: Decreased body weights, decreased urinary pH, salivary glands (histopatho-logy at terminal and interim kill, organ weight ↑ at interim kill); evidence of weak liver toxicity (clinical chemistry AP ↑, organ weight ↓)
	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/05 [redacted] 1981 (MON 1)	26-month, oral diet Rat, Sprague-Dawley	♂ 0, 3, 10, 31 ♀ 0, 3.4, 11, 34 (0, 30, 100, 300 ppm)	31/34 ♂/♀	No treatment-related effects
	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/06 [redacted] 1990 (MON 2)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 89, 362, 940 ♀ 0, 113, 457, 1183 0, 2000, 8000, 20000 ppm)	362/457 ♂/♀ (940/1183)	940/1183: Systemic effects: cataracts ♂, reduced body weight in ♀, increased liver weight. Local effects: inflammation of gastric mucosa
Studies not reviewed in the 2001 evaluation	IIA 5.5.2/07 [redacted] 1997 (EXC)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 150, 780, 1290 ♀ 0, 210, 1060, 1740 (0, 3000, 15000, 30000 ppm)	1290/1740 ♂/♀ (1290/1740)	> 1290/1740: Only mild toxic effects without histopathological changes
	IIA 5.5.2/08 [redacted] 2009a (NUF 1)	2-year, oral diet Rat, Wistar	0, 95, 317, 1230	1230 (1230)	> 1230: No treatment-related effects

* NOAEL for carcinogenicity
** Number refers to the data presented in Figure 5.11-1.
↓ = decreased; ↑ = increased

Tier II summaries are presented for all available chronic rat studies to provide a robust weight of evidence and an appropriate endpoint selection for ADI determination.

Annex point	Author(s)	Year	Study title
IIA, 5.5.1/01	[redacted]	1996	Glyphosate Acid: One Year Dietary Toxicity Study in Rats [redacted] Data owner: Syngenta Study No.: CTL/P/5143 Date: 1996-10-02 GLP: yes not published

Guideline: OECD 452, US EPA 83-1

Deviations: Several organ weights not determined

Dates of experimental work: 1995-04-03 - 1996-06-03

Executive Summary

The chronic toxicity potential of glyphosate acid was assessed in a 12-month feeding study in 24 male and female Wistar rats per group with 0, 2000, 8000 and 20000 ppm (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). Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

A reduction in bodyweight was evident in animals receiving 20000 ppm glyphosate acid, together with a marginal reduction in bodyweight in rats receiving 8000 ppm. There were no toxicologically significant or treatment-related effects on haematology, blood and urine clinical chemistry or organ weights.

The treatment-related pathological finding, increased incidence of mild focal basophilia of the acinar cells of the parotid salivary gland in both sexes which had received 20000 ppm glyphosate acid, is considered an adaptive response due to oral irritation from the ingestion of glyphosate, an organic acid, in the diet.

In conclusion, the NOAEL for gyphosate acid is 8000 ppm (corresponding to 560 mg/kg bw/day in males and 671 mg/kg bw/day in females).

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:AP$_f$SD)
Source:	Zeneca Pharmaceuticals, Alderley Park, Cheshire, UK
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

B: STUDY DESIGN AND METHODS

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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY AND CLINICAL SIGNS

There were no treatment-related death.

C. 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.

D. BODY WEIGHT

Bodyweights 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.

E. 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 5.5-2: 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.

F. OPHTHALMOSCOPY

There were no treatment-related effects observed.

G. 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 5.5-3: 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**

* $p < 0.05$; ** $p < 0.01$

H. URINALYSIS

There were no consistent treatment- and dose-related effects seen in the any urinary parameters.

I. 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.

III. CONCLUSION

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).

IIA 5.5.2 Carcinogenicity study in the rat

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/01	[redacted]	1996	Combined Chronic Toxicity and Carcinogenicity Study with Glyphosate Technical in Wistar Rats [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: 886.C.C-R Date: 1996-07-18 GLP: yes not published

Guideline:	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
Dates of experimental work:	1992-03-04 - 1994-03-04

Executive Summary
The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 24-month feeding study in male and female Wistar rats. Groups of 50 rats per sex received daily dietary doses of 0, 100, 1000, and 10000 ppm glyphosate technical (equivalent to mean achieved dose levels of 0, 7.4, 73.9 and 764.8/740.6 mg/kg bw/day for 12/24 months respectively). 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. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.
There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on body weight gain or food consumption noted. All dose- or treatment-

related significant changes observed in haematological, biochemical parameters as well as the urinalysis were within the range of the historical control data and hence appear to be of no biological significance.
Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related and dose-dependent effects.
In conclusion, glyphosate technical was not carcinogenic in the Wistar rats following continuous dietary exposure of up to 740.6 mg/kg bw/day (average for both sexes, 595.2 / 886 mg/kg bw/day (♂/♀)) for 24 months. Given the lack of correlating organ weights and histopathological data for the biochemical parameters the NOAEL for toxicity is considered to be 595 mg/kg bw/day for males, and 886 mg/kg bw/day for females (740.6 mg/kg bw/day for combined sexes).

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Rallis Research Centre, Bangalore, India
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), *ad libitum*
Water:	Deep bore well water treated with charcoal filter and UV rays, *ad libitum*
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

B: STUDY DESIGN AND METHODS

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 additional 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, prostrate, 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, 1980). When 'F' value was significant, Dunnett's pair wise comparison (Scheffe, 1953) 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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 5.5-4.

Table 5.5-4: 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

B. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs of toxicity observed during the study.

C. BODY WEIGHT
There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

D. 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 5.5-5: 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.

E. HAEMATOLOGY AND CLINICAL CHEMISTRY

The following significant dose related changes of the blood chemistry parameters were seen at the high dose:

a) decrease in GGT level at 12 months in male rats
b) decrease in Albumin level at 6 months in female rats
c) 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 5.5-6: 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

* $p < 0.05$;

F. URINALYSIS

There were no treatment-related findings.

G. 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 5.5-7: Summary of neoplastic histopathological findings

	Dose group (ppm)							
	Males				Females			
Findings for dead and moribund sacrificed animals	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
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 5.5-7).

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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/02	[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 GLP: yes not published

Guideline:	OECD 453 (1981), JMAFF 59 NohSan 3850 (1984), US-EPA (1989)
Deviations:	None
Dates of experimental work:	1994-12-19 - 1996-12-25

Executive Summary

The chronic toxicity and carcinogenic potential of HR-001 was assessed in a 24-month feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 10000, and 30000 ppm HR-001 (equivalent to 0, 104, 354 and 1127 mg/kg bw/day for males and 0, 115, 393 and 1247 mg/kg bw/day for females). In addition, 30 rats/sex/group were included for interim sacrifice at 26, 52 and 78 weeks to study non-neoplastic histopathological changes. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths in any of the dose-groups. Clinical observations consisted of loose stool together with soiled fur in the perianal region in the high dose group as well as increased incidences of tail mass in the mid and high dose group. Moreover, decreases in body weight were observed in both sexes in the mid and high dose group along with a lower food consumption. Ophthalmological examinations, urinalysis and haematological and blood biochemical analyses did not demonstrate apparent toxicity of the test substance in either sex or group.

Necropsy supported the clinical signs of loose stool by increased incidences of distension of the caecum in the high dose group together with increased absolute and relative caecum weights in the mid and high dose group. Moreover, the increased incidences of thickened areas in the skin of the tail, corresponding to the increased incidences of tail mass, were histopathologically diagnosed as follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose group.

In conclusion, HR-001 was not carcinogenic in the Sprague-Dawley rats following continuous dietary exposure of up to 30,000 ppm for 24 months. The NOAEL for toxicity is 3000 ppm, corresponding to 104 mg/kg bw/day for males and 115 mg/kg bw/day for females.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley (Crj:CD)
Source:	Tsukaba Breeding Center, Charles River Japan, Inc.
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

B: 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 phosphatise, 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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY AND CLINICAL SIGNS

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 5.5-8:

Table 5.5-8: 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)

C. 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 5.5-9.
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 5.5-9: Statistically significant changes in clinical signs

Parameters	Dose group (ppm) 0 ♂	0 ♀	3000 ♂	3000 ♀	10000 ♂	10000 ♀	30000 ♂	30000 ♀
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*

* $p < 0.05$; ** $p < 0.01$

D. 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.

E. 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 5.5-10: 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.

F. OPHTHALMOLOGIC EXAMINATIONS

No abnormalities were observed.

G. 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 5.5-11 and Table 5.5-12.

Table 5.5-11: Statistically significant changes in haematology

Parameters	Dose group (ppm) 3000 ♂	3000 ♀	10000 ♂	10000 ♀	30000 ♂	30000 ♀
Haematocrit	108[a]	99	111	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 5.5-12: Statistically significant changes in blood chemistry

Parameters		Dose group (ppm) 3000 ♂	3000 ♀	10000 ♂	10000 ♀	30000 ♂	30000 ♀
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.
* $p < 0.05$; ** $p < 0.01$

G. URINALYSIS

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 5.5-13.

Table 5.5-13: Statistically significant changes in urinalysis

Parameters		Dose group (ppm) 3000 ♂	3000 ♀	10000 ♂	10000 ♀	30000 ♂	30000 ♀
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.

H. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/03	[redacted]	2001	Glyphosate Acid: Two Year Dietary Toxicity and Oncogenicity Study in Rats [redacted] Data owner: Syngenta Study No.: CTL/PR1111 Date: 2001-03-15 GLP: yes not published

Guideline:	OECD 453 (1981), EEC B.33 (1988), MITI (1992), US OPTTS 870.4300 (1998)
Deviations:	None
Dates of experimental work:	1998-04-07 - 2000-10-16

Executive Summary

The chronic toxicity and carcinogenic potential of glyphosate acid was assessed in a 24-month feeding study in 52 male and 52 female Wistar rats with 0, 2,000, 6,000 and 20,000 ppm (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). In addition, three satellite groups with 12 rats per sex each were included for interim sacrifice at the 12th month to study non-neoplastic histopathological changes.
Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.
Treatment related findings in this study were found in the liver and kidney and were confined to animals (predominantly males) fed 20,000 ppm glyphosate acid. There were a number of changes in males and females fed 20000 ppm, notably renal papillary necrosis, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance. Despite the findings at 20000 ppm, survival was better in males fed 20,000 ppm than in the controls and lower dose groups.

This improved survival was associated with lower food consumption, lower bodyweights and a decreased severity of renal glomerular nephropathy.
In conclusion, glyphosate acid was not carcinogenic in the Wistar rats following continuous dietary exposure of up to 20,000 ppm for 24 months (corresponding to 1214 mg/kg bw/day in males and 1498 mg/kg bw/day in females). The NOAEL for toxicity is 6,000 ppm (corresponding to 361 mg/kg bw/day in males and 437 mg/kg bw/day in females). In addition, there was no evidence of neurotoxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:AP$_f$SD)
Source:	AstraZeneca UK Ltd., Alderley Park, Cheshire, UK
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

B: STUDY DESIGN AND METHODS

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 20000 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 20000 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, prostrate, 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 (Kaplan and Meier, 1958) 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 described in Gart *et al*. (1986). Analyses were carried out for all animals, intercurrent deaths and at terminal kill.

II. RESULTS AND DISCUSSION

A. 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.

B. 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 5.5-14.

Table 5.5-14: 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).

C. 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.

D. BODY WEIGHT

The bodyweights 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.

E. 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 5.5-15: 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.

F. OPHTHALMOSCOPY

There were no treatment-related effects observed.

G. 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 5.5-16).

Table 5.5-16: 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	15.7	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 5.5-17). 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 5.5-17: 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)								
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**

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
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	096	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	5.03	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

* $p < 0.05$; ** $p < 0.01$

H. URINALYSIS

Urinary pH was lower throughout the study in males fed 20000 ppm glyphosate acid (Table 5.5-18). 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 5.5-18: Urinanalytical findings

	Dose group (ppm)							
	0		2000		6000		20000	
	♂	♀	♂	♀	♂	♀	♂	♀
Urine pH								
Week 13	6.85	6.00	6.77	6.00	6.92	6.08	6.31**	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

I. 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 5.5-19) .
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 5.5-19: 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
Liver Proliferative cholangitis	-	56	57	55	64	-	55	58	59	61
Hepatitis	4.7 [2 - 8]	8	6	9	13	-	6	7	4	6
Kidney Papillary necrosis	0.4 [0 – 2]	0	1	0	14	-	0	1	2	5
Transitional cell hyperplasia	-	2	3	0	5	-	3	1	0	1
Prostate Prostatitis	23.4 [13 – 35]	13	22	23	37	-	-	-	-	-
Testis 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.

III. CONCLUSION

Based on the study results the NOAEL in rats after chronic exposure to glyphosate acid for 24 month is 6000 ppm (corresponding to 361 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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/04	[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: yes not published

Guideline: US-EPA Pesticide Assessment Guidelines Subdivision F, 83-5 (1982)

Deviations: None

Dates of experimental work: 1990-02-16 to 1992-03-09

Executive Summary
The chronic toxicity and carcinogenic potential of glyphosate technical was assessed in a 104-week feeding study in male and female Sprague-Dawley rats. Groups of 50 rats per sex received daily dietary

doses of 0, 10, 100, 300, or 1000 mg/kg bw/day glyphosate technical for 24 months. In addition, five groups of 35 rats/sex, receiving daily dietary doses of, 0, 10, 100, 300 or 1000 mg/kg bw/day, were included for interim sacrifice at the 12th month for evaluation of chronic toxicity. Observations covered clinical signs, body weight, food and water consumption, ophthalmoscopy, haematology, clinical chemistry and urinalysis, as well as organ weights, necropsy and histopathological examination.
Achieved doses throughout the study period were generally close to nominal. There were no treatment-related deaths or clinical signs in any of the dose-groups. Opthalmoscopic examinations showed no inter-group differences. At 1000 mg/kg bw/day 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. Food and water consumption did not differ significantly from the controls. Moreover, there were no treatment-related changes in haematological parameters. Clinical chemistry evaluation indicated a treatment-related increase of ALP in males of the 1000 mg/kg bw/day dose group and females of the 100, 300 and 1000 mg/kg bw/day dose groups, as well as reduced urinary pH in males at 1000 mg/kg bw/day.
At necropsy no treatment-related gross lesions were observed. Organ weight data showed reduced liver weights in females at 100, 300 and 1000 mg/kg bw/day at interim kill in week 52, but not after 104 weeks. At week 52 salivary gland weights were increased in 100, 300 and 1000 mg/kg bw/day dose group males. Combined sublingual and submaxillary gland weights were also increased in males and females treated with 1000 mg/kg bw/day. However there were no significant inter-group differences by week 104. Histopathological examination noted cellular alteration of in submaxillary and parotid salivary glands in males and females of the 300 and 1000 mg/kg bw/day dose groups (week 52) and the 100, 300 and 1000 mg/kg bw/day dose groups of both sexes at week 104. These changes followed a dose-related pattern and are considered treatment related. However, these cellular alterations are similar to those seen occasionally in other subchronic or long-term studies and are considered to be an adaptive response to acidic diet and are of no adverse consequence.
No treatment-related neoplastic lesions were observed at termination.
In conclusion, glyphosate technical was not carcinogenic in male and female Sprague-Dawley rats following continuous dietary exposure of up to 1000 mg/kg bw/day (the limit dose for this type of study) for 104 weeks. The NOAEL for toxicity is considered to be 300 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	229-Jak-5-1; 229-Jak-142-6
Purity:	98.9%; 98.7%
Stability of test compound:	At least two years at ambient temperature in the dark

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley
Source:	Charles River Limited, Margate, Kent, UK
Age:	Approx. 4 weeks upon arrival at testing facility
Sex:	Males and females
Weight at dosing:	Males: 85 ± 5 g, females: 60 ± 5 g
Acclimation period:	14 days

Diet/Food:	SQC Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 (Special Diet Services Limited, UK), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	In groups of five per sex in suspended polypropylene cages with stainless steel wire grid tops and bottoms
Environmental conditions:	Temperature: 20 ± 2 °C
	Humidity: 55 ± 10 %
	Air changes: 15 – 20 / hour
	12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

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 opthalmoscopic 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, creatinie 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 oncogenicicty study: adrenals, brain, heart, kidneys, liver, lungs, ovaries (with fallopian tubes), parotid salivary glands, pituitary, prostrate, 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, prostrate, 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 epididymes, 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 oncogenicicty 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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Analyses for achieved concentrations showed that the diet preparations of all dose groups were within an acceptable degree of accuracy (± 10%).

B. 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 5.5-20 below.

Table 5.5-20: 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

C. 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.
Opthalmoscopy examinations demonstrated no inter-group differences.

D. 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 5.5-21 below.

Table 5.5-21: 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

E. 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 5.5-22 below.

Table 5.5-22: 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.

F. 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 5.5-23).

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 5.5-24). All other changes in clinical chemistry parameters were not considered to be treatment-related.

Table 5.5-23: 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*	22.1	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 5.5-24: 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*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

G. URINALYSIS

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.

H. 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.

III. CONCLUSION

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 phosphatise 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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/05	[redacted]	1981	A Lifetime Feeding Study of Glyphosate (ROUNDUP Technical) in Rats [redacted] Data owner: Monsanto Study/Project No.: 77-2062 Date: 1981-09-18 GLP: no not published

Guideline:	Not stated; In general accordance with OECD 453 (1981)
Deviations:	None
Dates of experimental work:	In-life: 1978-07-12 to 1980-09-04

Executive Summary

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 at least 24 months. Males received treatment for 775 - 776 days and females 784 - 785 days before termination

Observations were made for mortalities, clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis. Scheduled and unscheduled terminal investigations included organ weights, necropsy and histopathological examination.

The findings for mortality, food consumption and water consumption data, haematology, clinical chemistry, urinalysis and terminal organ and body weights, organ/body weight ratios and organ/brain weight ratios did not indicate any effect attributable to the administration of glyphosate
However there was during most of the growth period, a slight but consistent trend toward reduced body weights in the treated males. However, 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, it was considered to be not toxicologically significant.
The treated females showed no statistically significant differences in mean body weights as compared to the controls through Month 19 of the study. However, for the following 2 months, the treated groups showed statistically significant reductions in group mean body weights, especially in the low and mid-dose groups, although not in a dose-related fashion. Thereafter, the treated females gained weight relative to the control group resulting in nearly identical group mean body weights at termination of the study.

The incidence of interstitial cell tumours of the testes in high dose males was elevated compared to the controls. Although an effect on the incidence of this tumour due to the administration of the test substance cannot be ruled out, the incidence in high dose males is within the range observed in recent historical control data. In addition, the data suggest that the incidence in all test substance groups is within the normal biological variation observed for tumours at this site in this strain of rat. Other gross and microscopic changes occurred sporadically in the control and/or treated rats and were considered unrelated to the administration of the substance.

The highest dose in this study is considered the NOAEL for toxicity, 300 ppm (corresponding to 31.5 mg/kg bw/day in males and 34.0 mg/kg bw/day in females). 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley CD
Source:	Charles River Breeding Laboratories, Wilmington, Massachusetts, USA
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

B: STUDY DESIGN AND METHODS

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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Results of diet analyses were not reported.

B. 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 5.5-25.

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 5.5-25: 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

C. FUNCTIONAL OBSERVATIONS

A functional observational battery of tests was not performed. It is not considered to affect the validity of this study.

D. 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 %.

E. 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.

F. WATER CONSUMPTION

There were no treatment-related effects observed.

G. 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..

H. 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 5.5-26).

Table 5.5-26: 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 5.5-27: Summary of the interstitial cell tumour findings in the testes of rats after 26-month dietary exposure to glyphosate technical

	Dose group (mg/kg bw/day)*			
Parameter	**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 5.5-28: 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/06	[redacted]	1990	Chronic study of glyphosate administered in feed to Albino rats [redacted] Data owner: Monsanto Project No.: ML-87-148 Date: 1990-09-26 GLP: yes not published

Guideline:	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%)
Dates of experimental work:	1987-08-05 – 1989-08-10

Executive Summary

The chronic toxicity and carcinogenic potential of glyphosate was assessed in a 24-month feeding study in 50 male and 50 female Sprague-Dawley rats with 0, 2,000, 8,000 and 20,000 ppm (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). In addition, 10 rats per sex per dose were included for interim sacrifice after 12 month. Observations covered clinical signs, ophthalmic examinations, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related effects on survival, clinical signs, food consumption, and haematology and clinical chemistry parameters. Observed increased urine specific gravity, decreased urinary pH, as well as increased absolute and relative liver weights in high-dose males were not considered to be of toxicological significance, since there were no correlated findings in clinical chemistry or histopathology. Increased incidences of inflammation of the stomach mucosa in mid-dose females, and pancreatic islet cell adenomas in low-dose males were not dose-related and considered incidental findings.

Treatment-related findings in this study were statistically reduced body weights in high-dose females, as well as increased incidences of cataractous lens changes in high-dose males. There was no evidence of a carcinogenic effect observed in any dose group of either sex.
In conclusion, glyphosate was not carcinogenic in Sprague-Dawley rats following continuous dietary exposure of up to 20,000 ppm for 24 months (corresponding to 940 mg/kg bw/day in males and 1183 mg/kg bw/day in females). The NOAEL for toxicity is 8,000 ppm (corresponding to 362 mg/kg bw/day in males and 437 mg/kg bw/day in females), based on reduced body weights in females and cataractous lens changes in males at 20,000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Albino Rat
Strain:	Sprague-Dawley (CD)
Source:	Charles River Breeding Laboratory, Portage, MI, USA
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

B: STUDY DESIGN AND METHODS

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 month. 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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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 5.5-29: 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

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical sings noted except the ophthalmological findings (see below).

D. 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.

E. 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 5.5-30: 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

F. 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 5.5-31: Incidences of cataract and lens fibre degeneration in males observed during ophthalmic examinations

	Dose group (ppm in diet)*			
	0	**2000**	**8000**	**20000**
1st examination	0/15	1/22	3/18	5/20**
2nd 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 5.5-32 below).

Table 5.5-32: 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.

G. 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.

H. URINALYSIS

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.

I. 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 5.5-33).

Table 5.5-33: 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 5.5-34). 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 5.5-34: 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≤ 0.01 (Fisher exact test with Bonferroni inequality)

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/07	[redacted]	1997	Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat [redacted] Study No.: 1231 Date: 1997-02-15 GLP: yes not published

Guideline:	OECD 453 (1981)
Deviations:	Organ weights were not determined for all animals; weights of heart, spleen and (para)thyroids are missing
Dates of experimental work:	1994-06-09 - 1996-06-12

Executive Summary

The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in male and female Sprague Dawley rats. Groups of 50 rats per sex received daily dietary doses of 0, 3000, 15000, and 25000 ppm Glyphosate technical (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)). In addition 20 rats/sex/group were included for interim sacrifice at Week 52, to study non-neoplastic histopathological changes with a different high dose level of 30000 ppm. 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. Observations covered clinical signs, body weight, food consumption, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.

There were no treatment-related deaths or clinical signs in any of the dose-groups. Moreover, there were no treatment-related effects on food consumption noted. Significantly reduced body weight gain that lasted throughout study until termination was observed in males receiving the highest dose. In all other groups body weight gain was comparable to the control at termination. Apart from increased alkaline phosphatase levels in the high dose of the carcinogenicity study at study termination, all other significant changes observed in haematological, biochemical and physio-pathological parameters of urine were within the range of the historical control data and hence appear to be of no biological significance.

Gross pathology and histopathological examination revealed no treatment-related and dose-dependent effects. Regarding organ weights, significant and dose-dependent effects after 52 weeks were found only

in animals dosed at 30000 ppm. After 104 weeks the effects were seen as well in the mid dose group. Increased organ weights that were observed after 52 weeks but not after 104 weeks could be due to the different high dose level, e.g. 25000 ppm and 30000 ppm, respectively.

In conclusion, Glyphosate technical was not carcinogenic in the Sprague Dawley rats following continuous dietary exposure of up to 1.29 g/kg bw/day for males and 1.74 g/kg bw/day for females for 24 months. The NOAEL for toxicity is 1.29 g/kg bw/day for males and 1.74 g/kg bw/day for females.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Diet

3. 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

B: STUDY DESIGN AND METHODS

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, prostrate, 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 (1958). 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.

II. RESULTS AND DISCUSSION

A. 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%.

B. 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 5.5-35:

Table 5.5-35: 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.

C. CLINICAL OBSERVATIONS
No significant toxic signs were observed in treated or control groups.

D. 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.

E. 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 5.5-36: 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)

F. OPHTHALMOLOGICAL EXAMINATION

Ophthalmological examinations revealed no abnormalities.

G. 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 5.5-37).

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 5.5-37: 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*

* $p < 0.05$

H. URINALYSIS

Urinalysis did not reveal any abnormalities attributable to the treatment.

I. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.2/08	[redacted]	2009a	Glyphosate Technical: Dietary Combined Chronic Toxicity/Carcinogenicity in the Rat [redacted] Study No.: 2060-0012 Date: 2009-04-23, amended 2009-05-08 GLP: yes not published

Guideline:	OECD 453 (1981), JMAFF Guideline 2-1-16 (2005), US OPTTS 870.4300 (1996)
Deviations:	None
Dates of experimental work:	2005-09-01 - 2008-03-19

Executive Summary
The chronic toxicity and carcinogenic potential of Glyphosate technical was assessed in a 24-month feeding study in 51 male and 51 female Wistar rats at dietary concentrations 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.
Observations covered clinical signs, behavioural assessment, functional observations, body weight, food consumption, ophthalmology, haematology, clinical chemistry and urinalysis as well as organ weights, necropsy and histopathological examination.
There were no treatment-related deaths or clinical signs in any of the dose-groups. Effects noted only in clinical biochemistry and histopathology were considered to not represent adverse toxic changes.
In conclusion, Glyphosate technical was not carcinogenic in the Wistar rats following continuous dietary exposure of up to 1229.7 mg/kg bw/day (average for both sexes) for 24 months. The NOAEL for toxicity is 1229.7 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate Technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7% w/w
Stability of test compound:	No data

2. Vehicle and/ or positive control:

Diet

3. Test animals:

Species:	Rat
Strain:	Wistar Han Crl:WI
Source:	Charles River Ltd., Margate, Kent, UK
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

B: STUDY DESIGN AND METHODS

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 phosphatise, 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 (thoraic), 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, prostrate, 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 ProvantisTM 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 Bartletts'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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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 in Table 5.5-38:

Table 5.5-38: 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

C. 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.

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

E. 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 5.5-39: 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.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption during the study.

G. OPHTHALMOSCOPY

There were no treatment-related effects observed.

H. 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 5.5-40: 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 5.5-41: 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.

I. URINALYSIS

There were no treatment-related effects observed.

J. 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.

III. CONCLUSION

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.

IIA 5.5.3 Carcinogenicity study in the mouse

Carcinogenicity studies that were not assessed during the 2001 glyphosate evaluation are summarised below.
A combined toxicity and carcinogenicity study in mice ([redacted] 2001, 5.5.3/01) demonstrated a slightly higher mortality in the high dose group. Mortality was within the upper end of the the historical control range. However, treatment with glyphosate might slightly have affected the mortality at the highest dose of 10000 ppm, and because a relationship to treatment was unclear a conservative NOAEL for toxicity at the mid dose of 1000 ppm (150.5 mg/kg bw/day for combined sexes) was set for this study. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and was not considered to be related to treatment. However it should be noted that the high dose group received a daily achieved dose of 1460 mg/kg bw/day which is in excess of the limit dose recommended by most current international guidelines.
In the study by [redacted] 1997 the low effect level was 8000 ppm (equivalent to 787 mg/kg bw/day) in females only based on a reduction in body weight gain. At the top dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females respectively) signs of toxicity included loose stools, reduced body weight gain, food consumption and food utilisation, caecum distention and increased absolute and relative caecum weight (without corollary histopathological findings), increased incidence of anal prolapsed consitent with histopathological erosion/ulceration of the anus.
The most recent 80-Week dietary mouse study was conducted by [redacted] 2009. There were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 810/1081 mg/kg bw/day in males and females respectively.
Overall the lowest effect level observed in the long-term mouse studies was 787 mg/kg bw/day in females in the [redacted] (1997) study and the wide range of NOEL/NOAELs of 151 – 1081 mg/kg bw/day is an artifact of dose selection (see Table 5.5-42).

Table 5.5-42: Summary of long-term toxicity and carcinogenicity studies in mice

	Reference (Owner**)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.5.2.2 Glyphosate Monograph [redacted] 1993b (CHE 2)	2-year, oral diet Mouse, CD-1	0, 100, 300, 1000	1000 (1000)	> 1000 Not clearly identified
	Annex B.5.5.2.1 Glyphosate Monograph [redacted] 1983 (MON 3)	2-year, oral diet Mouse, CD-1	♂ 0, 157, 814, 4841 ♀ 0, 190, 955, 5874	157/190 (4841/5874)	841/955 Decreased body weight, histological changes in liver and urinary bladder (epithelial hyperplasia)
Studies not reviewed in the 2001 evaluation	IIA 5.5.3/01 [redacted] 2001 (FSG 2)	18-month, oral diet Mouse, Swiss albino	0, 15, 151, 1460 (0, 100, 1000, 10000 ppm)	151 (1460)	1460 Increased mortality
	IIA 5.5.3/02 [redacted] 2009b (NUF 2)	18-month, oral diet Mouse, CD-1	0, 500, 1500, 5000 ppm	810/1081 (♂/♀) 946 (♂+♀) (810/1081 (♂/♀)	No treatment-related effects
	IIA 5.5.3/03 [redacted] 1997 (ALS 2)	18-month, oral diet Mouse, ICR	0, 1600, 8000, 40000 ppm	8000 / 1600 ppm (= 838/153 mg/kg bw/day (♂/♀) (4348/4116 (♂/♀))	8000 ppm (≅ 787 mg/kg bw/day) (♀): retarded growth 40000 ppm: pale-coloured skin ♂, loose stool, retarded growth, reduced food consumption and food efficiency, caecum distension and increased absolute and relative caecum weight without histopathological findings increased incidence of anal prolapse, in consistent with histopathological erosion/ulcer of the anus

* NOAEL for carcinogenicity
** Number refers to the data presented in Figure 5.11-1.

Tier II summaries are presented for the studies not previously evaluated in the 2001 EU glyphosate evaluation.
For details regarding studies reviewed during the 2001 EU evaluation we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.5.3/01	[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 GLP: yes not published

Guideline:	OECD 451 (1981)
Deviations:	None
Dates of experimental work:	1997-12-18 - 1999-06-29

Executive Summary
The carcinogenic potential of glyphosate technical was assessed in an 18-month feeding study in male and female Swiss albino mice. Groups of 50 mice per sex received daily dietary doses of 0, 100, 1000, and 10000 ppm glyphosate technical (equivalent to an average intake of 14.7, 150.5 and 1460.3 mg/kg bw/day). Observations covered survival, clinical signs, neurological changes, body weight, food- and water consumption, ophthalmological examinations, masses formation, blood smears with differential count analysis, organ weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues and lesions for all control and high dosage group animals died, sacrificed moribund or killed at termination.
The survival after 18-month of treatment was 56, 60, 56 and 46% in males and 68, 68, 60 and 60% in females in the control through high dosage groups, respectively. The mortality (combined for both sexes) was slightly increased at the high dose level with 38, 36, 42 and 47% for the control, low, mid- and high-dose group, respectively. Despite being in the upper range of the historical control data for mortality, the mortality in the high dose is considered to represent a LOAEL for safety reasons. There were no treatment-related effects on clinical signs, behaviour, eyes, body weight, body weight gain, food consumption, and differential white blood cell counts in both sexes. Gross pathology, organ weight data and histopathological examination demonstrated no treatment-related effects. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and in agreement with the study director.
Based on the slightly higher mortality and lower survival rates in the high dose groups, the NOAEL was considered 1000 ppm.

In conclusion, glyphosate technical was not carcinogenic in Swiss albino mice following continuous dietary exposure of up to 1460.3 mg/kg bw/day (average for both sexes) for 18 months. The NOAEL for toxicity was 149.7 mg/kg bw/day for male mice and 151.2 mg/kg bw/day for female mice (150.5 mg/kg bw/day for combined sexes).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification: Glyphosate
Description: Solid white, odourless crystals
Lot/Batch #: 01/06/97

Purity:	> 95.14 % (w/w)
Stability of test compound:	Expiry: December 1999

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	Swiss albino, HsdOla: MF1
Source:	Harlan UK, Ltd., UK
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), *ad libitum*
Water:	Well water passed through activated charcoal filter and exposed to UV rays, *ad libitum*
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

B: STUDY DESIGN AND METHODS

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.

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.

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.

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, prostrate, 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

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

Stability analyses indicated that the dose preparations were stable for up to 30 days with a loss 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 ± 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.

B. MORTALITY

The cumulated pre-terminal deaths (including moribund sacrifice) are summarised in Table 5.5-43 below.

Table 5.5-43: Cumulated mortalities after 78-week dietary exposure to glyphosate technical

	Historical control[#]		Dose group (ppm)**			
Sex	**min- max***	**Mean ± 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 5.5-44: 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, even though at the upper end, but within the historical control range. Although the treatment with glyphosate technical might slightly have affected the mortality at the highest dose of 10000 ppm, and a relationship to treatment is unclear, the worst-case NOAEL is set at 1000 ppm, corresponding to 150.5 mg/kg bw/day (combined sexes, see Table 5.5-45).

C. CLINICAL OBSERVATIONS

There were no significant treatment-related clinical signs of toxicity observed.

D. 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.

E. 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, 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 5.5-45 below.

Table 5.5-45: 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)	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

F. 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.

G. OPHTHALMOLOGICAL EXAMINATION

There were no treatment-related findings observed at the ophthalmological examinations performed at 6, 12 and 18 month of treatment.

H. 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.
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 significant 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. Cystic glands of the stomach were significantly increased in high dose mice of 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 significant decreased. All these findings were also observed at lower doses and/or were not dose dependent. Thus, these findings were also considered incidental.

In mice sacrificed at termination the following more frequent observed changes were observed: Cystic glands of the stomach were significantly increased in low-, mid- and high-dose males. However, this finding was not dose dependent.
Degenerative heart changes were higher in high-dose males and females, and significant higher in combined sex. Since the incidences were similar or slightly higher than historical controls, and no dose-dependency was observed this finding is considered incidental. 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 incident is considered incidental and not treatment-related. In addition, there was no increase with dose, and the incidences of this tumour varied with sex and fate (i.e. pre-terminal and terminal deaths).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 (see tables below).

Table 5.5-46: Summary of non-neoplastic histopathological findings for dead and moribund animals

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**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
Stomach Cystic glands (n)	8	8	9	16	1	4	5	6	9	12	14	22+
Kidney Nephropathy (n)	9	7	10	12	5	1	1	3	14	8	11	15
Bone (femur) Increased haematopoises (n)	1	1	8+	5	0	1	2	3	1	2	10+	8+
Epididymes Cell debris in tubules (n)	0	1	4	0	--	--	--	--	--	--	--	--
Epididymes Lymphocyte infiltration (n)	4	1	0	3	--	--	--	--	--	--	--	--
Heart Degenerative changes (n)	11	14	13	16	4	2	4	1	15	16	17	17
Adrenals sub-capsular cell hyperplasia (n)	3	8+	7	10	12	11	13	15	15	19	20	25
Mandibular LN 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

Table 5.5-47: Summary of non-neoplastic and neoplastic histopathological findings at termination

	Dietary concentration of glyphosate (ppm)											
	Males				Females				Combined sex			
Finding	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Stomach(N)	28	30	28	23	34	-	-	30	62	64	58	53
Cystic glands (n)	9	19+	22+	17+	22	-	-	19	31	-	-	36
Kidney(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
Bone (femur) (N)	28	-	-	23	34	2	1	30	62	--	--	53
Increased haematopoises (n)	1	-	--	0	1	0	0	2				
Epididymes (N)	28	1	-	23	--	--	--	--	--	--	--	--
Lymphocyte infiltration (n)	0	0	--	1	--	--	--	--	--	--	--	--
Mandibular LN (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
Heart (N)	28	2	--	23	34	--	-	30	62	--	--	53
Degenerative changes (n)	14	1	--	17	2	--	-	6	16	--	--	23+
Adrenals (N)	28	--	--	23	34	-	-	29	62	--	--	53
sub-capsular cell hyperplasia (n)	15	--	--	13	27	--	--	22	42	--	--	35
Hemolymphoreticular system (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 5.5-48: Incidences of lamlignant 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.3/02	[redacted]	2009b	Glyphosate technical: Dietary Carcinogenicity Study in the Mouse [redacted] SPL Project No.: 2060-0011 Date: 2009-04-22 GLP: yes not published

Guideline:	OECD 451 (1981), JMAFF guideline 2-1-15 (2005), US-EPA OPPTS 870.4200 (1996)
Deviations:	None
Dates of experimental work:	2005-10-10 - 2007-11-19

Executive Summary

The carcinogenic potential of Glyphosate technical was assessed in an 18-month feeding study in male and female CD-1 mice. Groups of 51 mice per sex received daily dietary doses of 0, 500, 1,500, and 5,000 ppm Glyphosate technical (equivalent to an average intake of 84.7, 266.8 and 945.6 mg/kg bw/day). Observations covered clinical signs, body weight, food and water consumption, palpation of masses, organ

weights, necropsy and histopathological examination. The latter involved examination of all sampled organ tissues for all control and high dosage group animals killed at termination. In addition, differential white blood cell counts were performed for animals that were killed or died in extremis and for selected animals at twelve and eighteen month of treatment. The dose-levels were chosen based on available toxicity data.
There were no treatment-related deaths or clinical signs in any of the dose-groups. In the carcinogenicity study, survival after 78 weeks of treatment was 76, 80, 76 and 69% in males and 73, 75, 75 and 78% in females in the control through high dosage groups, respectively.
There were no treatment-related effects on body weight gain or food and water consumption noted. No significant treatment-related effects were noted on differential white blood cell counts in both sexes. There were no treatment-related trends in the proportion of masses observed, number of mice affected or time to appearance of palpable masses. Gross pathology, organ weight data and histopathological examination revealed no treatment-related effects.
In conclusion, Glyphosate technical was not carcinogenic in the CD-1 mouse following continuous dietary exposure of up to 945.6 mg/kg bw/day (average for both sexes) for 18 months. The NO(A)EL for toxicity was 810 mg/kg bw/day for male mice and 1081 mg/kg bw/day for female mice, the highest dosage tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Glyphosate technical

Identification:	Glyphosate
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7%
Stability of test compound:	Expiry: 2008-03-25

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	CD-1, Crl:CD-1 (ICR) BR
Source:	Charles River (UK) Limited, Margate, Kent, UK
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

B: STUDY DESIGN AND METHODS

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 palped 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. Bodyweight 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, aortic (thoracic), bone & bone marrow (sternum and femur (incl. stifle joint)), brain (incl. cerebrum, cerebellum 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 ProvantisTM 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 $p < 0.1$.

II. RESULTS AND DISCUSSION

A. 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 ± 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 5.5-49: Group mean achieved dose levels

Dose group	Dietary concentration (ppm)	Achieved dose level (mg/kg bw/day)*				Overall mean
		Males		Females		
		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 males 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 is therefore 84.7, 266.8 and 945.6 mg/kg bw/day for 500, 1500, and 5000 ppm, respectively.

B. 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 5.5-50: 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 5.5-51: 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

C. 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 5.5-52) 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 5.5-52: 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

D. BODY WEIGHT

There were no treatment-related effects on male and female overall body weight gain during the conduct of study.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no treatment-related effects on food consumption for either sex noted during the study.

F. WATER CONSUMPTION

There were no treatment-related effects on water consumption for either sex noted during the study.

G. HAEMATOLOGY

There were no significance differences in the proportions of white blood cell counts for either sex at both 12 and 18 month.

H. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.5.3/03	[redacted]	1997	HR-001: 18-Month Oral Oncogenicity Study in Mice. [redacted] Data owner: Arysta LifeScience Study No.: IET 94-0151 Date: 1997-06-18 GLP: yes not published
Guideline:			Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984 OECD 451 (1981).
Deviations:			None
Dates of experimental work:			1995-02-21 to 1996-09-06

Executive Summary

In order to evaluate the oncogenic potential of HR-001 in mice, the test substance was administered to SPF ICR mice –Crj:CD-1) by incorporating it into a basal diet at a concentration of 0, 1600, 8000 or 40000 ppm for a period of 18 months (78 weeks). During the treatment period, all animals were observed for clinical signs and measured body weights as well as food consumption. At week 21, urinalysis was carried out on 20 males from all groups. Differential leukocytes counts were determined on the blood smears from 10 males and 10 females of all groups at week 52 and after 78 weeks of treatment, organ weight analysis was conducted on 10 males and 10 females which were served to the determination of differential leukocytes counts. All animals of both sexes were subjected to necropsy and histopathological examinations.

- 40,000 ppm groups In clinical observations, the incidence of pale-coloured skin was increased in males. In addition, loose stool was observed in all cages beginning at week 21 in males and at week 20 in females. Retarded growth was persistently observed during treatment period showing

statistically significant differences in weight from week 16 to 36 in males and from week 6 to end of treatment in females. These changes were associated with depressed food consumption and food efficiency. At necropsy, the increased incidences of distention of caecum were noted in males and females at terminal kill and in all animals examined, which were consistent to increase in absolute and relative weights of the caecum. However, no abnormalities were recorded in the caecum histopathologically. In males a significant increase was noted for the overall incidence of anal prolapsed which was correspondent to erosion/ulcer of the anus in histopathology.

- 8,000 ppm group: Retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9-24. No treatment related changes were seen in males.

- 1,600 ppm: There were no treatment related changes in either sex in any parameters.

Histopathological examinations failed to show increases of any types of neoplastic lesions in all treatment groups of both sexes.

Based on the results, the No-Observable Adverse Effect level (NOAEL) was set at 8,000 ppm (equivalent ot 838.1 mg/kg/da y) for males and 1,600 ppm (equivalent to 153.2 mg/kg/day) for females.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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	

2. Vehicle and/ or positive control: Diet

3. Test animals:

Species:	Mouse
Strain:	SPF ICR (Crj:CD-1)
Source:	Charles River Japan, Inc.
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

B: STUDY DESIGN AND METHODS

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 delibility 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 o 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 gallblader , 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 gallblader , 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.

II. RESULTS AND DISCUSSION

A. 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± 86.7, 7 790±394.4 or 38 783±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.

B. 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 5.5-53: 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)

C. CLINICAL OBSERVATIONS

Statistically significant changes in clinical signs observed in the treated groups of either sex are shown in the following table:

Table 5.5-54: 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**	1**	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.

D. 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.

E. 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 5.5-55: 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

F. HAEMATOLOGY

Statistically significant changes in differential leucocyte counts observed in the treated group of either sex are shown in the following table.

Table 5.5-56: 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.

G. 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 5.5-57: 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*

Sex	Male				Female			
Dose group (ppm)	0	1600	8000	40000	0	1600	8000	40000
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 icidence 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 cyct(s) in the kidney in thosenecropsied 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 foud 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 aither sex with statistically significant differences in incidence from those of the controls.

Table 5.5-58: Statistically significant changes in histopathology findings:

Sex	**Male**				**Female**			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
78tk (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 neoplatic 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 neoplatic lesions in the treated groups of both sexes.

Non-neoplatic 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 5.5-59: Statistically significant changes in non-neoplastic 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)*
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 cyct(s)	9	6	9	0*	2	1	5	0
Tibio-femoral joint: Proliferation of cartilageous tissue	14	17	11	15	18	14	11*	15

Sex	Male				Female			
Dose group (ppm)	**0**	**1600**	**8000**	**40000**	**0**	**1600**	**8000**	**40000**
78tk (N=)	*(26)*	*(34)*	*(27)*	*(29)*	*(32)*	*(36)*	*(40)*	*(35)*
Ke/fd (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
All (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
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**	3
Liver: Micro-granuloma	1	6	5	5	16	16	14	7*
Amyloid deposition	5	3	4	0*	12	3*	0**	1**
Kidney: Cortical cyct(s)	10	8	13	2*	5	1	5	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	1**	0**	2*
Eye: Cataract	4	5	5	5	5	2	0*	2
Skin: Skin subacutaneous 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, repscetively.

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 eithelium 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 cartilageous 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

III. CONCLUSION

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).
No observable effect level and sure toxic level in the present study were established as follows.

	No-observable effect level	Based on
Males	8 000 ppm (838.1 mg/kg/day)	- Increased incidences of tactile hair loss, pale-colored skin and mass(es) of anus at 40 000 ppm - Decrease in food efficiency at 40 000 ppm - Decrease in urinary pH at 8000 and 40000 ppm - Increase of lymphocytes and decrease in neutrophil (segmented form) at 40 000 ppm - Increase in mass(es) of the lung and loss of hair of skin at 1600 ppm but not observed at 40 000 ppm. - Increase in distension and absolute and relative weight of the cecum at 40 000 ppm - Increase in incidence of alveolar epithelial cell hyperplasia in the lung at 1600 ppm but not observed at higher doses.
Females	1 600 ppm (153.2 mg/kg/day)	- Increase in tactile hair loss at 1600 ppm but not observed in higher dose groups - Decrease in food efficiency at 8000 ppm - Increase in swelling of the lymph nodes and in mass(es) of the lung

IIA 5.5.4 Mechanism of action and supporting data

Considered not necessary. Glyphosate showed no carcinogenic potential in the longterm toxicity studies.

IIA 5.6 Reproductive toxicity

The potential of glyphosate to cause toxic effects on **reproduction** (reproductive performance, fertility, development) was examined in several multi-generation studies in rats. In the previous 2001 EU glyphosate evaluation no specific reproductive toxicity potential was shown for the active substance. Weak effects on the offspring consisting of a reduced pup weight were seen only at high dose levels and was associated with signs of paternal toxicity. Treatment-related effects in parent animals were similar to those seen in sub-chronic and chronic toxicity studies and occurred at comparable dose levels. Since the last review three new studies have been performed. The results of all studies are summarised in Table 5.6-1 and described below.

Developmental toxicity studies were performed in rats and rabbits.
Glyphosate does not cause teratogenicity. Adverse effects on the number of viable foetuses and the foetal weight were noted in rats and rabbits at higher dose levels also causing maternal toxicity. A reduced ossification and a higher incidence of skeletal and/or visceral anomalies at these dosages were also indicative of foetotoxicity. Overall, there is an inconsistent pattern of the most commonly occurring cardiac defects at maternally toxic doses without a clear dose effect. The lowest NOAEL for developmental effects was 300 mg/kg bw/day in rats ([redacted] 1991; IIA 5.6.11/05) and 175 mg/kg bw/day in rabbits ([redacted] 1996; IIA 5.6.11/03).
An endocrine disruption potential of glyphosate can be excluded based on the relevant endpoints evaluated in the reproductive and developmental toxicity studies. This conclusion is confirmed by the absence of any treatment-related findings in reproductive tissues and organs related to the endocrine system in repeated dose toxicity studies conducted with glyphosate (see IIA 5.3. and IIA 5.5).
In addition, dominant lethal assays performed in rats and mice (see IIA 5.4, Table 5.4-21) were negative.

IIA 5.6.1 Two generation reproductive toxicity in the rat

In the 2001 EU glyphosate evaluation a number of multigeneration studies were reviewed. It was concluded that glyphosate acid did not indicate a specific hazard for reproduction. It concluded that weak effects on the offspring as evidenced by reduced pup weight were confined to high dose levels where compound related effects were observed in the parent animals. Since the last review three additional studies have been conducted. Study summaries are available for these new studies below.
In the first additional study by [redacted] (1997) parental toxicity was evident at doses of 30000 ppm and consisted of reduced body weight, soft stool and distension of the caecum (see Table 5.6-1) which was consistent with findings in the sub-chronic and chronic rats studies conducted at this laboratory. In this study, effects in offspring consisted mainly of reduced body weight and distension of the caecum at 30000 ppm only.
In the [redacted] (2000) study the only effect of treatment was a reduction in the bodyweight of the F1A pups in the 10000 ppm group (1063/1634 mg/kg bw/day in males and females respectively) with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the mating period. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected by treatment.
In the most modern study by [redacted] (2007) there was no treatment-related effects on reproductive performance, parents or offspring.
In the previously reviewed study ([redacted] 1992) there were minimal histopathological changes on the salivary glands in parental and offspring animals noted at the highest dose (i.e., 10000 ppm) and to a lower extent at the mid-dose (i.e., 3000 ppm). This observation was also noted in other repeated dose studies with glyphosate but is considered an adaptive response to high dietary doses of glyphosate, which is a strong organic acid, and can therefore cause irritation of the oral cavity leading to increased salivary excretion (see chapter IIA 5.10). Overall the lowest effect level for parental toxicity was 668-771 & 752-841 mg/kg bw/day in males and females respectively based on slightly reduced body weight in F1 males,

increased food and water consumption F1 females in the [redacted] (1992) study The relevant parental NOEL/NOAELs ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females. There were no effects on reproduction (reproductive performance, fertility, parturition, lactation, sperm parameters and oestrus cycle) noted in any of the dose groups in any of the studies.
The lowest effect level for the offspring was 1063/1634 mg/kg bw/day in males and females respectively based on reduced body weight of first generation pups during lactation. The relevant NOEL/NOAELs for reproductive toxicity ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females. The range of NOEL/NOAELs is large as a consequence of variation in dose level selection between studies.

Table 5.6-1: Summary of reproductive toxicity

	Reference (Owner)	Type of study / Species	Dose levels (ppm)*	NOAEL (mg/kg bw/day (ppm))		LOAEL Targets / Main effects
				Parental	Offspring / reproductive	
Studies not reviewed in the 2001 evaluation	IIA 5.6.1/01 [redacted] 2007 (NUF)	2-generation, diet, rat, Sprague-Dawley	0, 1500, 5000, 15000	1063/1634 ♂/♀ (15000)	1063/1634 ♂/♀ (15000)	No treatment-related effects on parents, offspring and reproduction
	IIA 5.6.1/02 [redacted] 2000 (SYN/MON)	2-generation, diet, rat, Alpk:AP$_f$SD	0, 1000, 3000, 10000	NOAEL: 322 / 459 ♂/♀ (3000)	NOAEL: 322 / 459 ♂/♀ (3000)	1063/1634 ♂/♀ (10000 ppm): Parental: body weight of F1 males ↓ during pre-mating Offspring: reduced body weight of F1A pups during lactation No effects on reproduction
	IIA 5.6.1/03 [redacted] 1997 (ALS)	2-generation, diet, rat, Sprague-Dawley	0, 1200, 6000, 30000	417-458 and 485-530 ♂/♀ (6000)	417-458 and 485-530 ♂/♀ (6000)	2150-2411 & 2532-2760 ♂/♀ mg/kg bw/day (30000 ppm): Parental: loose stool, slight decrease in mean body weight in F1 ♂ at 2nd generation selection, caecum distension Offspring: reduced body weight (F0 ♂ and F1 ♀ during lactation), caecum distension No effects on reproduction
Studies from the 2001 evaluation	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/04 [redacted] 1993a (FSG)	2-generation, diet, rat, Wistar	0, 100, 1000; 10000	ca. 700-800 (10000)	ca. 700-800 (10000)	No treatment-related effects

	Reference (Owner)	Type of study / Species	Dose levels (ppm)*	NOAEL (mg/kg bw/day (ppm))		LOAEL Targets / Main effects
				Parental	Offspring / reproductive	
Studies from the 2001 evaluation	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/05 [redacted] 1981 (MON)**	3-generation, diet, rat, CD	0, 3, 10, 30 mg/kg bw/day	30	30	No treatment-related effects
	Annex B.5.6.1.2 Glyphosate Monograph IIA 5.6.1/06 [redacted] al., 1992 (CHE)	2-generation, diet, rat, Sprague-Dawley	0, 1000, 3000; 10000	197/226 (♂/♀) (3000)	197/226 (♂/♀) (3000)	668-771 & 752-841♂/♀ mg/kg bw/day (10000 ppm): Parental: slightly reduced body weight in F1 males, increased food and water consumption F1 females Offspring: no effects No effects on reproduction
	Annex B.5.6.1.2 Glyphosate Monograph [redacted] al., 1991a (CHE)	1-generation dose range finder, diet, rat, Sprague-Dawley	0, 3000, 10000, 30000	Not established	Not established	Parental: soft faeces, increased urination, reduced body weight gain and food consumption, increased water intake, GI disturbances, salivary gland changes Offspring: soft faeces, and reduced food consumption and pup weight, GI disturbances, salivary gland changes No effects on reproduction
	Annex B.5.6.1.1 Glyphosate Monograph IIA 5.6.1/07 [redacted], 1990 (MON)	2-generation, diet, rat, Sprague-Dawley	0, 2000, 10000, 30000	ca. 772/757 ♂/♀ (10000)	ca. 772/757 ♂/♀ (10000)	1983-2322 & 2320-2536 mg/kg bw/day (30000 ppm): Parental: reduced body weight, soft faeces, equivocal effect on litters size Offspring: reduced body weight No effects on reproduction
	Annex B.5.6.1.2 Glyphosate Monograph [redacted] 1985 (Alkaloida)	3-generation, diet, rat, Wistar	0, 200, 1000, 5000	ca. 460/500 ♂/♀ (5000)	ca. 462/502 ♂/♀ (5000)	No effects

* except stated otherwise
** Study was considered supplementary data in the 2001 EU glyphosate evaluation

Tier II summaries are presented for all available studies on reproductive toxicity to allow for a robust weight of evidence evaluation of endpoints.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/01	[redacted]	2007	Glyphosate technical: Dietary Two Generation Reproduction Study in the Rat [redacted] Data owner: Nufarm SPL project no.: 2060/0013 Date:2007-10-31 (amended 2008-04-08 and 2008-08-08) GLP: yes not published

Guideline:	OECD 416 (2001), JMAFF 2-1-17 (2001), US-EPA OPPTS 870.3800 (1998)
Deviations:	None
Dates of experimental work:	2005-11-18 to 2006-11-06

Executive Summary

Glyphosate Technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each, at dietary concentrations of 1500, 5000 and 15000 ppm (equivalent to a mean achieved dosage of 104, 351 and 1063 mg/kg bw/day for males and 162, 530 and 1634 mg/kg bw/day for females respectively). A further group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.

Clinical signs, bodyweight development, food and water consumption were monitored during the study. After 10 weeks of treatment, pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 24 male and 24 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.

Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. The ano-genital distance was recorded for all F2 generation offspring on Day 1 *post partum*. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*.

All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selected tissues was performed.

The following treatment-related effects were observed:

During the end of the lactation phases, females showed less bodyweight loss when compared to controls for the F0 and F1 generations. There was no adverse effect on bodyweight change for males throughout the treatment period, or for females during the pre-pairing and gestation phases of the study.

An increase in liver weights was noted for females treated with 15000 ppm from both generations. No such effect was noted for males treated with 15000 ppm or for animals of either sex treated with 5000 or 1500 ppm. However, this finding was considered as an adaptive response to treatment and not as an adverse health effect.

There were no treatment-related histopathological changes for F0 generation animals. Treatment-related changes in the F1 generation were confined to the presence of lower incidences and severities of cortical vacuolation of the adrenal glands for treated males when compared to controls.

Conclusion:
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. Therefore the NOAEL was considered to be 15000 ppm 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White crystalline solid
Lot/Batch #:	H05H016A
Purity:	95.7% (w/w)
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) IGS BR
Source:	Charles River (UK) Limited, Margate, Kent, UK
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), *ad libitum*
Water:	Tap water, *ad libitum*
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 house 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 ± 2°C Humidity: 55 ± 15% Air changes: at least 15/hour 12 hours light/dark cycle

B: 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, prostrate, 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, prostrate, 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, pair wise 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.

II. RESULTS AND DISCUSSION

A. 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.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in Table 5.6-2 below.

Table 5.6-2: Group mean achieved dose levels

Group	Dietary concentration (ppm)	Estimated dose level (mg/kg bw/day)	Mean achieved dose level (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

C. 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.

D. 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 5.6-3 and Table 5.6-4 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 5.6-3: Observed clinical signs in F0 generation

Clinical sign	Number of rats affected in dose group*							
	Control (0 ppm)		Low (1500 ppm)		Mid (5000ppm)		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	/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	028	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	/28	0/28	0/28	0/28	0/28	0/28	1/28

* x/y: number affected / total number of animals in group

Table 5.6-4: 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

E. BODY WEIGHT

No adverse effect of bodyweight 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 5.6-5). During the final week of lactation, both the F0 and F1 generations showed statistically significant less bodyweight loss in comparison to controls ($p < 0.001$ and $p < 0.01$ respectively).

Table 5.6-5: 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 $p < 0.001$

F. 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.

G. 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.

H. 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.

I. 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 bodyweight ($p < 0.001$). 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 bodyweight ($p < 0.01$) (see Table 5.6-6). No such observations were detected for males treated with 15000 ppm from either generation.

Table 5.6-6: 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

Dietary concentration (ppm)	No. of animals		Organ weight (g)			
			Liver		Kidney	
			Absolute	Relative	Absolute	Relative
			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 5.6-7: 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/02	[redacted]	2000	Glyphosate acid: Multigeneration reproduction toxicity study in rats [redacted] Data owner: Syngenta, Monsanto Report No.: CTL/P/6332 Date:2000-06-16 GLP: yes not published
Guideline:			OECD 416 (2001), Annex V 67/548/EEC, 9.ATP 87/302/EEC OJEC, L133, 47-50 (1988), US-EPA OPPTS 870.3800 (1998)
Deviations:			None
Dates of experimental work:			1998-09-01 to 1999-12-10

Executive Summary
Glyphosate acid was administered by dietary admixture to three groups of 26 male and female F0 generation Alpk:AP$_f$SD rats each, at dietary concentrations of 1000, 3000 and 10000 ppm. A further group of 26 male and 26 female F0 animals was exposed to basal laboratory diet to serve as a control.

After 10 weeks, the animals were mated and allowed to rear the F1A litters to weaning. The regime was repeated with the F1 parents (26 per sex and dose) selected from the F1A litters to produce the F2A litters after a 10 week pre-mating period. The remaining surviving F0 females and unselected offspring were terminated at Day 29 *post partum*, followed by the termination of all F0 male dose groups. At weaning of the F2 litters all surviving adults and their offspring were killed, followed by the termination of all F1 male dose groups. Diets containing glyphosate acid were fed continuously throughout the study. Clinical signs, bodyweight development, food and water consumption were monitored during the study.
Oestrous cycle assessment was performed daily for three weeks prior to mating for both the F0 and F1 generations. Observations for positive evidence of mating were recorded together with the start and completion of parturition. During the maturation phase of the F1 generation offspring, males and females were evaluated for sexual maturation. During the lactation phases daily clinical observations were performed on all surviving offspring, together with litter size. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*.
All animals at termination were subjected to a gross necropsy examination and histopathological evaluation of selective tissues was performed if necessary.

The bodyweights of the F1A pups in the 10000 ppm group were lower in comparison with the control group from Day 2 to Day 29 *post partum* although a similar effect was not observed for the F2A pups. In line with this, compound-related reductions in bodyweight and food consumption were evident only in F1 males given 10000 ppm. No further treatment related effects were observed.

Conclusion:
The oral administration of glyphosate acid to rats by dietary admixture at a maximum dose level of 10000 ppm for two successive generations of the Alpk:APfSD rat resulted in possible treatment-related changes at 10000 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. The effects however were considered not to represent an adverse health effect, therefore the NOAEL was considered to be 3000 ppm, for adult toxicity for both the F0 and F1 generations (equivalent to 322 mg/kg bw/day for males and 459 mg/kg bw/day for females).
The NOAEL for reproductive and developmental toxicity, for both generations and offspring was considered to be 10000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Alpk:AP_fSD (Wistar-derived)
Source:	Rodent Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK
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), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Rats were house in pairs (same sex) in multiple rat racks (with rats of the same group in adjacent cages). During mating animals were house 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 house 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

B: STUDY DESIGN AND METHODS

In life dates: not reported

Animal assignment and treatment:
In a two-generation reproduction study groups of 26 Alpk:AP$_f$SD 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, prostrate, 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 Bouins fixative: adrenals, brain, coagulating gland, left epididymis, ovary, left testis, pituitary, prostrate, 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 formation, 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.

II. RESULTS AND DISCUSSION

A. 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.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in Table 5.6-8 below.

Table 5.6-8: 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	113.9	90.4	227.9
Intermediate	3000	322.2	346.8	277.9	752.4
High	10000	1072.9	1136.2	910.9	2424.8

C. 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).

D. 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.

E. BODY WEIGHT

There was no effect of glyphosate acid on bodyweight adjusted for initial weight for the F0 rats, males and females, during the pre-mating period. For the F1 males given 10000 ppm, bodyweight was slightly lower at week 1, in comparison with the control group. Thereafter, bodyweights 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 5.6-9). There was no effect of 10000 ppm on the bodyweight of the Fl females and no effect of 3000 or 1000 ppm on the bodyweight of the F1 males or the F1 females (see Table 5.6-9). There was no effect of glyphosate acid on bodyweight adjusted for initial weight for either the F0 or F1 rats during gestation or lactation.

Table 5.6-9: Body weight during the pre-mating period-F1 generation (Group mean values)

F1 generation	body weight (g)							
	Control (0 ppm)		Low (1,000 ppm)		Mid (3,000ppm)		High (10,000 ppm)	
week	♂ (n=25)	♀ (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 $p < 0.01$

F. 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 10000ppm 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.

G. 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.

H. 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 groups 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.

I. 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 10,000 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 5.6-10). 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 5.6-10). 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 5.6-10: 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/03	[redacted]	1997	HR-001: A two-generation reproduction study in rats [redacted] Data owner: Arysta Life Sciences Study No.: IET 96-0031 Date: 1997-06-19 GLP: yes not published

Guideline:	OECD 416 (1981), US-EPA FIFREA Guidelines Subdivision F (1984), Japan MAFF Guideline 59 NohSan No. 4200 (1985)
Deviations:	None
Dates of experimental work:	1996-04-16 to 1997-03-31

Executive Summary

To evaluate the potential effects of HR-001 on reproduction groups of 24 Sprague-Dawley rats per sex were fed diets containing test substance concentrations of 0, 1200, 6000 and 30000 ppm for two consecutive generations. Clinical signs, bodyweight development, food consumption were monitored during the study. Reproductive parameters (oestrus cycle, mating, fertility and gestation indices, sperm assessment) were also evaluated. Gross pathological examinations were performed on all animals. Organ weight determinations and histopathological examinations were also performed on designated animals.

Litter data determined covered the total number of live and dead pups, the number of males and females, viability indices, body weights and clinical signs.

There were no treatment-related signs of toxicity noted in parental animals of the low- and mid-dose groups. At 30000 ppm treatment-related adverse effects consisted of defecation of loose stool in F0 and F1 males and females, and decreased body weights in F0 and F1 males. Also in the high-dose group distension of the caecum and increased liver and kidney weights in F0 and F1 males and a decreased prostrate weight in F1 males were observed at necropsy.

Reproductive performance was not affected by the treatment in any dose group. The slightly lower gestation indices observed in F1 females of the mid- and high-dose group were considered unrelated to treatment, as shown by the results of the reciprocal crosses of F1 animals with untreated rats.

No treatment-related alterations were observed in offspring of the low- and mid-dose groups. In the high-dose group pups of both sexes of the F1 and F2 generation showed significant decreased body weights and a significant increase in the incidence of distension of the caecum.

Oral dietary administration of 0, 1200, 6000 and 30000 ppm HR-001 to Sprague-Dawley rats for two successive generations resulted in treatment-related signs of toxicity in parental rats at 30,000 ppm. Therefore, the NOAEL for maternal toxicity is considered to be 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley; Crj:CD (SD)
Source:	Charles River Japan, Inc.
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 o 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 house 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

B: 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 Crj: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, prostrate, 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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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.

C. 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.

D. BODY WEIGHT

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.

E. 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 5.6-11 below.

Table 5.6-11: Group mean achieved dose levels F0 and F1-generation

Group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)			
		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	2411	2532	2760

F. 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.

G. 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.

I. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/04	[redacted]	1993	Two Generation Reproduction Study in Wistar Rats. [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: TOXI 885-RP-G2 Date: 1993-08-27 GLP: yes not published
Guideline:			OECD 416 (1983)
Deviations:			None
Dates of experimental work:			May 1991 - April 1992 (not further specified)

Executive Summary
To evaluate the potential effects of glyphosate technical on reproduction groups of 30 Wistar rats per sex were fed diets containing test substance concentrations of 0, 100, 1000, and 10000 ppm (equivalent to 0, 7.7, 77 and 770 mg/kg bw/day) for two consecutive generations up to weaning of third (F2) generation.

First parental (F0) animals were treated for at least 8 weeks before mating. The first pairing produced the F1 litter from which the second parental generation was selected. All F2 litters were sacrificed at weaning. All groups were observed for clinical signs, body weight development, food consumption, mating behaviour, vaginal smear and pups observation (number, sex, survival and body weight). During gestation and lactation periods the dams were observed for body weight and food consumption. The data were statistically analysed. From all groups of parental (F0 and F1) rats, major visceral organs and reproductive organs were subjected to detailed gross necropsy. Lesions if any found in reproductive organs/tissues in all groups were subjected to histopathological examination. All pups were subjected to detailed gross pathology.

Dietary administration of glyphosate at up to 10000 ppm for two successive generations in Wistar rats showed no treatment and dose related significant and consistent changes in the incidence of clinical signs, mortality rate, body weight change and food consumption during treatment, gestation and lactation periods. No treatment and dose related consistent effects on number of pups born (combined and individual sex) and their growth were observed; however the total litter weight and female pup weight tended to be higher in treatment groups occasionally.

The reproductive performance parameters like (a) number of dams littered (b) number of dead pups at first observation (c) mean litter size; pup survival parameters like live birth index and survival index for 24 hours, day 4, 7, 14 and 21 and fertility index parameters for sires and dams have though shown some incidental significant changes compared to the control group; however, these changes were not consistent

in both the generations and there was no relationship with treatment or the dose of the test compound in the diet.

In the two generation reproduction study conducted on glyphosate at dietary dose levels of 0, 100, 1000 and 10000 ppm no major effects on general health, growth of parents, gestation/lactation period, body weight and food consumption, gross necropsy findings of pups and parents were observed. The test compound did not cause any treatment or dose related consistent changes in parental mortality, parturition performance, mean litter size, pup weight and male and female fertility index. The NOAEL for maternal toxicity was therefore considered to be 10000 ppm. The NOAEL for offspring was found to be 10000 ppm.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Wistar rats (Random bred)
Source:	Toxicology Department, Rallis Agrochemical Research Station, Bangalore, India
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 *ad libitum*
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

B: 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 1.0 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*, offsprings 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 t/r test (for dose-response relationship).

II. RESULTS AND DISCUSSION

A. 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.

B. 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.

C. 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.

D. 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.

E. 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.

F1 females

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.

F. REPRODUCTIVE PARAMETERS

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.

F0 generation
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.

F1 generation
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.

Table 5.6-12: Reproductive parameters of F0- and F1-generation

	Group 1 - control 0 ppm		Group 2 100 ppm		Group 3 1000 ppm		Group 4 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

G. 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.

I. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/05	[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 GLP: no, pre-GLP not published
Guideline:			None (pre-guideline)
Deviations:			Not applicable
Dates of experimental work:			1978-06-13 to 1980-04-09

Executive Summary
Rats were administered glyphosate oral via diet continuously for three successive generations. Each generation (F0, F1 and F2) consisted of 12 male and 24 female Charles River CD® rats. Dietary concentrations were adjusted weekly during growth, and between mating rest periods, to achieve dose levels of 3, 10, and 30 mg/kg bw/day. A concurrent control group (plain diet) was included in the study.
Each parent generation was mated to produce two litters. Offspring from the second litters of the F0 and F1 parents (F1b and F2b litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F1a, F2a, and F3a) were sacrificed at weaning and given a gross post-mortem examination. Pathological and histopathological examinations were conducted from control and high-dose parent generations (F0, F1 and F2) and from control and high dose F3b offspring at weaning (10/sex/group).
Parameters evaluated for each generation included: mortality, body weight, food consumption, clinical observation, maternal body weights (gestation/lactation), reproduction-fertility indices (mating, pregnancy and fertility indices), litter data at parturition and organ weight data. Offspring from each litter interval were evaluated during a 21-day lactation period for growth, survival, sex distribution data and gross post-mortem observations including organ weight data (F3b offspring only).

No treatment-related effect was evident in adult mortality data, body weight and food consumption data (growth and rest periods), and clinical observation data throughout the study (F0, F1 and F2 generation). Considerable variability was seen in the mating-fertility indices during this study in both, the control and treated groups, in particular during the F1 and F2 generations. In the control group, mating indices were

low for both mating intervals of the F1 generation and in the F2 generation these indices were higher than normally encountered. Similar fluctuations were seen in mating indices for some of the treated groups during these same intervals. Throughout the study no consistent effect was seen in mating indices, fertility indices (male) or pregnancy rate data to suggest an adverse effect of treatment.

No adverse effects of treatment were evident in maternal weight data, gestation length, parturition data (number of live/dead pups at birth) or litter survival indices throughout the study. Concerning the offspring, no treatment-related effects were indicated in sex distribution data, body weights, survival or gross post-mortem findings. Likewise, no effect of treatment was evident in mean organ weight data (absolute and relative to body or brain weight) for randomly selected Day 21 F3b offspring.
No adverse effect of treatment was evident in organ weight data for the F0, F1 generations and F2 adult males. In the F2 treated females, a non-dose related, albeit statistically significant, decrease in mean liver/body weight ratio was evident; liver/brain weight ratios for these females showed a similar reduction; however, these differences from control were not statistically significant.
Gross post-mortem evaluations of the adult generations and histological evaluations of tissues from randomly selected F0, F1 and F2 high-dose animals and F3b high-dose offspring did not indicate a treatment-related effect.

Conclusion:
Oral dietary administration of 0, 3, 10 and 30 mg/kg bw/day glyphosate to CD-rats for three successive generations resulted in no treatment-related signs of toxicity in parental rats. Therefore, the NOAEL for parental toxicity is considered to be 30 mg/kg bw/day a for males and females, respectively.
The NOAEL for reproduction and the NOAEL for offspring was found to be 30 mg/kg bw/day, since the reproductive performance was not affected in any dose group and no adverse effects on offspring were observed.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	CD® (Sprague-Dawley derived)
Source:	Charles River, Wilmington, Massachussetts
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*
Housing:	Individually (except during mating and lactation), in elevated stainless steel wire mesh cages; nesting material: 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

B: STUDY DESIGN AND METHODS

In life dates: 1978-06-14 - 1980-04-09

Study design

Table 5.6-13: 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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. 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.

C. 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.

D. 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.

E. 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.

F. 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.

G. 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 l2 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.

I. 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/06	[redacted]	1992	The Effect of Dietary Administration of Glyphosate on Reproductive Function of Two Generations in the Rat. [redacted] Data owner: Cheminova Project no.: CHV 47/911129 Date: 1992-05-14 GLP: yes not published

Guideline:	OECD 416 (1983), US-EPA FIFRA 83-4 (1982)
Deviations:	None
Dates of experimental work:	1990-03-29 - 1991-03-22

Executive Summary
Glyphosate technical was administered by dietary admixture to three groups of 28 male and female F0 generation Sprague-Dawley rats each at dietary concentrations of 1000, 3000 and 10000 ppm (equivalent to a mean achieved dosages of 66.4 – 76.1, 196.8 – 230.2 and 668.1 – 771.3 mg/kg bw/day for males and 75.3 – 82.1, 226 – 244.9 and 752.3 – 841.1 mg/kg bw/day for females, respectively). An additional group of 28 male and 28 female F0 animals was exposed to basal laboratory diet to serve as a control.
Each parent generation was mated to produce two litters. Offspring from the first litters of the F0 parents (F1A litters) were selected to be parents for the F1 generation (24/sex/group). Offspring not included in the selection procedure and offspring from the second litter of each generation (F1B and F2B) were sacrificed at Day 21 *post partum* and given a gross post-mortem examination. Parent animals were sacrificed shortly after termination of the second litter.
Clinical signs, bodyweight development, food and water consumption, mating performance, pregnancy rate, and length of gestation of adults were monitored during the study. Litter weight, individual offspring weights and landmark developmental signs were also recorded on specific days *post partum*. All animals at termination were subjected to a gross necropsy examination and weighing of selected organs and histopathological evaluation of selected tissues for the F0 and F1 adults was performed.

Treatment at 10000 ppm produced marginal signs of toxicity in parent animals and minimal histological changes in the target organ (salivary gland). Histological changes in the salivary gland were also noted at 3000 ppm. This finding 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. In the offspring no treatment-related effects were apparent at dietary administration up to 10000 ppm.

Conclusion:
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 bodyweights of F1 males and minimal histological changes in the salivary glands in F0 and F1 adults at 10000 ppm. The only finding associated with treatment at 3000 ppm were minimal histopathological changes in the salivary glands of F0 and F1 adults. 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rat
Strain:	Sprague-Dawley Crl:CD (SD) BR VAF/Plus
Source:	Charles River (UK) Limited, Margate, Kent, UK
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, *ad libitum*
Water:	Tap water, *ad libitum*
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

B: 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, prostrate (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.

II. RESULTS AND DISCUSSION

A. 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.

B. TEST COMPOUND INTAKE
The group mean intakes of glyphosate are summarised in Table 5.6-14 and Table 5.6-15 below.

Table 5.6-14: 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 5.6-15: 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

C. 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.

D. 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.

E. 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, bodyweight 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.

F. 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.

G. REPRODUCTIVE PARAMETERS

There were no treatment-related effects on mating performance, fertility and gestation length for both F0 and F1 generation animals.

H. 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.

I. 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 5.6-16.

Table 5.6-16: Incidence of salivary gland findings

Observation	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10000	0	1000	3000	10000
F0 Generation								
Animals examined	27	28	28	26	28	27	28	28
Hypertrophy of acinar cells with prominent granular cytoplasm (minimal)								
parotid	2	2	3	12	0	2	5	17
submaxillary	0	-	-	0	0	1	4	14
F1 Generation								
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.

III. CONCLUSION

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 bodyweights 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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.1/07	[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 GLP: yes not published

Guideline:	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,
Dates of experimental work:	1988-10-24 to 1989-10-13

Executive Summary

30 Sprague-Dawley rats sex/dose group (F0 and F1a generation) were fed daily with glyphosate at concentrations 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)) through two generations for approximately 11 (F0-generation) and 14 weeks (F1a-generation), respectively. Animals of the F1a-generation were mated twice to produce the F2a and F2b-generations.
Pairing of animals within each dose group was undertaken on a 1:1 basis. At weaning of offspring from the F0 mating phase, groups of 30 male and 30 female offspring from each dose group were selected to form the F1a generation. The remaining surviving F0 females and unselected offspring were terminated at Day 21 *post partum*. Males were sacrificed after completion of mating phase. The offspring selected for the F1a generation were dosed for approximately 14 weeks and then paired within each dose group to produce the F2a and F2b litters. At weaning of the F2 litters all surviving adults and their offspring were killed, whereas F1 males were sacrificed after completion of mating phase.

Clinical observations for mortality and moribundity were performed twice daily, and detailed observations for signs of toxicity once weekly, when diets were prepared. Body weights were weekly determined for

adults and on day 0, 4 (pre- and post-culling), 14 and 21 of lactation for offspring. Food consumption was determined weekly for adults and on days 0-7, 7-4 and 14-21 of gestation and lactation.
All animals at termination and which died, or were sacrificed moribund, were subjected to a gross necropsy. Histopathological evaluation of selected tissues was performed for control and highest dose level animals.

No significant changes concerning mortality, mating, fertility, organ weights, gross pathology and histopathology were observed. In the high dose group at 30000 ppm, soft stool in adults were observed in both sexes, being accompanied by reduced body weights of adult animals (about 8%) and pups (about 10%) when compared to controls. This effect was assumed to be treatment-related. Furthermore, decreased pup weights were observed when pups began supplementing their milk with the glyphosate-containing food. In the 10000 ppm-dose group, decreased pup body weights were observed, too, but the effect was less pronounced and occurred not in both sexes of all generations.
At the highest dose level of 30000 ppm a slightly, and statistically not significant, reduced average litter size was observed in the F0 and to a lesser degree in the F1 dams. A reduction was not noted when F1 animals were re-mated and treatment-relation was considered to be equivocal.

Conclusion:
Daily oral administration of glyphosate to Sprague-Dawley rats via the diet at concentrations of 2000, 10000 and 30000 ppm to two generations identified treatment-related effects at 30000 ppm, which became manifest in soft stools in adults and consequently reduced body weights in the parent and offspring animals. This effect was less pronounced in the pups of the 10000 ppm dose group and not in both sexes of all generations.
Therefore, the NOAEL was considered to be 10000 ppm for adult toxicity for both the F0 and F1 generations.
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate (Identification code: T880068)
Description:	White powder
Lot/Batch #:	XLI-203
Purity:	97.67%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Albino Rat
Strain:	Sprague-Dawley
Source:	Charles River Breeding Laboratory, Portage, MI
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 premating 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

B: 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 became 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 where 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 chosed 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.

II. RESULTS AND DISCUSSION

A. 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.

B. TEST COMPOUND INTAKE

The group mean achieved dosages are summarised in the table below.

Table 5.6-17: 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

C. 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.

D. 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.

E. 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 5.6-18).
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 5.6-19 and Table 5.6-20).
Terminal body weights were significantly decreased for both sexes at the highest exposure level (see Table 5.6-18).

Table 5.6-18: 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	11.56	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 5.6-19: 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 5.6-20: 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

F. 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.

G. 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.

H. 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 5.6-21: Mean pup weights

Dietary concentration (ppm)	No. of animals		Mean group body weight (g) at Day 0	Mean group body weight (g) at Day 21	Mean group body weight (g) at Day 0	Mean group body weight (g) at Day 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.

I. 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.

III. CONCLUSION

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.

IIA 5.6.2 Separate male and female studies

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.3 Three segment designs

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.4 Dominant lethal assay for male fertility

Studies considered not necessary. Information provided in chapter IIA 5.4.6.

IIA 5.6.5 Cross-matings of treated males with untreated females and vice versa

Not required according to Regulation 1107/2009/EEC and Directive 91/414/EEC.

IIA 5.6.6 Effects on spermatogenesis

Studies considered not necessary. Effects on spermatogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.7 Effects on oogenesis

Studies considered not necessary. Effects on oogenesis are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.8 Sperm motility and morphology

Studies considered not necessary. Parameters are assessed in the two-generation reproductive toxicity studies (see IIA 5.6.1).

IIA 5.6.9 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).

IIA 5.6.10 Teratogenicity test by the oral route in the rat

The 2001 EU glyphosate review concluded that in rats the lowest relevant NOEL for both maternal and developmental effects was 300 mg/kg bw/day and the lowest effect level was 1000 mg/kg bw/day. The evaluation found there was no evidence of teratogenicity. A summary of the teratogenicity studies conducted in the rat are available in Table 5.6-22. Two additional teratogenicity studies have been performed in the rat that have not been previously reviewed in the 2001 EU glyphosate evaluation. These studies are considered to be confirmatory data and are summarised below.

Materntal toxicity in rats at 1000 mg/kg bw/day was characterised by GI disturbances (loose stool/diarrhoea), reduced body weight gain and noisy respiration and in addition at 3500 mg/kg bw/day maternal toxicity was characterised by mortality, post dose salivation and lower food consumption (Table 5.6-22 Non-specific signs of foetotoxicity were observed at 1000 mg/kg bw/day only in the [redacted] study (1991) which was characterised by a slight increase above the historical control range of foetuses showing skeletal variations. Reduced foetal weight, delayed ossification and a lower number of viable foetuses was observed at the extremely high top dose of 3500 mg/kg bw/day in the [redacted] (1991) and [redacted] (1980) studies. The toxicological relevance of these findings are uncertain because of the clear increase in mortality in the dams at this dose level suggest it was in excess of the maximum tolerated dose.

Table 5.6-22: Summary of developmental toxicity in rats

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / develop-mental	
Studies from the 2001 evaluation	Annex B.5.6.2.1.1 Glyphosate Monograph [redacted] 1991b (CHE)	Developmental toxicity, rat, CD	0, 300, 1000, 3500	300	300	Maternal: 3500 mg/kg bw/day: mortality, salivation, loose stool, noisy respiration, reduced body weight, slightly reduced food intake, increased water intake 1000 & 3500 mg/kg bw/day: noisy respiration, reduced body weight gain Developmental: 3500 mg/kg bw/day: reduced mean foetal weight 1000 & 3500 mg/kg bw/day: reduced / delayed ossification, increased incidence of skeletal variations
	Annex B.5.6.2.1.2 Glyphosate Monograph [redacted] 1991g (FSG)	Developmental toxicity, rat, Wistar	0, 1000 (Limit test)	1000	1000	Maternal: no effects Developmental: No treatment related effect
	Annex B.5.6.2.1.2 Glyphosate Monograph [redacted] 1980 (MON)	Developmental toxicity, rat, CD	0, 300, 1000, 3500	1000	1000	Maternal: mortality, diarrhoea, reduced body weight gain Developmental: reduced number of viable foetuses

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / develop-mental	
Studies not reviewed in the 2001 evaluation	IIA 5.6.10/01 [redacted] 1996b (SYN)	Developmental toxicity, rat, Alpk: AP_fSD	0, 250, 500, 1000	1000	1000	Maternal: no effects Developmental: no effects
	IIA 5.6.10/02 [redacted] 1995 (ALS)	Developmental toxicity, rat	0, 30, 300, 1000	300	1000	Maternal: slightly loose stool Developmental: No effects

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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.6.10/01	[redacted]	1996b	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: yes not published

Guideline: OECD 414 (2001): OPPTS 870.3700 (1998): 2004/73/EC B.31 (2004)

Deviations: None

Dates of experimental work: 1995-05-17 to 1996-03-26

Excecutive summary

In a developmental study, groups of time-mated female rats of the Alpk:APfSD (Wistar-derived) strain were dosed by gavage with 0, 250, 500 or 1000 mg glyphosate acid/kg/day using deionised water as a vehicle. The day of mating was designated day 1 of gestation. The rats were dosed on days 7-16 (inclusive) of gestation which thus included the period of major organogenesis. On day 22 of gestation the rats were killed and their uteri examined for live foetuses and intra-uterine deaths. The foetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

There was no evidence of maternal toxicity attributable to glyphosate acid as assessed by the clinical condition of the animals during the study, their bodyweight gain and food consumption and the type and incidence of macroscopic findings *post mortem*.

There was no evidence of developmental toxicity attributable to glyphosate acid as assessed by the number, growth and survival of the foetuses. Observation of the external appearance of the foetuses, examination of the viscera and assessment of the skeletons revealed no treatment-related findings.

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.

I. MATERIALS AND METHODS

Materials:

Test Material:	Glyphosate acid
Description:	Technical, white solid
Lot/Batch number:	P24
Purity:	95.6% w/wa.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	Barriered Animal Breeding Unit, Alderley Park, Macclesfield, Cheshire UK.
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

B: 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 5.6-23: 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***) 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 1mL 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 bodyweight. 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 bodyweight during the dosing and post-dosing periods was considered by analysis of covariance on initial (day 7) 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 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:-

- Percentages were analysed by analysis of variance following the double arcsine transformation of ***Freeman and Tukey (1950)***
- the proportion of foetuses affected 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 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.

Bodyweight: There was no effect of glyphosate acid on maternal bodyweight.

Table 5.6-24: Intergroup comparison of maternal bodyweight (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 5.6-25: Intergroup comparison of food consumption (g/day) (selected timepoints)

day	Dose level of glyphosate acid (mg/kg/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
19226	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 5.6-26: 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
Corpora Lutea/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	4.0**	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*.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.10/02	[redacted]	1995	HR-001: Teratogenicity Study in Rats. [redacted] Data owner: Arysta LifeScience Study No.: IET 94-0152 Date: 1995-07-21 GLP: yes not published

Guideline:	Japan MAFF Guidelines 59 NohSan No.4200, 1985 U.S. EPA FIFRA Guidelines Subdivision F, 1984
Deviations:	None
Dates of experimental work:	1995-03-23 to 1995-06-26

Executive Summary

A teratogenicity study was conducted to evaluate the potential maternal and developmental toxicity of HR-001 in rats. The test substance was suspended in 0.5% aqueous solution of sodium carboxymethylcellulose and was administered orally with stomach tube to 24 copulated Crj:CD (SD) female rats per group at dose levels of 0, 30, 300 or 1000 mg/kd/day from days 6 to 15 of gestation.
No adverse effects related to test substance treatment were observed for maternal rats in the 30 and 300 mg/kg groups. In the 1000 mg/kg group, 20 of 22 pregnant females showed slightly loose stool during the dosing period and/or on the following day of last dosing, and the incidence was statistically significantly high. No treatment related changes were observed in the body weights and body weight gains. Food consumption in this group was significantly decreased at an interval of days 6-9 of gestation (early dosing period).
Observation at cesarian section revealed no treatment related adverse effects in any of the parameters tested, i.e. gross pathology findings, gravid uterine weights, numbers of corpora lutea, implants and live foetuses, percent incidences of resorptions and foetal deaths, foetal sex ratio, foetal body weights and placenta weights.
In the teratological examination foetuses, no treatment related malformations or variations were noted in any of the treated group.

Based on the results, the No-Observable Adverse Effect level (NOAEL) was set at 300 mg/kg/day for maternal rats, and that 1000 mg/kg/day is not teratogenic to SD rat foetuses.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: Diet

3. 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

B: 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 copora 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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 group.
No significant differences were found in the mean body weight gains of maternal rats between the control group and any of the treated groups.

C. 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.

D. 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 group.

E. 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 group.

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 foetuses in the control, 300 mg/kg and 1 000 mg/kg groups, respectively, asymmetry of the sternebrae with sterno-costal joint displacement in a foetus of the 300 mg/kg group, and fusion of the sternebrae in a foetus of the 300 mg/kg group, and fusion of the sternebrae 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 foetuses were cervical ribs shortening of the 13th ribs, lumber ribs, sacralization of the lumber vertebra and asymmetry and/or splitting of the sternebrae in 0-1, 0-1, 1-11, 0-1 and 3-5, respectively

III. CONCLUSION

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 foetuses.

IIA 5.6.11 Teratogenicity test by the oral route in the rabbit

The 2001 EU glyphosate review concluded that the NOEL for developmental effects was 350 mg/kg bw/day ([redacted] 1980) and that effects on the foetuses were only observed in the presence of marked maternal toxicity. Overall the previous evaluation determined that glyphosate was not teratogenic in rabbits. Three additional studies have been included in this submission. The results from these studies are consistent with the data that has been previously reviewed, the pattern of maternal toxicity is consistent and effects on the foetuses were only observed in the presence of maternal toxicity.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity in does consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality. Table 5.6-22 details maternal toxicity observed following glyphosate treatment via oral gavage. These effects are consistent with gastro-intestinal stasis (ileus) likely caused by the mucosal membrane irritation potential of glyphosate acid. Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors can lead to the normal muscular contractions of the stomach and intestines being greatly diminished, which in turn leads to disruption of the normal intestinal/ceacum bacterial flora. It is likely that the mucosal membrane of the rabbit gastro-intestinal tract is irritated by bolus administration of glyphosate acid. Consequently the associated stress leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995), [redacted] (1996) and Moxon (1996), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. This finding appears to be relevant to only hindgut fermenters as it is not seen in rats or dogs following administration of an oral bolus dose.

Further evidence, that these findings are related to gastro-intestinal disturbance comes from the [redacted] (2012) study that measured dermal absorption *in vitro* through rabbit skin. This study demonstrated that systemic exposure to glyphosate acid following percutaneous administration in the [redacted] study (1982) was equivalent to the systemic exposure following oral gavage administration in many of the rabbit developmental toxicity studies where clinical signs of soft faeces, reduced faecal output, reduced body weights and mortality were observed.

Table 5.6-27: Summary of maternal toxicity in glyphosate acid developmental toxicity in rabbits

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality#	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
Japanese White ([redacted] 1995)	300	1/18	4/17	0/17	Lower than control	Erosion in the stomach, hair bolus in stomach, watery contents in large intestine/caecum	15/18
NZW [redacted] 1996)	400	2/18	10/16	2/18	Initial loss and then statistically significant lower bodyweight gain than control	Fluid filled large intestines, haemorrhage, ulceration and sloughing of the stomach, duodenum congested and colon, rectum and appendix gas distended.	16/18
	200	1/18*	0/16	2/18	Lower body weight gain than control	-	16/18

Strain (Reference)	Dose level (mg/kg bw/day)	Maternal Mortality#	Diarrhoea/ loose faeces #	Reduced faecal output #	Body weight effect	Necropsy findings	Number of dams with live young or litters at Day 29 #
NZW ([redacted] 1996)	300	2/20	19/20	9/20	Reduction in maternal body weight gain	Hair-like substance in the stomach	17/20
	175	2/20	11/20	9/20	Reduction in maternal body weight gain	-	17/20
Dutch Belted ([redacted] 1980)	350	10/16	16/16	not recorded	No effect	-	6/16
	175	2/16	slight increase in incidence	not recorded	No effect	-	11/16
NZW ([redacted] *al.*, 1991)	450	1/20	13/20	12/20	No effect	-	13/20
	150	0/16	5/16	11/16	No effect	-	15/16
NZW ([redacted] 1993)	500	8/15	12/15	0/15	Statisticaly significant lower body weights than controls	-	6/15
NZW ([redacted] 1989)	500	0/15	0/15	0/15	Statisticaly significant lower body weight gain	-	12/15

- x/y: number of animals affected/total number of animals in group
* - due to mal-dosing.

Foetotoxicity/developmental toxicity occurred at or above doses that caused maternal toxicity. Most indications of developmental toxicity were reduced ossifications of skull, phalangeal and sternebral bones, which are typically seen in the litters of pregnant animals that do not eat well and lose weight during pregnancy (see expert statement of Williams et al., 2012[6]). The importance of this observation should not be misconstrued to mean that maternal toxicity in those cases was the proximate agent that injured the fetus, but rather that if exposures to the causative agent are kept below the doses that cause maternal toxicity, the developing offspring are protected. The lowest observed effects on the foetuses occurred at 300 mg/kg bw/day and were characterized by delayed ossification and decreased foetal weights (Moxon, 1996). The relevant NOAEL for foetotoxicity is 250 mg/kg bw/day.

A report from an independent source (Antoniou et al., 2011) has claimed that congenital malformations, especially of the cardiovascular system, were caused by glyphosate exposure in this same series of studies. A variety of malformations were reported across the database of glyphosate studies; these included:

- Dilated aorta/narrow pulmonary artery
- Narrow aorta/dilated pulmonary artery
- Interventricular septal defect
- Cardiomegaly
- Single ventricle
- Retro-esophageal right subclavian artery
- Interrupted aorta
- Right subclavian artery arising from aortic arch
- "Seal-shaped" heart

[6] Williams, A.L., Kimmel, G.L.,, Kimmel, C.A., DeSesso, J.M. 2012:Review and evaluation of the rabbit developmental toxicity database on glyphosate, with particular attention to cardic malformations, Exponent, 2012-04-30, 1200798.000.A0T0.0412.AW01

If glyphosate does cause congenital heart defects, it would be anticipated that the prevalence of congenital heart defects would be increased and one would expect the malformation rate to increase with increasing dose until the pregnant does would become intoxicated or the fetuses would die. The malformations occurred at a low incidence across all dose groups; they did not exhibit a positive dose-response; and often clusters of the malformations occurred in the same fetuses.

The incidence of aorticopulmonary septum-related defects in the combined control groups was 1/879 (0.1%); in the combined glyphosate-treated groups the incidence was 12/2250 (0.5%). One half of the malformed fetuses was found in litters exposed to the highest doses (450 and 500 mg/kg/day), which also experienced severe maternal toxicity including maternal deaths, abortions, and weight loss. If these groups are not considered because of the potential confounding factor introduced by maternal health issues, the incidence of the defects is 6/2049 (0.3%). These data show that the overall incidence of aorticopulmonary septum-related defects in offspring from mothers exposed to glyphosate at doses below those that cause severe maternal toxicity is similar to that seen in non-exposed rabbits.

The other prominent cardiovascular malformation is dilated heart. All observations of this finding (among both control and treated groups) occurred in a study conducted in a single laboratory (Suresh, 1993). This study has several weaknesses including a small number of litters available for examination due to low pregnancy rates and maternal deaths in the mid- and high-dose groups. None of the other six studies reported dilated hearts. Neither the criteria used to diagnose dilated heart nor measurements of the hearts were provided, so it is not possible to directly compare the dilated heart findings to the hearts of the more than 2800 fetuses in the other studies. It is possible that the observation of dilated hearts is due to overly stringent inspection compared to criteria used by other laboratories.

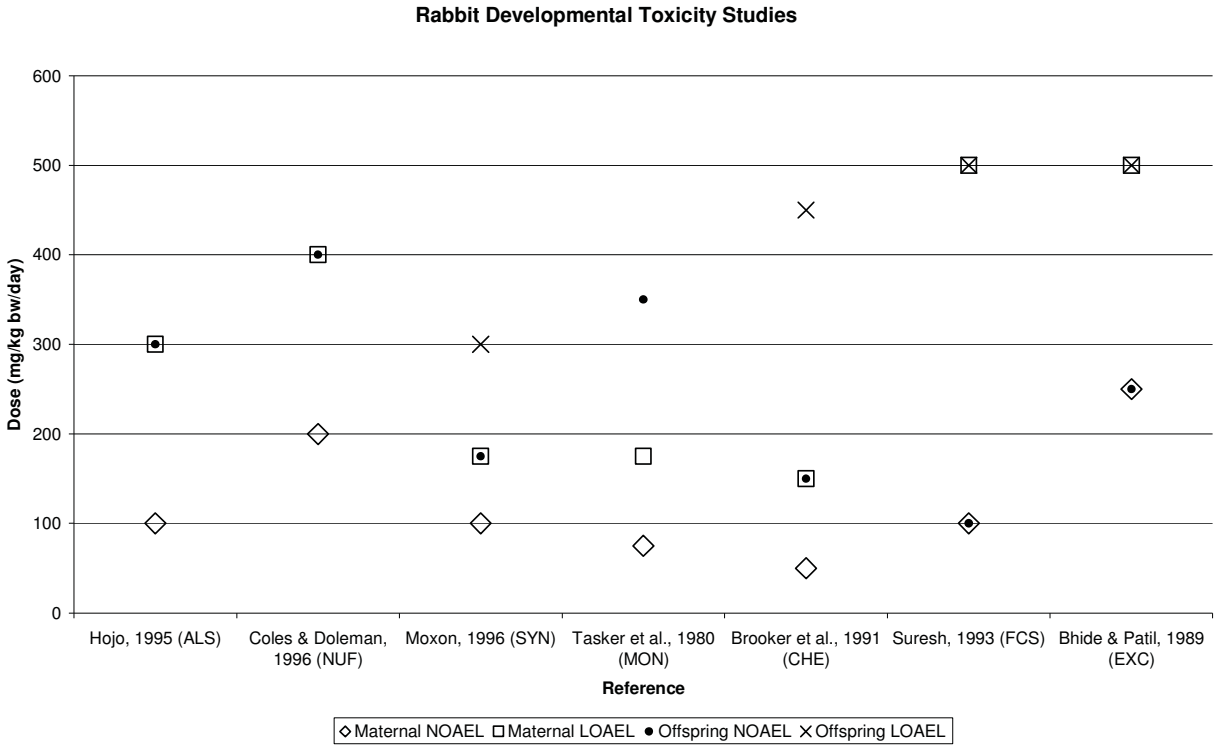
Taken together, overall data regarding potential cardiovascular malformations in the seven rabbit developmental toxicology studies do not support the contention that there is a clear compound related effect on the foetal heart. A comprehensive review of all GTF rabbit developmental toxicity studies was conducted by experts in the field of developmental toxicology and this report is referenced in Doc L and submitted in Doc K (see expert statement of Williams et al., 2012b)

Table 5.6-28: Summary of developmental toxicity in rabbits

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / developmental	
Studies not reviewed in the 2001 evaluation	IIA 5.6.11/01 [redacted] 1995 (ALS)	Developmental toxicity, gavage, rabbit, Japanese White	0, 10, 100, 300	100	300	Maternal: defecation of loose stool and subsequent abortion or premature delivery and reduced body weight Offspring: no effects
	IIA 5.6.11/02 [redacted] 1996 (NUF)	Developmental toxicity, gavage, rabbit, NZW	0, 50, 200, 400	200	400	Maternal: increased mortality, reduced body weight and diarrhoea (at the high dose), clinical signs (reduced faecal output, scours), reduced body weight at the mid-dose Offspring: no treatment-related effects

	Reference (Owner)	Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL [mg/kg bw/day (ppm)]		LOAEL Targets / Main effects
				Maternal	Offspring / developmental	
Studies not reviewed in the 2001 evaluation	IIA 5.6.11/03 [redacted] 1996a (SYN)	Developmental toxicity, gavage, rabbit, NZW	0, 100, 175, 300	100	175	Maternal: diarrhoea, reduced faecal output, staining of genital area at the high and mid-dose dose Offspring: delayed ossification, decreased body weights at the high dose
Studies from the 2001 evaluation	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/04 [redacted] 1980 (MON)	Developmental toxicity, gavage, rabbit, Dutch Belted	0, 75, 175, 350	75	350	Maternal: diarrhoea and soft stool, and increased mortality at the high dose Offspring: No treatment-related effects
	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/05 [redacted] 1991a (CHE)	Developmental toxicity, rabbit, NZW	0, 50, 150, 450	50	150	Maternal: reduced food consumption, soft/liquid faeces, reduced body weight Developmental: increased embryo/foetal deaths and post-implantation loss
	Annex B.5.6.2.2.1 Glyphosate Monograph IIA 5.6.11/06 [redacted] 1993 (FSG)	Developmental toxicity, rabbit, NZW	0, 20, 100, 500	100	100	Maternal: increased mortality, soft stool/ liquid faeces Developmental: general signs of secondary toxicity (e.g. incomplete ossification)
	Annex B.5.6.2.2.2 Glyphosate Monograph IIA 5.6.11/07 [redacted] 1989 (EXC)	Developmental toxicity, gavage, rabbit, NZW	0, 125, 250, 500	250	250	Maternal: body weights and food consumption signify-cantly reduced; 2 abortions were noted in the high dose group Developmental: mean number of viable im-plants significantly reduced, mean number of external, visceral and skeletal malfor-mations, and mean number of variations significantly increased.

Figure 5.6-1: Results of developmental toxicity studies in rabbits



Tier II summaries are presented for all available studies on developmental toxicity in the rabbit to allow for a robust weight of evidence evaluation of endpoints.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/01	[redacted]	1995	HR-001: A Teratogenicity Study in Rabbits [redacted] Data owner: Arysta Life Sciences Study no.: IET 94-0153 Date: 1995-07-21 GLP: yes not published

Guideline:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:	None
Dates of experimental work:	1995-03-31 to 1995-06-09

Executive Summary

HR-001 was administered by gavage to three groups of 18 artificially inseminated Japanese White rabbits each, at doses of 10, 100 and 300 mg/kg bw/day from Gestation Day 6-18 (artificial insemination = Day1). A further group of 18 animals was exposed to the vehicle to serve as control.

No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. In the high dose group, defecation of loose stool and subsequent abortion or premature delivery was considered to be related to test substance treatment with regard to the results of the preliminary study.

Mean maternal body weights and body weight gains on Days 16-24 of gestation in the high dose group decreased slightly although the differences from controls were not statistically significant.
Examinations at caesarean sectioning demonstrated no significant differences in the gravid uterine weights and the numbers of corpora lutea and implants between the control and the treated groups. The mean number of live foetuses, mean percent incidences of resorptions and foetal deaths, foetal sex ratios, mean foetal body weights, and mean placental weights in the treated groups were comparable to those in the control group.
Teratological examinations demonstrated no test substance treatment-related external, visceral and skeletal abnormalities in any foetuses in any treated groups.

Conclusion:
The oral administration of HR-001 to artificially inseminated rabbits by gavage Gestation Day 6-18 resulted in treatment-related changes at 300 mg/kg bw/day. Therefore the 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical, Code: HR-001
Description:	White crystal
Lot/Batch #:	T-941209
Purity:	97.56%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.5% carboxymethylcellulose

3. 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

B: 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 euthanatized 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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The test substance was detected at levels of 95-105% of the target concentrations in each dosing solution.

B. FOOD CONSUMPTION

Mean food consumption in the treated groups was comparable to that in the control group throughout the study period.

C. MORTALITY

One rabbit in the high dose group died on Day 20 of gestation without showing any clinical signs.

D. 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 5.6-29). 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 5.6-29: 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.

E. 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.

F. 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.

G. DEVELOPMENTAL PARAMETERS

Number and viability of foetuses

No statistically significant differences were noted in the mean number of live foetuses 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 foetuses, 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 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 number of litters with malformations was significantly higher than that in the control group (see Table 5.6-30). This increased malformation rate was due to an increase in skeletal malformations, as no external or visceral malformations were noted in foetuses 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 foetuses 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 (see Table 5.6-30). This high value was due to a significantly high incidence (87.5% of litters, 27.3% of the foetuses) of lumbar ribs in this dose group when compared with the control (72.2% of litters, 16.4% of foetuses). 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 foetuses), and because no such increase was observed in the 300 mg/kg bw/day group (13.4%).

Table 5.6-30: 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 sternebrae with sternocostal joint displacement	0	2	0	0
Total no. of foetuses with skeletal malformations **Percentage of foetuses with skeletal malformations (%)**	1 0.7	4 3.1	6 4.0	5 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 13th ribs	12	9	15	12
Cervical ribs	1	3	1	1
Lumbar ribs	23	19	41*	15
Extra ossification centre anterior to the 1st 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 $p < 0.05$.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/02	[redacted]	1996	Glyphosate technical: Oral gavage teratology study in the rabbit [redacted] 2GD, UK Data owner: Nufarm SPL project no.: 434/020 Date: 1996-07-04 GLP: yes not published

Guideline:	OECD 414 (1981), JMAFF 59 NohSan 4200 (1985), US-EPA 83-3 (1984)
Deviations:	None
Dates of experimental work:	1995-10-13 - 1995-12-12

Executive Summary
Glyphosate technical was administered by gavage to three groups of 18 mated New Zealand White rabbits each, at doses of 50, 200 and 400 mg/kg bw/day from gestation Day 7-19 (mating = Day 0). A further group of 18 animals was exposed to the vehicle to serve as control.
Individual clinical observations, bodyweight and food consumption were recorded during the study. The females were killed on Day 29 of gestation, examined macroscopically for external and internal malformation. The uteri were examined number of corpora lutea, implantation number, position and type, foetal weights, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.
At the high dose level there was evidence of treatment-related effects resulting in one treatment-related death. Clinical signs of toxicity, particularly scours, reduced faecal output and diarrhoea, reduced bodyweight gain and reduced food consumption were seen. At the intermediate dose level similar, but less severe, effects were seen on bodyweight gain. At the low dose level, no treatment related effects were observed. There were no treatment-related effects on the uterine or foetal parameters examined in any dose group.

Conclusion:
The oral administration of glyphosate technical to time-mated 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 NOAEL was considered to be 200 mg/kg bw/day for maternal toxicity. The NOAEL for developmental toxicity was considered to be 400 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	H95D161A
Purity:	95.3%
Stability of test compound:	not reported

2. **Vehicle and/ or positive control:** 1% carboxymethyl cellulose
3. **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

B: 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 analyzed. 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 (Van Julsingha and Bennett 1977). 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 (Dawson 1926). 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 Kruskall-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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The test substance was detected at the levels of 81-102% of the target concentrations in each dosing solution.

B. 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 to19 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.

C. 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.

D. 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 5.6-31). 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 5.6-31: 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)
Hypthermia	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.

E. 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 bodyweight 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 bodyweight gain from Day 9 to Day 29 *post coitum* was noted. In the low dose level group bodyweight gain was comparable to controls throughout the study period (see Table 5.6-32).

Table 5.6-32: 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 $p < 0.01$.

F. 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 foetuses 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.

G. 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 5.6-33: 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/03	[redacted]	1996	Glyphosate acid: Developmental toxicity study in the rabbit [redacted] Data owner: Syngenta Report No.: CTL/P/5009 Date: 1996-07-02 GLP: yes not published

Guideline: OECD 414 (1981), EEC B.31 (1988),US-EPA 83-3

Deviations: None

Dates of experimental work: 1996-01-01 to 1996-02-09

Executive Summary

Glyphosate acid was administered by gavage to three groups of 20 mated New Zealand White rabbits each, at doses of 100, 175 and 300 mg/kg bw/day from gestation Day 8-20 (mating = day 1). A further group of 20 animals was exposed to the vehicle (deionised water) to serve as control.

Individual clinical observations, bodyweight and food consumption were recorded during the study. The females were killed on Day 30 of gestation, examined macroscopically for external and internal malformation. The uteri were examined number of corpora lutea, implantation number, position and type, foetal weights, foetal sex, external appearance and internal visceral anomalies/abnormalities were recorded. All live foetuses were preserved, processed and subsequently examined for skeletal anomalies with the heads of half the offspring preserved and examined for visceral anomalies.

Administration of 175 or 300 mg/kg bw/day was associated with dose-related maternal toxicity. This toxicity was manifested as signs of diarrhoea, reduction in faecal output as a result of reduced food consumption and a corresponding reduction in body weight. There was no maternal toxicity attributable to the administration of 100 mg/kg bw/day. At the high dose level, when maternal toxicity was seen, a reduction in mean foetal weight and very minor alterations in foetal ossification were evident. There was no effect on the number of foetuses or their survival and there was no evidence of teratogenicity.

Conclusion:
The oral administration of glyphosate acid to time-mated rabbits by gavage from Gestation Day 8-20 resulted in 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 NOAEL was considered to be 100 mg/kg bw/day for maternal toxicity, and 175 mg/kg bw/day for offspring.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Deionised water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Harlan Interfauna Ltd., Huntington, 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

B. 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 l50 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 where 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 of Freeman and Tukey (1950),
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.

II. RESULTS AND DISCUSSION

A. 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.

B. FOOD CONSUMPTION

During the dosing period, does receiving 175 or 300 mg/kg bw/day showed reduced food consumption compared to the controls.

C. 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.

D. 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 5.6-34).

Table 5.6-34: Observed clinical signs during the dosing period

	Number of rabbits affected in dose group			
Clinical sign	**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

E. 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, bodyweight gain was comparable to controls throughout the study period (see Table 5.6-35).

Table 5.6-35: 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 $p < 0.01$.

F. 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.

G. 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 foetuses 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 foetuses 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 foetuses 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 5.6-36 and Table 5.6-38).

The proportion of foetuses 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 foetuses in the high dose level group with partially ossified transverse processes on the 7th cervical vertebra (8 foetuses in 2 litters), unossified transverse processes on the 7th lumbar vertebra (14 foetuses in 4 litters) or partially ossified 6th sternebra (16 foetuses in 7 litters). None of the specific minor defects were statistically significantly increased in the low or intermediate dose level groups. None of the foetuses were found to have an external/visceral variant.

The proportion of foetuses 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 foetuses in this group with partially ossified odontoids (62 foetuses in 15 litters) or with 27 pre-sacral vertebrae (37 foetuses 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 foetuses scoring 4 or 5. A similar response was apparent from the *pes* scores.

Table 5.6-36: Summary of the type and incidence of major defects

Major foetal defects	Number of foetuses affected in dose group*			
	Control (0 mg/kg/day)	**Low (100 mg/kg/day)**	**Intermediate (175 mg/kg/day)**	**High (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 5.6-37: Summary of the type and incidence of major defects (litter incidences)

Major foetal defects	Number of litters affected in dose group*			
	Control (0 mg/kg/day)	Low (100 mg/kg/day)	Intermediate (175 mg/kg/day)	High (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 5.6-38: 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 ($p < 0.05$)

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/04	[redacted]	1980	Technical Glyphosate: Teratology study in rabbits [redacted] Monsanto Report No.: IR-79-018 Date: 1980-02-29 GLP: no (pre-GLP study) not published

Guideline:	Not stated. (pre-guideline; satisfies in general the requirements of OECD 414 (1981), but not of OECD 414 (2001))
Deviations:	Not applicable
Dates of experimental work:	1979-04-10 to 1979-05-11

Executive Summary
Glyphosate acid was administered by gavage to groups of 16 pregnant Dutch Belted rabbits each, at doses of 75, 175 and 350 mg/kg bw/day from gestation Day 6-27 (insemination = Day 0). A further group of 16 animals was exposed to the vehicle (0.5 % aqueous Methocel®) to serve as control.
Individual clinical observations and bodyweights were recorded during the study. The animals were either killed on Day 28 of gestation, or after they had aborted during the study period. All sacrificed animals or animals found dead were subjected to a gross necropsy. The uteri were examined for number of corpora lutea, early and late resorption, and total implantations. Foetuses were weighted, sexed and examined for external and internal malformations, as well as internal visceral anomalies/abnormalities.
At 175 mg/kg bw/day, a slight increase of soft stool and diarrhoea was observed in dams. At 350 mg/kg bw/day, definite signs of maternal toxicity were observed; this toxicity was manifest as signs of soft stool and/or diarrhoea in all high dose animals at least once, nasal discharge, as well as an increase in the number of dams that died. There was no maternal toxicity attributable to the administration of 75 mg/kg bw/day. In addition, there were no signs of developmental effects noted in any dose group.

Conclusion:
The oral administration of glyphosate acid to pregnant rabbits by gavage from Gestation Day 6-27 resulted in maternal toxicity at 175 and 350 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White powder
Lot/Batch #:	XHJ-64
Purity:	98.7%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.5 % aqueous Methocel®

3. Test animals:

Species:	Rabbit

Strain:	Dutch Belted
Source:	Langshaw Farms, Augusta, Michigan, USA
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

B. 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. Ther 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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There was an increased incidence of mortalities in the high dose group (see Table 5.6-39).

Table 5.6-39: 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.

D. 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.

E. BODY WEIGHT
There were no treatment-related effects on maternal body weights and body weight gain.

F. 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 5.6-40: 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

G. 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 5.6-41: 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 5.6-42).

Table 5.6-42: 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)	
Number of litters examined		12		15		11		6	
	%	x/y	%	x/y	%	x/y	%	x/y	%
Skeletal malformations		**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
Visceral malformation	--	**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**
Variations									
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
13th 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
13th 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/05	[redacted]	1991	The Effect of Glyphosate on Pregnancy of the Rabbit (Incorporates Preliminary Investigations) [redacted] Data owner: Cheminova Study/Project No.: CHV 45 & 39 & 40/901303 Date: 1991-10-14 GLP: yes not published

Guideline:	OECD 414, US EPA 83-3
Deviations:	None
Dates of experimental work:	1989-12-14 to 1990-03-02

Executive Summary
In a developmental toxicity study, groups of 16 - 20 time-mated female New Zealand White rabbits were administered glyphosate acid in 1% methylcellulose 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 (mating = Day 0). All animals were observed daily for clinical signs and mortality, and body weights and food consumption were measured on Days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation. On Day 29 of gestation, the does were sacrificed and a gross necropsy was performed. 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. All live foetuses were examined for external, visceral and skeletal abnormalities.
Observations recorded included one death at 450 mg/kg bw/day following abortion, reduced food intake during the treatment period and reduction in body weight gain from Days 11 – 19 of pregnancy. Clinical signs included a dose-related increase in the number of females showing soft/liquid faeces (gastrointestinal disturbances) and slight reductions in food consumption and body weight at 150 and 450 mg/kg bw/day.

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 food consumption and bodyweight gain and soft/liquid faeces during the dosing period. The NOAEL was 150 mg/kg bw/day for foetotoxicity and was 450 mg/kg bw/day for teratogenicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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

2. Vehicle and/ or positive control: 1% methylcellulose

3. 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

B: 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 analyzed 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.

II. RESULTS AND DISCUSSION

A. 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 renalysis on Day 19 showed the concentration to be 5% above the nominal dose.

B. 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 5.6-43).

Table 5.6-43: 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

C. 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.

D. 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 5.6-44).

Table 5.6-44: 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

E. BODY WEIGHT

A slight reduction in bodyweight 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 5.6-45).

Table 5.6-45: 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

F. 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 foetuses 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 foetuses.

There were no significant intergroup differences in the numbers of corpora lutea, implantations, pre-implantation loss, foetal sex ratios or foetal weights (see Table 5.6-46). 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 5.6-46). 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 5.6-46: 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 $P > 0.05$

G. 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 (see Table 5.6-47) 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 5.6-47).

Table 5.6-47: Summary of foetal parameters

Parameter	Dose Group (mg/kg bw/day)				Historical control range or x/y ◊ (mean)
	0(control)	50	150	450	
Number of does with live young or litters at Day 29	18	12	15	13	--
Mean foetal weight (g)	43.9	43.3	44.0	44.5	41.4 – 47.6 (44.1)
Sex (% males)	55.3	55.8	57.6	53.8	--
Malformations					--
Total number of foetuses examined	163	104	112	95	1511
No. of malformed foetuses	3	3	5	6	51
%	1.9	5.8	4.3	5.9 (F)	0.7 – 5.9 (3.8)
Number of Affected Litters	3	3	3	5	43/188
%	16.67	25	20	38.5	22.9
Thoracic region malformations					--
No. of foetuses with interventricular septal defect	1	1	1	4	10/1511
%	0.6	1.0	0.9	4.2	0.66
Litter incidence	1	1	1	4	10/188
%	5.56	8.3	6.67	30.8	5.32
Foetuses with enlarged left, reduced right ventricles	0	0	0	2	2/1511
%	0.0	0.0	0.0	2.1	0.13
Litter incidence	0	0	0	2	2/188
%	0	0	0	15.4	1.10
Foetuses with retro-oesophageal right subclavian artery	0	0	3	2	7/1511
%	0.0	0.0	2.7	2.1	0.46
Litter incidence	0	0	1	1	7/188
%	0	0	6.6	7.6	3.72
Foetuses with narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1	1	1	3	8/1511
%	0.6	1.0	0.9	3.2	0.52
Litter incidence	1	1	1	3	8/188
%	5.56	8.3	6.67	23.1	4.25
Anomalies					--
Total number of foetuses examined[#]	160	101	107	89	--
No. of foetuses with gross/visceral anomalies	9	14	14	6	--
%	6.4	19.5	12.9	9.6 (K)	--
No. of foetuses with skeletal anomalies	21	13	14	11	--
%	11.7	17.7	12.5	10.1 (K)	--
No. of foetuses with reduced ossification	7	4	5	4	--
%	4.4	4.0	4.7	4.5	--
Mean foetal weight of foetuses with reduced ossification (g)	37.9	43.6	37.7	26.1	--

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

III. CONCLUSION

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 bodyweight 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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/06	[redacted]	1993	Teratogenicity study in rabbits – Test compound: Glyphosate technical (FSG 03090 H/05 March 1990) [redacted] Data owner: Feinchemie Schwebda GmbH Study No.: TOXI: 884-TER-RB Date: 1993-04-17, amended 1994-06-18 GLP: not published

Guideline:	OECD 414 (1981)
Deviations:	None
Dates of experimental work:	1991-12-24 to 1992-03-06

Executive Summary

This developmental toxicity study in rabbits is not clearly or accurately documented and does not provide appropriate interpretation of the study findings. 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 (mating = Day 0). Dose volumes were calculated for individual animals from day 6 and adjusted according to body weight daily. The day of mating was considered as Day 0. Dose levels were based on the findings of preliminary studies. 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 on day 28 were subjected to post-mortem gross pathological examination. Individual body weights of dams were recorded on Days 0, daily from Days 6 – 18 and on Day 28 of gestation.

The ovaries and uteri were examined to determine the number of corpora lutea, implantations, the number of dead/abnormal/live foetuses, the number and distribution of embryonic and /foetal resorptions. Foetuses were examined for individual foetal weight and foetal abnormalities, sex and visceral organ malformations by foetal necropsy/modified Wilson's technique.

There were 4/16 and 8/15 mortalities at dose levels of 100 and 500 mg/kg bw/day, respectively. The deaths at 500 mg/kg bw/day were accompanied by clinical signs, including the increased incidence of soft liquid/diarrhoea or mucoid faeces, reduction in feed consumption and reduction in body weight gain during the treatment period. However, clinical signs were not evident for the 100 mg/kg bw/day dose group and only one incidence of soft faeces was recorded at 100 mg/kg bw/day. Further, a number of lung and tracheal findings in the dams at gross necropsy indicated possible gavage errors to which the deaths at this dose may be attributed. Thus, the NOAEL for maternal toxicity was set to 100 mg/kg bw/day.

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. Mortality and clinical signs of toxicity at 500 mg/kg bw/day included reduced feed consumption and soft faeces and reduced bodyweight gain during the dosing period one incidence of

complete resorptions. 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).

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: 0.5 % w/v carboxymethylcellulose

3. Test animals:

Species:	Rabbit
Strain:	Female New Zealand White
Source:	Toxicology Department, Rallis Agrochemical Research Station, Bangalore, India.
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 *ad libitum*
Water:	Protected deep bore well drinking water, treated via activated charcoal filter and UV in Aquaguard on-line water filter-cum-purifier provided *ad libitum*
Housing:	Individually in 3-tier all aluminium cages with wire mesh bottom and common self-draining litter trays.
Environmental conditions:	Temperature: 22 ± 3 °C Humidity: 40 – 70 % Air changes: 10 – 15/h Natural lighting supplemented with fluorescent lighting 12 hours light/dark cycle

B: 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 2ml/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 euthanized 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 $P \leq 0.05$.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
Not reported.

B. 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 5.6-48). Feed consumption during the post-treatment period did not show significant intergroup differences.

Table 5.6-48: Summary of food consumption

Parameter	Historical positive control#	Dose Group (mg/kg bw/day)			
		0 (control)	20	100	500
Food consumption (g/rabbit/day)					
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 $P \leq 0.05$

C. 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 5.6-49 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 and are not appropriately discussed in the report.

Table 5.6-49: 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

D. 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 5.6-50: 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

Parameter / clinical sign	Dose Group (mg/kg bw/day)			
	0 (control)	20	100	500
Weak	0	0	0	2
Ocular discharge	0	0	0	1
Dyspnoea	0	0	0	1

E. 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 5.6-51: 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 bodyweights (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 (bodyweight – uterine weight)	2.7*	3.0	3.0	2.9	2.7
Mean bodyweight 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 $P \leq 0.05$

F. 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 foetuses 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 5.6-52).

Table 5.6-52: 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

G. 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 5.6-53).

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 5.6-53).

Table 5.6-53: 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 foetuses	46	134	80	78	28
Mean litter size	8	7	6	7	6
Abnormal foetuses (%)	0 (0)	1 (1)	2 (3)	0	0
Dead foetuses (%)	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 foetuses	46	133	77	77	28
Mean weight of live foetuses (g ± SD)	29 ± 1.4	32 ± 5.3	35 ± 3.7*	35 ± 2.4*	33 ± 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 5.6-54). The exception was dilated heart, which was reported in four foetuses of 3 litters in the 20 mg/kg bw/day dose group, 4 foetuses (3 + 1) from 2 litters of the 100 mg/kg bw/day dose group and all foetuses (4) of one litter and one foetus of another litter at the 500 mg/kg bw/day (Statistically significant P≤ 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 foetuses 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 5.6-54: Summary of relevant external, visceral and skeletal findings (litter data)

Foetal findings	HC Data[#]	Dose level (mg/kg bw/day) 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 arteriousus (%)	--	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

Foetal findings	HC Data#	Dose level (mg/kg bw/day) 0	20	100	500
Minor skeletal malformations					
No. of foetuses with extra 13th rib		0	1	2	1
Percentage of foetuses with extra 13th 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 ($P \leq 0.05$)

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.6.11/07	[redacted]	1989	Rabbit Teratology Study with Glyphosate Technical [redacted] Data owner: Excel Study no.: IIT Project No. 1086 Date: 1989-11-03 GLP: no not published

Guideline:	OECD 414 (1981)
Deviations:	no uterine weight, no maternal necropsy findings
Dates of experimental work:	1989-07-03 to 1989-11-02

Executive Summary

This developmental toxicity study in rabbits is limited in its extent of documentation. Glyphosate Technical was administered by gavage to three groups of 15 successfully mated New Zealand albino rabbits each, at doses of 125, 250, and 500 mg/kg bw/day from Gestation Day 6-18 (mating = Day 1). A further group of 15 animals was exposed to the vehicle to serve as control.

No adverse effects related to test substance treatment were observed in any animals of the low and mid dose group. Mean maternal body weights and food consumption were lower in the high dose group, however clinical signs of systemic toxicity were not observed at any dose. In the high dose group, two cases of total abortion were considered to be related to test substance treatment.

The mean number of viable implants (foetuses) per litter was lower in the high dose group and the mean number of external, visceral and skeletal malformations as well as the mean number of variations was higher in the high dose group compared to the control group. No differences in the examined

developmental parameters were found in the low and mid dose groups, although a dose-related increase in the numbers of malformations and variations was noted.

Conclusion:
The oral administration of glyphosate to successfully mated rabbits by gavage from Gestation Day 6-18 resulted in treatment-related changes at 500 mg/kg bw/day. The NOAEL for reproductive and non-reproductive toxicity was considered to be 250 mg/kg bw/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate technical
Description:	White amorphous powder
Lot #:	38
Purity:	95%
Stability of test compound:	Not reported

2. Vehicle and/ or positive control: 0.1% gum acacia in water

3. Test animals:

Species:	Rabbit
Strain:	New Zealand White
Source:	Indian Institute of Toxicology, New Bombay
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

B: 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 SAll foetuses were decapitated and heads were fixed in Bouin's solution for examination of craniofacial structures.

Statistics
Not reported.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS
The analytical purity of test substance was stated to be 95%.

B. 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.

C. MORTALITY
None of the rabbits died during the study period.

D. CLINICAL OBSERVATIONS
No toxic symptoms were observed in any of the animals during the study.

E. 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 stastical comparison was provided in the report.

F. 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 5.6-55).

Table 5.6-55: 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

G. 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 5.6-55), 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 5.6-55). However, stasticial 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 5.6-56). 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, viscerally 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, stasticial analyses were provided in the report.

Table 5.6-56: 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)
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)

III. CONCLUSION

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.

IIA 5.7 Neurotoxicity

The 2001 glyphosate evaluation concluded that there was no evidence of neurotoxicity in acute, subchronic or chronic studies in rodents and dogs. Glyphosate is often erroneously called an organophophate pesticide. However, it is important to note that glyphosate is not an organophosphate ester but a phosphonoglycine, that does not inhibit cholinesterase activity. Therefore, studies for delayed neurotoxicity are not considered essential. Despite this, two studies for delayed neurotoxicity in hens ([redacted] 1987 and 1988) have been conducted and were reviewed during the 2001 EU glyphosate evaluation confirmed the absence of neurotoxic effects. The NOAEL derived from these studies is 1000 mg/kg bw/day.

An acute neurotoxicity in rats was performed by [redacted] (1996a) that was not reviewed during the 2001 glyphosate evaluation. Administration of glyphosate acid produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 hours after dosing on day 1 in 3/10 females, only, which received 2000 mg/kg. One of these females was subsequently found dead on day 2. These clinical signs were considered to reflect general toxicity associated with the administration of high dose levels of glyphosate acid. Quantitative assessment of landing foot splay, sensory perception, muscle weakness and locomotor activity revealed no changes indicative of neurotoxic potential. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving 2000 mg/kg.

The no-observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg and the NOEL for systemic toxicity was 1000 mg/kg.

In addition a sub-chronic neurotoxicity study was also performed by [redacted] (1996b). In this study administration of glyphosate acid produced no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system revealed no evidence of any changes 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 (equivalent to 1547/1631 mg/kg bw/day in males and females respectively). There was a treatment-related effect on growth and food utilisation in males receiving 20000 ppm (1547 mg/kg bw/day). In this study the NOEL for systemic toxicity was 8000 ppm (617 mg/kg bw/day) in males and 20000 ppm (1631 mg/kg bw/day) in females.

In a study performed by [redacted] (1996, see IIA 5.10/03) *ex vivo* investigations with isolated rat gastrocnemius muscle were performed. Evaluation of innervated muscle response showed that glyphosate technical, when administered at the maximum solubility concentration in physiological saline (12 mg/mL), did not cause any neuromuscular blocking activity.

Overall, across a wide database of both specific neurotoxicity and repeat dose studies there is no evidence that glyphosate has neurotoxic potential.

Table 5.7-1: Summary of neurotoxicity studies with glyphosate acid

	Reference (Data owner)	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.7 Glyphosate Monograph [redacted] 1987 (EXC)	21-day, oral diet Hen, White leghorn	0, 250, 500, 1000 mg/kg bw/day	Toxicity: 500 mg/kg bw/day Neurotoxicity: 1000 mg/kg bw/day	1000 mg/kg bw/day: slight ataxia in 1 hen; all hens appeared hunched, lethargic, red liquid and mattening of feathers in anogenital region, body weight and food consumption ↓, haematological changes
	Annex B.5.7 Glyphosate Monograph [redacted] 1988 (EXC)	21-day, oral diet Hen, White leghorn	0, 400, 800, 1600 mg/kg bw/day	Toxicity: 800 mg/kg bw/day Neurotoxicity: 1600 mg/kg bw/day	1000 mg/kg bw/day: slight ataxia in 1 hen; all hens appeared hunched, lethargic, red liquid and mattening of feathers in anogenital region, body weight and food consumption ↓, haemato-logical changes, reduced egg number
Studies not reviewed in the 2001 evaluation	IIA 5.7.1/01 [redacted] 1996a (SYN)	Acute, oral gavage, Rat Alpk:AP_fSD	0, 500, 1000, 2000 mg/kg bw/day	Toxicity: 1000 mg/kg bw/day Neurotoxicity: 2000 mg/kg bw/day	2000 mg/kg bw/day: on day 1 subdued behaviour, decreased activity, hunched posture, sides pinched in, tip-toe gait and hypothermia in females only
	IIA 5.7.4/01 [redacted] 1996b (SYN)	13-week oral diet, Rat Alpk:AP_fSD	0, 2000, 8000, 20000 ppm (≅ 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)	Toxicity: 617.1 / 1630.6 mg/kg bw/day (♂/♀) Neurotoxicity: 1546.5 / 1630.6 mg/kg bw/day (♂/♀)	20000 ppm (1546.5 mg/kg bw/day) males: reduced body weights and food utilisation

↓ = decreased; ↑ = increased;

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 we refer to the Monograph and the former dossier.

IIA 5.7.1 Acute neurotoxicity – rat

Annex point	Author(s)	Year	Study title
IIA, 5.7.1/01	[redacted]	1996a	Glyphosate acid: Acute neurotoxicity study in rats [redacted] Data owner: Syngenta Report No. CTL/P/4866 Date: 1996-03-01 GLP: yes unpublished

Guideline:	No guideline stated in the report but in general compliance with OECD 424 (1997).
Deviations:	None
Dates of experimental work:	Not reported. The study was conducted during May and June 1995.

Executive Summary

Groups of 10 animals Alpk:APfSD rats per sex were administered 0, 500, 1000 and 2000 mg/kg bw glyphosate acid. Two weeks post administration all animals were observed daily for any changes in clinical condition. A detailed clinical observation was performed at weekly intervals. At scheduled termination 5 rats/sex/group were subjected to full *post mortem* examination. Selected nervous system tissues were examined microscopically.

Clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) occurred during Day 1 but were limited to 3 females in the highest dose group. One of these females was subsequently found dead on Day 2.
Slight reductions in food consumption, without any associated effects on body weight, were also observed during Week 1 for both sexes in the highest dose group.
Quantitative assessment of neurotoxic parameters and histopathological evaluation of the central and peripheral nervous confirmed no neurotoxic potential for glyphosate.

In conclusion, the NOAEL for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg bw.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid
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.

2. Vehicle and/or positive control:

Deionised water

3. Test animals:

Species:	Rats
Strain:	Alpk:APfSD (Wistar-derived)

Source:	Barriered Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, England (UK)	
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	

B: STUDY DESIGN AND METHODS

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. Locomoter 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.

II. RESULTS AND DISCUSSION

A. 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.

B. 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.

C. BODY WEIGHT

No treatment-related effects were observed.

D. 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 5.7-2). There was no evidence of treatment-related effects in animals receiving 500 or 1000 mg/kg bw.

Table 5.7-2: Intergroup comparison of food consumption (g/rat/day) during Week 1

Dose level of glyphosate (mg/kg bw)			
0 (control) **Mean ± SD**	**500** **Mean ± SD**	**1000** **Mean ± SD**	**2000** **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)

E. FUNCTIONAL OBSERVATIONAL BATTERY

Examinations of the functional observational battery did not identify any conclusive treatment- and dose-related effects

F. PATHOLOGY

Necropsy

No macroscopic findings were detected.

Histopathology

No microscopic findings were considered to be treatment-related.

III. CONCLUSION

Based on the study results the NOAEL for acute neurotoxicity, following single oral administration of glyphosate acid is 2000 mg/kg bw.

IIA 5.7.2 Delayed neurotoxicity following acute exposure

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity toxicity studies. Therefore, a study for the assessment of delayed neurotoxicity following acute exposure is considered not necessary.

IIA 5.7.3 28-day delayed neurotoxicity

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity, as well as in reproductive and developmental toxicity studies. Therefore, a 28-day delayed neurotoxicity studies is not required.

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IIA 5.7.4 Subchronic neurotoxicity – rat - 90 day

Annex point	Author(s)	Year	Study title
IIA, 5.7.4/01	[redacted]	1996b	Glyphosate Acid: Subchronic Neurotoxicity Study In Rats [redacted] Data owner: Syngenta Report No.: CTL/P/4867 Date: 1996-03-11 GLP: yes unpublished

Guideline:	Study was pre-guideline, but satisfies in general the requirements of OECD 424 (1997)
Deviations:	None
Dates of experimental work:	1995-04-25 to August 1995

Executive Summary

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.

All animals were observed prior to the study start and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of

landing foot splay, sensory perception and muscle weakness, were performed at intervals. Locomotor activity was also monitored at intervals. At the end of the study, 6 rats/sex/group were killed and subjected to a full post mortem examination. Selected nervous system tissues were removed, processed and examined microscopically.

Administration of glyphosate acid produced treatment-related effects on growth and food utilisation in males receiving 20000 ppm, with no associated effects on food consumption. There were no treatment-related effects on bodyweight, food consumption or food utilisation for males receiving 2000 or 8000 ppm, or for females from all treated groups.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system showed no evidence of any changes 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate acid (technical)
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Stability of test compound:	Confirmed for the study period

2. Vehicle and/or positive control: Plain diet

3. Test animals:

Species:	Rats	
Strain:	Alpk:APfSD	
Source:	Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK	
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

B: STUDY DESIGN AND METHODS

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)[7].

The levels of probability chosen as significant different from control were $p < 0.01$** and $p < 0.05$* (Student's t-test, two-sided).

II. RESULTS AND DISCUSSION

A. 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.

B. MORTALITY
No deaths occurred during the study.

C. CLINICAL OBSERVATIONS
There were no treatment-related clinical signs of toxicity.

[7] SAS Institute Inc. SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 2. Cary, NC: SAS Institute Inc., 1989

D. FUNCTIONAL OBSERVATIONS

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 5.7-3: 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)

E. 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 bodyweight 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 bodyweight was essentially similar to that of concurrent controls throughout the study.

Table 5.7-4: Intergroup comparison of bodyweights (g)

	Dietary concentration (ppm)							
	Males				Females			
Week	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)

F. 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 5.7-5: Intergroup comparison of food utilisation (g growth/100 g food)

	Dietary concentration (ppm)							
	Males				Females			
Week	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

G. PATHOLOGY

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.

III. CONCLUSION

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.

IIA 5.7.5 Postnatal development neurotoxicity

There were no indications for a neurotoxic potential of glyphosate observed in acute and subchronic neurotoxicity, acute, short-, and long-term toxicity, as well as in reproductive and developmental toxicity studies. Therefore, it is not necessary to conduct a postnatal development neurotoxicity study.

IIA 5.8 Toxicity studies on metabolites

The metabolite aminomethyl phosphonic acid (AMPA) was investigated for acute and subchronic effects, mutagenicity and teratogenicity. These studies previously evaluated in the 2001 EU glyphosate evaluation have shown that AMPA has a lower toxicity than the parent compound and is devoid of any mutagenic or teratogenic potential.
Studies conducted after the 2001 EU evaluation, further investigated the potential dermal and oral toxicity, as well as skin sensitizing potential of AMPA. From these studies a dermal LD_{50} of > 2000 mg/kg bw/day for rat and an oral LD_{50} of > 5000 mg/kg bw/day for rat and mice was found ([redacted] 2002, IIA 5.8/03; [redacted] 1996, IIA 5.8/01; [redacted] 1988, IIA 5.8/02). In a Magnusson Kligman Maximisation Test, no sensitizing effects of AMPA were observed ([redacted] 2002, IIA 5.8/04).

Table 5.8-1: Summary of toxicological studies with metabolites of glyphosate

Reference (Data owner)		Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1973 (MON)	Metabolism Rat	AMPA	not available	Metabolism after single oral dosing	Of 6.7 mg [14C]-AMPA, 74% appeared in faeces, 20% in urine, <0.1% in exhaled air; 0.06 of the total dose recovered from carcass; liver, kidney, and muscle exhibited residues of 6, 6 and 3 ppb
	Annex B.5.8.1.1 Glyphosate Monograph [redacted], 1993a (CHE)	Acute oral toxicity Rat	AMPA	99.2	Limit test	LD_{50} > 5000 mg/kg bw
Studies not reviewed in the 2001 evaluation	IIA 5.8/01 [redacted] 1996 (ALS)	Acute oral toxicity Mice	AMPA	99.33	Limit test	LD_{50} > 5000 mg/kg bw
	IIA 5.8/02 [redacted] 1988 (SYN)	Acute oral toxicity Rat, Wistar	AMPA	> 99	Limit test	LD_{50} > 5000 mg/kg bw
	IIA 5.8/03 [redacted] 2002 (FSG)	Acute dermal toxicity Rat, CD	AMPA	98	Limit test	LD_{50} > 2000 mg/kg bw

Reference (Data owner)		Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993b (CHE)	Acute dermal toxicity Rat	AMPA	99.2	Limit test	LD_{50} > 2000 mg/kg bw
	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1993c (CHE)	Skin sensitisation Guinea pig	AMPA	99.2	MKT	Not sensitising
Studies not reviewed in the 2001 evaluation	IIA 5.8/04 [redacted] 2002 (FSG)	Skin sensitisation Guinea pig	AMPA	98	MKT	Not sensitising
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph Heath, [redacted] 1993 (CHE)	4-week oral toxicity Rat	AMPA	99.2	gavage	NOAEL = 100 / 1000 mg/kg bw/day (♂/♀)
	Annex B.5.8.1.1 Glyphosate Monograph Strutt, Atkinson, [redacted] 1993	13-week oral toxicity Rat	AMPA	99.2	gavage	NOAEL > 1000 mg/kg bw/day
	Annex B.5.8.1 Glyphosate Monograph [redacted] 1979 (MON)	90-day oral toxicity Rat	AMPA	99.96	diet	NOEL = 400 mg/kg bw/day
	Annex B.5.8.1. Glyphosate Monograph [redacted] 1991 (MON)	90-day oral toxicity Dog	AMPA	87.8	capsule	NOAEL = 300 mg/kg bw/day
	Annex B.5.8.1.1 Glyphosate Monograph Jensen, 1993a (CHE)	Genotoxicity in bacteria	AMPA	99.2	Reverse mutation in bacteria (Ames test)	negative

Reference (Data owner)		Type of study Species, Strain	Metabolite	Purity [%]	Exposure conditions / test method	Results
Studies not reviewed in the 2001 evaluation	IIA 5.8/05 Callander, 1988 (SYN)	Genotoxicity in bacteria	AMPA	99.2	Reverse mutation in bacteria (Ames test) in *S. typhimurium & E. coli*	negative
	IIA 5.8/06 Mie, 1996 (ALS)	Genotoxicity in Bacteria	AMPA	99.33	Reverse mutation test in *S. typhimurium & E. coli*	negative
	IIA 5.8/07 Nesslany, 2002 (ALS)	Genotoxicity *in vitro*	AMPA	99.9	UDS in rat hepatocytes	negative
Studies from the 2001 evaluation	Annex B.5.8.1.1 Glyphosate Monograph Jensen, 1991d (CHE)	Genotoxicity	AMPA	99.2	Micronucleus test	negative
	Annex B.5.8.1.1 Glyphosate Monograph [redacted] 1992 (CHE)	Develop-mental toxicity Rat	AMPA	99.2	0, 100, 350, 1000 mg/kg bw/day; gavage gestation days 6-16	NOAEL > 1000 mg/kg bw/day (maternal and developmental)
	Annex B.5.8.1 Glyphosate Monograph [redacted] 1991 (MON)	Develop-mental toxicity Rat	AMPA	94.38	0, 150, 400, 1000 mg/kg bw/day; gavage gestation days 6-15	NOAEL 150 / 400 mg/kg bw/day (maternal / developmental)

AMPA = Aminomethyl Phosphonic Acid
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 we refer to the Monograph and the former dossier.

Annex point	Author(s)	Year	Study title
IIA, 5.8/01	[redacted]	1996	AMPA: Acute Oral Toxicity Study In Mice. [redacted] Data owner: Arysta Life Sciences Report No.: IET 96-0075 Date: 1996-11-11 GLP: yes not published

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

Deviations: None

Dates of experimental work: 1996-09-24 to 1996-10-08

Executive Summary
The test substance, AMPA, was evaluated for its acute oral toxicity potential in ICR mice when administered as a gavage dose at a level of 5,000 mg/kg bw. No mortality occurred during the study. Neither clinical signs nor macroscopic lesions at necropsy were observed in any animals. All animals gained body weights 7 and 14 days after administration when compared with the body weights on the day of administration. The acute oral LD_{50} was calculated to be

LD_{50}, oral, mice > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	AMPA
Description:	White powder
Lot/Batch #:	A-960719
Purity:	99.33%
Stability of test compound:	Stable for 1 year at RT.

2. Vehicle and/or positive control: 1% carboxymethyl-cellulose (CMC)

3. Test animals:

Species:	Mice
Strain:	ICR (Crj:CD-1), SPF
Source:	Atsugi Breeding Center, Charles River Japan, Inc.
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 ± 3°C Humidity: 55 ± 15% Air changes: 12/hour 12-hour light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-09-17 to 1996-10-08

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
No body weight losses were recorded in any animal 7 and 14 days after the administration.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/02	[redacted]	1988	Aminomethyl Phosphonic Acid: Acute Oral Toxicity to the Rat. [redacted] Data owner: Syngenta Report No.: CTL/P/2266 Date: 1988-08-10 GLP: yes not published

Guideline:	Not stated, but method is in accordance with OECD 401.
Deviations:	None
Dates of experimental work:	No date given in the report.

Executive Summary
The test substance, Aminomethyl Phosphonic Acid (AMPA), was evaluated for its acute oral toxicity potential in Wistar rats when administered as a gavage dose at a level of 5000 mg/kg bw. No mortality due to the test substance occurred during the study. Signs of slight toxicity were observed following dosing, but all animals appeared normal by Day 4. Observed body weight losses were not regarded to be treatment-related since no associated clinical abnormalities or any abnormalities at necropsy were observed. The acute oral LD_{50} was calculated to be

LD_{50}, oral, rat > 5000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute oral toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: 0.5% (w/v) aqueous polysorbate 80

3. Test animals:

Species:	Rat
Strain:	Wistar (Alpk:APfSD), SPF
Source:	Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park Macclesfield, Cheshire, UK
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.), *ad libitum* except for approx. 24 h before dosing
Water:	Tap water, *ad libitum*
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

B: STUDY DESIGN AND METHODS

In life dates: No date given in the report.

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. 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.

C. 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.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The oral LD_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/03	[redacted]	2002a	Acute Toxicity Study of AMPA (Aminomethyl Phosphonic Acid) in CD Rats by Dermal Administration – LIMIT TEST [redacted] Data owner: Feinchemie Schwebda GmbH Report No.: 16168/02 Date: 2002-12-03 GLP: yes not published

Guideline:	OECD 402 (1987), EEC B.3 (1992)
Deviations:	None
Dates of experimental work:	2002-10-21 to 2002-11-01

Executive Summary
The test substance, Aminomethyl Phosphonic Acid (AMPA), was evaluated for its acute dermal toxicity potential in CD rats when administered at a level of 2000 mg/kg bw. No mortality occurred during the study. Neither clinical signs nor macroscopic lesions at necropsy were observed in any animals. All animals gained body weights 7 and 14 days after administration when compared with the body weights on the day of administration. The acute dermal LD_{50} was calculated to be

LD_{50}, dermal, rat > 2000 mg/kg bw

According to EU and OECD Globally Harmonized System (GHS) classification criteria the test substance AMPA is not to be classified for acute dermal toxicity.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: 0.5% aqueous hydroxypropylmethyl cellulose gel

3. Test animals:

Species:	Rat
Strain:	CD / Crl:CD
Source:	Charles River Deutschland, Sulzfeld, Germany
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 dosing
Water:	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

B: STUDY DESIGN AND METHODS

In life dates: 2002-10-21 to 2002-11-01

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
There were no mortalities during the study.

B. CLINICAL OBSERVATIONS
No clinical signs were observed during the study.

C. BODY WEIGHT
No body weight losses were recorded in any animal 7 and 14 days after the administration.

D. NECROPSY
The gross necropsy conducted at termination of the study noted no observable abnormalities.

III. CONCLUSION

The dermal LD_{50} 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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/04	[redacted]	2002b	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 GLP: yes unpublished

Guideline:	OECD 406 (1992); EEC B.6 (1996)
Deviations:	None
Dates of experimental work:	2002-10-12 to 2002-11-26

Executive Summary

AMPA (Aminomethyl Phosphonic Acid) was tested for its sensitizing effect on the skin of the guinea pig in the Maximisation Test. The test-substance concentrations for the main test were selected based on the results of the pre-test. The intradermal induction was performed with a 5% dilution of the test item in purified water and an emulsion of Freund's Complete Adjuvant (FCA)/purified water. The epidermal induction was conducted for 48 h under occlusion with the test item at 50% one week after the intradermal induction.

Two weeks after induction the animals were challenged by epidermal application of the test item at 50% under occlusive dressing. The study was performed using a control group consisting of five animals, one test group consisting of ten animals and a positive control group consisting of 20 animals.

None of the vehicle control or test animals exhibited a positive skin reaction (defined as scores of ≥ 1) after the challenge treatment. 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).

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.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Purified water

3. Test animals:

Species:	Guinea pig
Strain:	Dunkin Hartley
Source:	Charles River Laboratories GmbH, Kißlegg, Germany
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), *ad libitum*
Water:	Tap water, *ad libitum*
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

B: STUDY DESIGN AND METHODS

In life dates: 2002-10-12 to 2002-11-26

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.

II. RESULTS AND DISCUSSION

A. MORTALITY
No deaths occurred.

B. CLINICAL OBSERVATIONS
No signs of systemic toxicity were observed.

C. BODY WEIGHT
All animals showed the expected gain in body weight.

D. 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).

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/05	Callander, R.D.	1988	Aminomethyl Phosphonic Acid: An Evaluation of Mutagenic Potential Using *S. Typhimurium* and *E.Coli* ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK Data owner: Syngenta Report No.: CTL/P/2206 Date: 1988-09-21 GLP: yes not published

Guideline:	Comparable to OECD 471 (1997): OPPTS 870.5100 (1998): 2000/32/EEC B.13/B.14 (2000)
Deviations:	None
Dates of experimental work:	1988-03-01 to 1988-09-21

Excecutive summary
In a reverse gene mutation, plate incorporation assay in bacteria (***Maron and Ames, 1983***), five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2 *uvrA* pKM101) were exposed to aminomethyl phosphonic acid.

In at least two separate experiments, the compound did not induce any significant, reproducible increase in the observed numbers of revertant colonies in any of the tester strains used, either in the presence or absence of an auxiliary metabolising system (S9). Although slight effects were observed in strain TA1537 in the first experiment, these were not reproducible in two further experiments with this strain. In each experiment, the positive controls responded as expected, indicating that the assay was working satisfactorily.

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.

I. MATERIALS AND METHODS

A: MATERIALS:

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 (final concentration): Dimethylsulphoxide – DMSO (10 µL/plate)

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-Aminothracene 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: Na_2HPO_4 (150 mM), KCl (49.5 mM), glucose-6-phosphate (7.5 mM), NADP (Na salt) (6 mM) and $MgCl_2$ (12 mM).

Test organisms:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537	X	TA1538		list any others		

E. coli 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.

B: STUDY DESIGN AND METHODS:

In-life date: Start: 10 May 1988 End: 23 May 1988

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 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.

REPORTED 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/06	Mie, A.	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 GLP: yes not published

Guideline:	U.S. EPA FIFRA Guidelines, Subdivision F OECD guidelines 471, 472 (1983) Japan MAFF guidelines 59 NohSan N° 4200 (1985)
Deviations:	None
Dates of experimental work:	1996-09-09 to 1996-10-11

Executive Summary

Reverse mutation tests were performed on AMPA in *Escherichia coli* WP2 uvrA and four tester strains of *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537). Experiments were carried out with and without metabolic activation system (S9 mix) at dose levels up to 5000 µg/plate. The mean number of revertant colonies did not exceed the factor of 2 above that of the corresponding solvent control in any strain at any dose with or without S9 mix.

Based on the results, AMPA is non-mutagenic to bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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 inDMSO; NaN_3: sodium azide dissolved in sterile water
2-AA: 2-aminoanthracene dissolved in DMSO; 9-AA: 9-aminoacridine hydrochloride dissolved in sterile water

3. 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.

4. test organisms: *Escherichia coli* WP2 uvrA
Salmonella typhimurium (TA100, TA1535, TA98 and TA1537)

5. test concentrations:

(c) 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.

(d) 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.

Re-tests: Not concerned

B: TEST PERFORMANCE

1. Preliminary cytotoxicity/plate incorporation mutation assay

Results of the range-finding are presented in table below.

Table 5.8-2: preliminary dose range finding test

		Concentration (µg/plate)	Revertants (n° colonies/plate) TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix		Solvent Control (H_2O)	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_2O)	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	0.1	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*

AMPA did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

2. Pre-incubation 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.

3. Statistics

Results were judged without statistical analysis.

4. 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:

1. A two-fold or greater increase above solvent control in the mean number of revertants is observed
2. This increase in the number of revertants is accompanied by a dose-response relationship
3. This increase in the number of revertants is reproducible.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. PRELIMINARY CYTOTOXICITY ASSAY

AMPA did not show any toxicity to any strain up to the highest dose of 5000 µg/plate with and without S9 Mix.

C. MUTATION ASSAYS

Results are shown in table hereafter

Table 5.8-3: Reverse mutation tests without metabolic activation– Experiment 1

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	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 ± SD

Table 5.8-4: Reverse mutation tests with metabolic activation– Experiment 1

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	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 5.8-5: Reverse mutation tests without metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
- S9 mix	Solvent Control (H_2O)	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 5.8-6: Reverse mutation tests with metabolic activation– Experiment 2

	Concentration (µg/plate)	Revertants (n° colonies/plate) *				
		TA100	TA1535	WP2 *uvr A*	TA98	TA1537
+ S9 mix	Solvent Control (H_2O)	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

III. CONCLUSIONS

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.

Annex point	Author(s)	Year	Study title
IIA, 5.8/07	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 GLP: yes not published

Guideline:	OECD guideline n° 482
Deviations:	None
Dates of experimental work:	2002-04-29 to 2002-07-02

Executive Summary
AMPA was examined for mutagenic potential by measuring its ability to induce Unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

Cytotoxicity of AMPA was estimated in a pre screen by exposing cells to 8 concentrations of AMPA for determination of survival. From these data a range of 5 test concentrations 0 (solvent control), 0.625, 1.25, 2.5, 5 and 10 µg/ml are selected for UDS analysis procedure.
2-acetamidofluorene prepared in DMSO was used as positive control.

Based on the results, AMPA did not have DNA-damaging activity in the bacteria.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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-acetamidofluprene

3. activation: none

4. test organisms: Rats hepatocytes

5. test concentrations: 5 dose level were tested: 10, 5, 2.5, 1.25 and 0.625 mM with and without S9 metabolic activation

B: TEST PERFORMANCE

1. 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 occuring. 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

2. Statistics

Results were judged without statistical analysis.

3. 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.

4. 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.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

None

B. 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 summarized in the tables below.

Table 5.8-7: UDS data of the first experiment

Concentration (µg/ml)	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 5.8-8: UDS data of the second experiment

Concentration (µg/ml)	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
	-4.62	5.81	5.37	0.21	3.74	2.08
	-4.77	5.46	6.90	0.60	1.78	0.00
	-4.04	5.35	6.87	1.00	4.49	1.53
Solvent control	-3.81	5.82	7.07	1.61	6.18	2.08
0.625	-3.47	5.83	7.83	0.29	7.73	1.73
1.25	-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 (Tables 5.8.1.1-1 and 5.8.1.1-2), group mean net nuclear grain count (NNG) values at the dose range tested from 10 to 6.25 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 valure 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 ta 10 mM to 6.90 at 0.625 mM vs. 5.37 in control in the second assay).

III. CONCLUSIONS

Under the conditions of this experiment, **AMPA did not reveal any genotoxicity activity** in the Unscheduled DNA synthesis assay.

IIA 5.9 Medical data

IIA 5.9.1 Report on medical surveillance on manufacturing plant personnel

Monsanto Glyphosate Manufacturing Industrial Hygiene Monitoring Data, Luling, Louisiana, USA

Industrial hygiene air monitoring data for glyphosate with workers at the Monsanto Luling, Louisiana manufacturing facility are available for the years 1981-1998 and are presented below. 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 is not considered necessary. These data are air concentration measurements which are conservatively applied as 100% bioavailable to calculations of mean and maximum daily exposures.

Table 5.9-1: 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 1	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 4	0.00034	0.00088
Lab	2	0.0003-0.0004	0.00035	N/A	0.00006	0.00007

* based on breathing 10 m^3 air/shift and 60 kg worker

Table 5.9-2: 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 m^3 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.

IIA 5.9.2 Report on clinical cases and poisoning incidents

See IIA 5.9.4. Clinical cases and poisoning incidents are referenced in order to address the clinical signs and symptoms of poisoning.

IIA 5.9.3 Observations on exposure of the general population and epidemiological studies

Please refer to the literature review, captured in section IIA 5.10 in regards to epidemiologic studies.

IIA 5.9.4 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 (Bradberry et al 2004), and a review of cases with assessment of clinical prognostic factors was more recently published (Lee et al. 2008).

GENERAL:
Glyphosate does not inhibit cholinesterase, and has no cholinergic effect. Animals do not have the shikimic acid pathway; and no direct target-mediated action in mammalian systems has been clearly identified to date (Bradberry et al. 2004). 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 (Goldstein et al. 2002).

CLASSIFICATION OF EXPOSURES:
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 is minor or significant include:

- The 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.
- The 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.
- The 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.
- Clinical condition of the patient.
- Known or suspected co-ingestants (if any).
- Professional judgment.

ROUTE AND ORGAN SYSTEM SPECIFIC SYMPTOMS OF EXPOSURE:

DERMAL
MINOR EXPOSURES:
- Contact with skin may produce a dermatitis similar to that of detergents (Bradberry et al. 2004)
- 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. This is believed due to an antimicrobial additive (benzisothiazolone) which is present in selected residential use (i.e. non- agricultural) products containing 10% glyphosate or less (Bradberry et al. 2004).

- Significant absorption through the skin does not occur (<0.2% for concentrates and <0.01% for dilute formulations; see section 5.9.9)
- Studies in farmers and farm family members during the machine spray application of glyphosate products indicates that farmer exposure is generally far below recommended maximal daily intakes and that urinary levels in children and spouses are largely non-detectable (limit of urinary detection 1 µg/L) (Acquavella et al. 2004). These studies do not provide a quantitative measure of dermal exposure, but are consistent with the primate data noted above.

SIGNIFICANT EXPOSURES:

- Skin exposures are not expected to cause systemic effects or serious cutaneous effects. 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 (Acquavella et al. 1999).
- 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" (Bradberry 2004).
- 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 (Acquavella et al. 1999; Bradberry et al. et al. 2004).

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 (Tominack 1989); however "moderate disorders of consciousness" have been reported within 48 hours of suicidal ingestions of the concentrate (Sawada and Nagai 1987; Sawada et al. 1988). This has occurred in patients with significant systemic illness and is not believed to be the result of reduced organ perfusion (Bradberry et al. 2004) or perhaps other factors such as metabolic disturbance, but the possibility of a direct toxicological effect cannot be excluded (Bradberry et al. 2004).
- There are two isolated case report of Parkinson's disease developing in individuals with a history of glyphosate product exposure. In one case, Parkinson's disease of relatively acute onset was diagnosed 6 months following incidental dermal exposure to a glyphosate-surfactant product (Barbosa et al. 2001). It appears that the same case was reported as part of a case series by daCosta et at (2003) [Similar list of authors on both publications, case descriptions and ages match (52 years old at diagnosis vs 54 year old with a 2 year history of Parkinsons) and the T2- weighted Axial MRI images shown appear to be identical]. The second case (Wang et al. 2011) reports the development of Parkinson's of a 44 year old woman who had been employed in a glyphosate manufacturing facility. In both instances, there is no evidence for causation other than a history of prior exposure. No other human or animal data support the contention that Parkinson's disease results from exposure to glyphosate, even following massive ingestion or prolonged exposure.

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 (Tominack et al. 1989).
- 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. Transmural injury and perforation have not been noted on panendoscopy (Chang et al. 1999).
- 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 (Tominack et al. 1989).
- Clinical, autopsy and experimental evidence (Mizuyama 1987) indicate a potential for gastrointestinal damage from glyphosate components of glyphosate formulations, but the frequency of severe injury appears to be low and early endoscopy is probably not indicated (see below).

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 from minor exposures.

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. (Tominack et al. 1989, Bradberry et al. 2004). 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 (Bradberry et al. 2004).

SIGNIFICANT EXPOSURES:

- Significant systemic exposures are not anticipated to occur via the inhalational route, see minor exposures within this subheading.

LOWER RESPIRATORY:

MINOR EXPOSURES:

- Because of the non-volatile nature of glyphosate and the surfactant, there are no vapour exposures possible. The spray equipment commonly used with the product produces 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 appear to be the result 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 (Pushnoy et al. 1998). However, the registrants do not believe that a credible mechanism of exposure was documented in this case, and the occurrence of pneumonitis in this individual was more likely coincidental in nature (Goldstein et al. 1999).
- There is also a case report out of Germany in which a glyphosate-surfactant product (tallowamine or "POEA" based) was applied by knapsack spayer in a 0.5ha forestry application at the registered application rate at 25° C for approximately 3 hours. 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" (significantly different than bacterial infection). After 7-days of drug treatments, changes in lung reversed. Six months after the incident the patient still experienced moderate respiratory complaints on exertion. In the X-ray findings lungs showed improved results, but still detectable changes. While it is possible to differentiate acute bacterial infections on histopathology (microorganisms and polymorphonuclear leucocytic inflammatory changes should be visible), characteristics of viral, mycoplasmal, or autoimmune (vasculitic, Wegoner's granulomatosis) induced pneumonitis or Bronchiolitis Obliterans with Organizing Pneumonia (BOOP, which closely mirrors the limited case information available) are not clinically distinguishable from "toxic" etiologies. Many cases occur, most being idiopathic (no identifiable cause). Agricultural aerosols are far larger than 10 microns (generally 200 microns or so in size) and not respirable to lung, and POEA is not volatile. Contrary to this isolated case, backpack applications of glyphosate-surfactant products occur regularly in forestry and in agriculture in the developing world, without known occurrence of serious lower respiratory disease.

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 from minor exposures.

SIGNIFICANT EXPOSURES:

- Hypotension and hypovolemic shock may result in oliguria and anuria, following severe ingestions (Bradberry et al. 2004). 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.

SIGNIFICANT EXPOSURES:

- Mild fever may be noted even in the absence of infection (Bradberry et al. 2004)
- Metabolic acidosis is often seen in a severely poisoned patient (Bradberry et al. 2004) and the acidosis may fail to respond to bicarbonate therapy. Although the exact cause of the acidosis is unknown, a lactic acidosis is 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.
- SIGNIFICANT EXPOSURES:
- Leukocytosis without evidence of bacterial infection has been noted in peripheral blood after ingestion of the concentrate (Bradberry et al. 2004).
- Hemoconcentration has been seen as a result of intravascular volume depletion (possibly indicating severe capillary fluid leakage) (Tominack et al. 1989).
- No primary toxic effects on bone marrow or formed elements have been seen 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 minor exposures.

SIGNIFICANT EXPOSURES:

- No direct hepatotoxic effects have been noted; however, minor elevations in transaminases and bilirubin are reported (Tominack et al. 1989; Bradberry et al. 2004).

ELECTROLYTES:

MINOR EXPOSURES:

- Severe or prolonged vomiting and diarrhoea may induce fluid and electrolyte imbalance. This degree of illness is not generally expected from 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 do indicate that with large ingestions of glyphosate-potassium salt concentrate solutions, clinically significant hyperkalemia may occur. Bando et al (2001) report 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 an attempt at suicide. 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.
- Kamijo et al (2012) report 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.

- Monsanto is aware of one additional inquiry (unpublished) of a similar ingestion with a 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 that the use of postassium 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 are generally not available in a time frame 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 concentrations of glyphosate will generally not impair clinical care. Levels may be helpful in addressing forensic issues following clinical recovery or in the event of a fatality of unclear cause.

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 (Lee 2008). These authors also developed a prognostic index based upon these factors. The use of prognostic criteria does not appear to add significantly to patient care. As symptom onset may be delayed, early use of such prognostic indicators may lead to an under-estimate of clinical severity.

IIA 5.9.5 First aid measures

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).

IIA 5.9.6 Therapeutic regimes

The registrants believe that the following represent general best practices for medical management of serious ingestions of glyphosate-surfactant products.

1. Establish respiration and assure adequacy of ventilation.

2. Eye exposure:
   A) Remove contact lens from the affected eye(s) if appropriate.
   B) 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.
   C) 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.

3. Ingestion exposure:
   A) 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.
   B) patient disposition:
      Concentrated preparations (Glyphosate 41% or greater):
      1) 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.
      2) 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.
      3) 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.

4. 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.)

A) 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.

B) 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.

C) 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.

5. 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). 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 hospitalization 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.

6. Monitor blood pressure:

Monitor the patient closely for signs of hemodynamic instability. The insertion of a Swan-Ganz catheter may be warranted.

7. 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, isoproterinol, etc.) if needed.

8. 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.

9. 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.

10. 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.

11. 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.

12. Monitor renal function closely:

Assure adequate urine output. Catheterize severely ill patients. Hemodialysis may be needed in the event of renal failure or electrolyte disturbances.

11. Enhanced elimination:

A) 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.
B) 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.

12. 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.

13. 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.

14. 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.

IIA 5.9.7 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 can require a more extended and highly variable time to recover.

IIA 5.9.8 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. The availability (or lack) of acute field management does not appear likely to impact severity of survival of most serious ingestions.

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IIA 5.9.9 Dermal penetration

In the 2001 EU glyphosate evaluation, dermal absorption of glyphosate was considered to be less than 3%. This value based on *in vivo* data in Rhesus monkeys, as well as on *in vitro* data in human skin, using the original glyphosate formulation Roundup ([redacted] 1991). *In vivo* dermal absorption in Rhesus monkeys ranged from 0.8 – 2.2% of applied dose ([redacted]., 1991; overall recovery was low with approx. 75-80%), whereas the *in vitro* penetration through human skin was at maximum 0.152% (Franz, 1983) and ≤ 2.2% (published data). Wester et al. (1991) *in vivo* studies in rhesus monkeys demonstrated very good mass balance for both oral and *intra venous* studies, which support almost complete urinary excretion of systemic doses. However, the dermal *in vivo* study in [redacted] (1991) demonstrated poor mass balance and the high dermal dose result showed an increase in faecal excretion (3.6% of dose); this contradicts the *intvra venous* high dose results with 98.8% excretion of the systemic dose in urine. Given the dermal study design with monkeys yielded poor mass balance, the results should not be considered to accurately represent dermal absorption for at least two reasons; (i) flaking and rubbing of unocculded application application sites of the highly stressed animals fully restrained for 12 hours, then unrestrained in metabolism cages; and (ii) given the very low dermal absorption of glyphosate, possible hand to mouth activity of monkeys with even very small oral exposures would confound the excretion profile for the dermal doses. Therefore, more value should be ascribed to modern *in vitro* studies using human skin for assigning dermal absorption values.

Since the last evaluation some new *in vitro* studies have been performed by individual task force members with glyphosate formulations containing glyphosate at 360 g/L, 450 g/L and 480 g/L (see Table 5.9-3). All studies were performed according to GLP and to the current OECD guideline 428 (2004). The tested concentrations correspond to the formulation concentrates, as well as typical in-use dilutions. As can be seen from the results, the dermal penetration through human skin is limited, with maximum values of 0.086%, 0.059% and 0.166% for the 360 g/L, 450 g/L and 480 g/L concentrates, respectively. The absorption values for the in-use dilutions are also very low ranging from 0.169% to 0.88%.

The new data below indicated that the dermal absorption of glyphosate through human skin is clearly below 1%, irrespective of glyphosate salt form (potassium, isopropylamine and ammonium salts) and surfactant type.

The low penetration potential is further supported by all five repeat dose dermal toxicity studies performed in rats and rabbits (see Table 5.3-51). In these repeated dose *in vivo* dermal toxicity studies, no signs of systemic toxicity were observed up to 5000 mg/kg/day.

Table 5.9-3: Summary of Results for Dermal Absorption of ^{14}C-Glyphosate - SL Formulation

Reference (Data owner)		Content of glyphosate acid (g/L)	Study design according to guideline / exposure duration	Tested concentrations (actual) (g glyphosate/L)	Mean % of applied dose potentially biologically available* (%)
Studies not reviewed in the 2001 evaluation	[redacted] 2010 (IIIA 7.6.2/01) (MON)	360	OECD 428 24 h exposure	360 29.6 2.51	0.086 0.169 0.342
	[redacted] 2010a (MON)	450	OECD 428 24 h exposure	459 29.3 2.49	0.059 0.821 0.302
	[redacted] 2010b (MON)	480	OECD 428 24 h exposure	491 30.4 3.19	0.166 0.267 0.824
	[redacted] (2012b) (NUF)	360	OECD 428 8 h exposure	366 2.72	0.155 0.663
	[redacted] 2003 (SYN)	360	OECD 428 6 h exposure	364 6.7	0.06 0.24
			OECD 428 24 h exposure	364 6.7	0.07 0.88

* Potentially biological available = amount in receptor fluid + amount in remaining skin
Only the first two tape strips (considered as stratum corneum) were excluded for calculation of potentially available dose.

Annex point	Author(s)	Year	Study title
IIA, 5.9.9/01	Ward, R.J.	2010a	450 g/L Glyphosate SL Formulation (MON 79545) – *In vitro* Absorption of Glyphosate Through Human Epidermis Dermal Technology Laboratory Ltd., Keele, Staffordshire, ST5 5NL, UK Data Owner: Monsanto Monsanto Report No.: DTL-09-093 Date: 2010-02-19 GLP: yes unpublished

Guideline: OECD 428

Deviations: None

Dates of experimental work: 2009-05-26 to 2009-06-02

Executive Summary

The objective of this study was to evaluate the potential dermal absorption of glyphosate from a 450 g/L SL formulation concentrate, as well as from two representative in-use dilutions, prepared as 1:15.6 (v/v) and 1:188 (v/v) aqueous dilutions.

^{14}C-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/cm^2 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).

The total amounts absorbed after 24 hours were 0.012, 0.129, and 0.082% of the applied doses for the concentrate, 1:15.6 (v/v) dilution, and 1:188 (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 0.049, 0.796, and 0.245%, respectively.

Conclusion
The results of this *in vitro* study indicate the dermal absorption of glyphosate through human skin is very slow, and that the vast majority of glyphosate will be washed off during normal washing procedures. The results predict that the dermal absorption of glyphosate from potential exposure to this 450 g glyphosate/L SL formulation (MON 79545) would be less than 1%.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

a) Non radio-labelled test substance:

Identification:	Potassium salt of glyphosate technical material (glyphosate-potassium)
Description:	Clear, colourless to pale yellow liquid (solution in water)
Lot/Batch #:	A9B50041K0
Chemical purity:	Glyphosate-potassium: 59.17% Glyphosate acid: 48.29%
Stability of test compound:	Expiry date: 2011-09-10

b) Analytical reference standard:

Identification:	Glyphosate analytical reference standard (glyphosate acid)
Description:	White solid
Lot/Batch #:	GLP-0810-19515-A
Chemical purity:	99.8 %
Stability of test compound:	Expiry date: 2011-01-31

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid)
Lot/Batch #:	53463-3-23
Chemical purity:	Not reported
Radiochemical purity:	99.8% (by HPLC from supplier) 97.5 % (confirmed by re-analysis, 2009-05-26)
Specific activity:	47 mCi/mmol; 1739 MBq/mmol; 277.9 µCi/mg; 10.28 MBq/mg
Stability of test compound:	Not reported

c) Blank formulation

Identification:	Proprietary surfactant blend
Concentration of a.i.:	0%
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Confidential
Stability of test compound:	Not reported

d) Formulated test substance

Identification: MON 79545

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.

2. Test skin source:

Species: Human

Source: Tissue bank (not further specified)

Age: Not reported

Sex: Not reported

Type of skin: Not reported

B: STUDY DESIGN AND METHODS

Preparation of skin samples:
Human skin samples 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 ºC, 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 450, 28.8, and 2.4 g glyphosate/L. The nominal radioactivity contained in the dose preparations was 3.3 MBq.

Radioactive stocksolution of ^{14}C-glyphosate
Dry ^{14}C-glyphosate was solubilised in 2 mL of water and mixed thoroughly.

High dose (formulation concentrate, 459 g/L)
A pre-mix was prepared by mixing 4750 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) of the radioactive stock solution was mixed with 519.9 mg of the pre-mix. Water was added to give a total weight of 654 mg. The solution was mixed well. Assuming a density of 1.308 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 459 g glyphosate/L.

Intermediate dose (1:15.6 [v/v] aqueous dilution, 29.3 g/L)
A pre-mix was prepared by mixing 298.7 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) of the radioactive stock solution was mixed with 32.73 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 29.3 g glyphosate/L.

Low dose (1:188 [v/v] aqueous dilution, 2.50 g/L)
A pre-mix was prepared by mixing 74.7 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) of the radioactive stock solution was mixed with 2.18 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 2.50 g glyphosate/L.

Analyses of dose preparations
The radioactivity content of the ^{14}C-glyphosate 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 was measured by HPLC analyses. The formulated ^{14}C-glyphosate was shown to be stable for 24 hours, the duration of the exposure period, in a previous study.

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 across the skin membranes. Membranes with a resistance of $\geq$ 10 kΩ 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/cm^2 exposed skin area (25.4 µL dose), corresponding to target concentration of 4589, 293, and 25.0 µg/cm^2 for the high, intermediate, and low dose level, respectively. The applications were left unoccluded for 24 hours.

Receptor fluid samples (500 µL) were taken by an autosampler 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.

To assess penetration through the stratum corneum, successive layers of the skin surface were removed by the repeated application of adhesive tape (Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips. Each strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove layers of stratum corneum. The adhesive 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 5 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/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/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.

II. RESULTS AND DISCUSSION

A. ANALYSES OF UNFORMULATED ^{14}C-GLYPHOSATE
HPLC analysis of the unformulated sample of ^{14}C-glyphosate confirmed a radiochemical purity of 97.5%.

B. ANALYSES OF DOSE PREPARATIONS
The achieved concentrations of glyphosate in the dose preparations were calculated to be 458.9, 29.3 and 2.50 g glyphosate/L in the formulation concentrate, 1:15.6 (v/v) dilution, and 1:188 (v/v) dilution, respectively. LCS analyses confirmed the dose solutions to be homogeneous.

C. DERMAL ABSORPTION OF GLYPHOSATE
The determined distribution of radioactivity for the different dose groups are summarised in Table 5.9-4 below.

Table 5.9-4: Summary of Results for Dermal Absorption of ^{14}C-Glyphosate - SL Formulation

Dose preparation	**High (concentrate)**		**Intermediate (1:15.6 [v/v] dilution)**		**Low (1:188 [v/v] dilution)**	
Nominal concentration [g/L]	459		29.3		2.50	
Actual concentration [g/L]	458.9		29.3		2.50	
Applied dose [µL/cm^2]	10		10		10	
Applied dose [µg/cm^2]	4589		293		25.0	
Number of cells accessed	4*		5*		6	
	Distribution of radioactivity (mean values)					
	µg/cm^2	**% of applied dose**	**µg/cm^2**	**% of applied dose**	**µg/cm^2**	**% of applied dose**
Surface compartment						
Stratum corneum (tape strips)	1.25	0.027	0.254	0.087	0.050	0.201
Skin wash	4647	101	303	103	26.2	105
Donor chamber	2.64	0.057	0.806	0.275	0.092	0.369
Receptor compartment						
Receptor fluid (0-24 h)	0.573	0.012	0.379	0.129	0.021	0.082
Total absorbed	**0.573**	**0.012**	**0.379**	**0.129**	**0.021**	**0.082**
Remaining epidermis	1.70	0.037	1.95	0.666	0.040	0.163
Total potentially absorbable**	**2.27**	**0.049**	**2.33**	**0.796**	**0.061**	**0.245**
Total recovery	**4653**	**101**	**307**	**105**	**26.4**	**106**
Absorption rates [µg/cm^2/h] (0-24h)	0.024		0.016		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

The overall total recovery for the three dose levels was good, with mean values of 101-106% of the applied dose.

Glyphosate absorption from the 450 g/L concentrate formulation was essentially constant over the entire 24 hour exposure period (mean rate = 0.024 µg/cm^2/h). By the end of the exposure period, the mean total amount of absorbed glyphosate was 0.573 µg/cm^2 (0.012% of applied dose).

From the 1:15.6 (v/v) and 1:188 (v/v) aqueous dilutions of the formulation, absorption was also essentially constant over the entire 24 hour exposure period (mean rates = 0.016 and 0.001 µg/cm^2/h, respectively). At the end of the exposure period, the mean total amounts of absorbed glyphosate were 0.379 and 0.021 µg/cm^2 (0.129 and 0.082% 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 101 - 105%). The mean total amount of glyphosate recovered from the epidermis (stratum corneum + remaining epidermis after tape stripping) was 0.064, 0.753, and 0.364% of the applied dose (concentrate, 1:15.6 [v/v] dilution, and 1:188 [v/v] dilution, respectively). The mean absorbed amounts were 0.012, 0.129, and 0.082% of applied dose, respectively. The amount of potentially biologically available glyphosate (absorbed + epidermis after tape striping) for the concentrate, 1:15.6 (v/v) and 1:188 (v/v) dilutions were 0.049, 0.796, and 0.245% respectively.

III. CONCLUSION

The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through human skin is 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 0.012, 0.129, and 0.082% of the applied dose for the formulation concentrate, the 1:15.6 (v/v) dilution, and 1:188 (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 0.049, 0.796, and 0.245%, respectively. These data predict that the dermal absorption of glyphosate from potential exposure to this 450 g glyphosate /L SL formulation (MON 79545) would be minimal, at less than 1% of any potential dermal exposure.

Annex point	Author(s)	Year	Study title
IIA, 5.9.9/02	Ward, R.J.	2010b	480 g/L Glyphosate SL Formulation (MON 79351) – *In vitro* Absorption of Glyphosate Through Human Epidermis Dermal Technology Laboratory Ltd., Keele, Staffordshire, ST5 5NL, UK Data Owner: Monsanto Monsanto Report No.: DTL-09-095 Date: 2010-02-19 GLP: yes unpublished

Guideline:	OECD 428
Deviations:	None
Dates of experimental work:	2009-06-15 to 2009-08-26

Executive Summary

The objective of this study was to evaluate the potential dermal absorption of glyphosate from a 480 g/L SL formulation concentrate, as well as from two representative in-use dilutions prepared as 1:16.7 (v/v) and 1:200 (v/v) aqueous dilutions.

^{14}C-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/cm^2 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).

The total absorbed amounts after 24 hour exposure were 0.007, 0.182, and 0.048% of the applied dose for the formulation concentrate, the 1:16.7 (v/v) dilution, and 1:200 (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 0.123, 0.262, and 0.799%, respectively.

Conclusion

The results of this *in vitro* study indicate the dermal absorption of glyphosate through human skin is very slow, and that the vast majority of glyphosate will be washed off during normal washing procedures. The results predict that the dermal absorption of glyphosate from potential exposure to this 480 g/L glyphosate SL formulation would be less than 1%.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

a) Non radio-labelled test substance:

Identification:	Potassium salt of glyphosate technical material (glyphosate-potassium)
Description:	Clear, colourless to pale yellow liquid (solution in water)
Lot/Batch #:	A9B50041K0
Chemical purity:	Glyphosate-potassium: 59.17% Glyphosate acid: 47.28%
Stability of test compound:	Expiry date: 2011-09-10

b) Analytical reference standard:

Identification:	Glyphosate analytical reference standard (glyphosate acid)
Description:	White solid
Lot/Batch #:	GLP-0810-19515-A
Chemical purity:	99.8 %
Stability of test compound:	Expiry date: 2011-01-31

c) Radio-labelled test substance

Identification:	^{14}C-glyphosate (as glyphosate acid)
Lot/Batch #:	53463-3-23
Chemical purity:	Not reported
Radiochemical purity:	99.8% (by HPLC from supplier) 97.6 % (confirmed by re-analysis, 2009-06-14)
Specific activity:	47 mCi/mmol; 1739 MBq/mmol; 277.9 µCi/mg; 10.28 MBq/mg
Stability of test compound:	Not reported

c) Blank formulation

Identification:	Proprietary surfactant blend
Concentration of a.i.:	0%
Description:	Not reported
Lot/Batch #:	Not reported
Purity:	Confidential
Stability of test compound:	Not reported

d) Formulated test substance

Identification:	MON 79351 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.

2. Test skin source:

Species:	Human
Source:	Tissue bank (not further specified)
Age:	Not reported

Sex: Not reported
Type of skin: Not reported

B: STUDY DESIGN AND METHODS

Preparation of skin samples:
Human skin samples 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 ºC, 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 480, 28.7, and 2.4 g glyphosate/L. The nominal radioactivity contained in the dose preparations was 3.3 MBq.

Radioactive stocksolution of ^{14}C-glyphosate
Dry ^{14}C-glyphosate was solubilised in 2 mL of water and mixed thoroughly.

High dose (formulation concentrate, 491 g/L)
A pre-mix was prepared by mixing 5067.02 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) (≡ 78 mg) of the radioactive stock solution was mixed with 592.16 mg of the pre-mix. Water was added to give a total weight of 670 mg. The solution was mixed well. Assuming a density of 1.34 g/mL, the total weight was equivalent of 0.5 mL at a nominal concentration of 491 g glyphosate/L.

Intermediate dose (1:16.7 [v/v] aqueous dilution, 30.4 g/L)
A pre-mix was prepared by mixing 303.47 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) (≡ 78 mg) of the radioactive stock solution was mixed with 36.00 mg of the pre-mix. Water was added to give a total weight of 501 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 30.4 g glyphosate/L.

Low dose (1:200 [v/v] aqueous dilution, 3.19 g/L)
A pre-mix was prepared by mixing 101.41 mg glyphosate-potassium technical material with an appropriate amount of proprietary surfactant blend. Seventy eight microliters (78 µL) (≡ 78 mg) of the radioactive stock solution was mixed with 3.09 mg of the pre-mix. Water was added to give a total weight of 501 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 3.19 g glyphosate/L.

Analyses of dose preparations
The radioactivity content of the ^{14}C-glyphosate 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 was measured by HPLC analyses. The formulated ^{14}C-glyphosate was shown to be stable for 24 hours, the duration of the exposure period, in a previous study.

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 across the skin membranes. Membranes with a resistance of ≥ 10 kΩ 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/cm^2 exposed skin area (25.4 µL dose), corresponding to target concentration of 4906, 304, and 32.0 µg/cm^2 for the high, intermediate, and low dose level, respectively. The applications were left unoccluded for 24 hours.

Receptor fluid samples (500 µL) were taken by an autosampler 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.

To assess penetration through the stratum corneum, successive layers of the skin surface were removed by the repeated application of adhesive tape (Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips. Each strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove layers of stratum corneum. The adhesive 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 5 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/cm^2. The amounts absorbed, rates of absorption (µg/cm^2/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.

II. RESULTS AND DISCUSSION

A. ANALYSES OF UNFORMULATED ^{14}C-GLYPHOSATE

HPLC analysis of the unformulated sample of ^{14}C-glyphosate confirmed a radiochemical purity of 97.6%.

B. ANALYSES OF DOSE PREPARATIONS

The achieved concentrations of glyphosate in the dose preparations were calculated to be 490.6, 30.4, and 3.20 g glyphosate /L in the formulation concentrate, 1:16.7 (v/v) dilution, and 1:200 (v/v) dilution, respectively. LCS analyses confirmed the dose solutions to be homogeneous.

C. DERMAL ABSORPTION OF GLYPHOSATE

The determined distribution of radioactivity for the different dose groups are summarised in Table 5.9-5 below.

Table 5.9-5: Summary of results for dermal absorption of ^{14}C-glyphosate - SL formulation

Dose preparation	**High (concentrate)**		**Intermediate (1:16.7 [v/v] dilution)**		**Low (1:200 [v/v] dilution)**	
Nominal concentration [g/L]	491		30.4		3.19	
Actual concentration [g/L]	490.6		30.4		3.20	
Applied dose [µL/cm^2]	10		10		10	
Applied dose [µg/cm^2]	4906		304		32.0	
Number of cells accessed	4*		4*		5	
	Distribution of radioactivity (mean values)					
	µg/cm^2	**% of applied dose**	**µg/cm^2**	**% of applied dose**	**µg/cm^2**	**% of applied dose**
Surface compartment						
Stratum corneum (tape strips)	6.85	0.140	0.061	0.020	0.045	0.139
Skin wash	4920	100	303	99.7	31.7	99.0
Donor chamber	53.9	1.01	2.72	0.894	<LOQ**	<LOQ**
Receptor compartment						
Receptor fluid (0-24 h)	0.342	0.007	0.553	0.182	0.015	0.048
Total absorbed	**0.342**	**0.007**	**0.553**	**0.182**	**0.015**	**0.048**
Remaining epidermis	5.70	0.116	0.244	0.080	0.241	0.752
Total potentially absorbable* **	**6.04**	**0.123**	**0.797**	**0.262**	**0.256**	**0.799**
Total recovery	**4987**	**102**	**307**	**101**	**32.0**	**100**
Absorption rates [µg/cm^2/h]	0.011 (0-10 h) 0.016 (10-24 h) 0.014 (0-24 h)		0.134 (0-1 h) 0.066 (1-24) 0.027 (0-24)		0.0016 (0-6 h) 0.0003 (6-24 h) 0.0006 (0-24 h)	

* Some cells for these applications were excluded from calculations as the analytical data indicated that the epidermal membrane may have been damaged during application.
** LOQ, Limit of quantitation. The LOQ for the donor chamber was 0.003 µg/cm^2 and 0.010% of applied dose.
*** Total potentially absorbable = total absorbed + remaining epidermis.

The overall total recovery for the three dose levels was good, with mean values of 100-102% of the applied dose.

Glyphosate absorption from the 480 g/L concentrate formulation increased slowly over the entire 24 hour exposure period (mean rate = 0.014 µg/cm^2/h). The mean rates during the first 10 hours and between

10-24 hours were 0.011 μg/cm^2/h and 0.016 μg/cm^2/h, respectively. At 10 hours, the mean amount of glyphosate absorbed was 0.105 μg/cm^2 (0.0021% of applied dose) and by the end of the 24 hour exposure period, the mean total amount of absorbed glyphosate was 0.342 μg/cm^2 (0.0070% of applied dose).

From the 1/16.7 v/v aqueous dilution of the formulation, glyphosate absorption was fastest during the first hour of exposure (rate of 0.134 μg/cm^2/h). The rate decreased to 0.066 μg/cm^2/h over the remainder of the 24 hour exposure, giving an average absorption rate of 0.027 μg/cm^2/h over the entire 24 hour exposure period. At the end of the exposure period, the mean total amount of absorbed glyphosate was 0.553 μg/cm^2 (0.182% of applied dose).

From the 1/200 v/v aqueous dilution of the formulation, glyphosate absorption was fastest during the first 6 hours of exposure (mean rate = 0.0016 μg/cm^2/h). The rate decreased to 0.0003 μg/cm^2/h over the remainder of the 24 hour exposure, giving an average absorption rate of 0.0006 μg/cm^2/h over the entire 24 hour exposure period. At the end of the exposure period, the mean total amount of absorbed glyphosate was 0.015 μg/cm^2 (0.048% of applied dose).

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 99-100%). The mean total amount of glyphosate recovered from the epidermis (statum corneum + remaining epidermis after tape stripping) was 0.256, 0.100, and 0.891% of the applied dose (concentrate, 1/16.7 [v/v] dilution, and 1/200 [v/v] dilution, respectively). The mean absorbed amounts were 0.007, 0.182, and 0.048% of applied dose, respectively. The amount of potentially biologically available glyphosate (absorbed + epidermis after tape striping) for the concentrate, 1/16.7 and 1/200 dilutions were 0.123, 0.262, and 0.799% respectively.

III. CONCLUSION

The results of this *in vitro* dermal absorption study indicate that the absorption of glyphosate through human skin is 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 0.007, 0.182, and 0.048% of the applied dose for the formulation concentrate, the 1:16.7 (v/v) dilution, and 1:200 (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 0.123, 0.262, and 0.799%, respectively. These data predict that the dermal absorption of glyphosate from potential exposure to this 480 g glyphosate /L SL formulation (MON 79351) would be minimal, at less than 1% of any potential dermal exposure.

Annex point	Author(s)	Year	Study title
IIA, 5.9.9/03	Hadfield, N.	2011	Glyphosate 360 IPA Salt (CA2273): In Vitro Absorption through Human Epidermis using [14C]-glyphosate Dermal Technology Laboratory Ltd. Med IC4, Keele University Science and Business Park, Keele, Staffordshire, ST5 5NL, UK Report No.: JV2147-REG Date: 2012 GLP: yes unpublished

Guideline: 1) OECD Test Guideline 428 (2004). Skin Absorption: In Vitro Method.
2) OECD (Guidance Document No. 28 (2004). The Conduct of Skin Absorption Studies.
3) European Commission Guidance Document on Dermal Absorption (2004).

Deviations: None

Dates of experimental work: 18th April 2011 to 15th June 2011

Executive Summary

The penetration of glyphosate from a glyphosate 360 IPA Salt (CA2273) formulation concentrate, containing a nominal 360 g glyphosate/L and a 1/133 w/v aqueous dilution of the concentrate, containing a nominal 2.7 g glyphosate/L, through human epidermis was measured in vitro over 24 hours. The doses were applied to the epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for an exposure period of 8 hours. The distribution of glyphosate within the test system (skin washes, donor chamber, stratum corneum and residual epidermal tissue) after 24 hours and time course penetration profiles were also determined. [^{14}C]-glyphosate was incorporated into the doses prior to application. The penetration process was followed by taking samples of the receptor fluid (physiological saline) at recorded intervals throughout the experimental period. All samples were analysed for radioactivity by LSC.

Penetration of Formulation concentrate glyphosate was fastest between 0-2 hours (0.914 µg/cm²/h). The mean penetration rate slowed to 0.074 µg/cm²/h between 2-24 hours. Between 0-8 hours the mean penetration rate was 0.283 µg/cm²/h. Between 0-24 hours, the penetration rate was, on average, 0.109 µg/cm²/h. The mean amount penetrated over the entire 24 hour exposure period was 3.51 µg/cm^2, corresponding to 0.096% of the applied dose. The mean total recovery of the applied test material was 110%. The vast majority of the applied glyphosate (mean 109%) was washed off the skin at 8 hours, with a further 0.417% washed off at 24 hours. A small proportion of the dose applied was recovered from the stratum corneum and remaining epidermis (0.034% and 0.043%, respectively).

Penetration of the 1/133 w/v aqueous spray strength dilution glyphosate was fastest between 0-1 hours (0.009 µg/cm²/h). The mean penetration rate slowed to 0.002 µg/cm²/h between 1-24 hours. Between 0-8 hours the mean penetration rate was 0.003 µg/cm²/h. Between 0-24 hours, the penetration rate, on average, was 0.002 µg/cm²/h. The mean amount penetrated over the entire 24 hour exposure period was 0.050 µg/cm^2, corresponding to 0.183% of the applied dose. The mean total recovery of the applied test material was 106%. The vast majority of the applied glyphosate (mean 100%) was washed off the skin at 8 hours, with a further 4.44% washed off at 24 hours. A small proportion of the dose applied was recovered from the stratum corneum and remaining epidermis (0.242% and 0.362%), respectively.

The results obtained in this study demonstrate that the penetration of glyphosate from this glyphosate 360 IPA Salt (CA2273) formulation concentrate and its 1/133 w/v dilution, through human epidermis is at a very slow rate. The extent of glyphosate penetration through human skin from the concentrate was below 0.1% and amounted to less than 0.2% of the applied dose, for the aqueous dilution, after 24 hours.

The vast majority of the applied dose could be removed by gentle skin washing after 8 hours. Only low proportions of the dose were associated with the skin at the end of the 24-hour experimental period.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	[^{14}C]-glyphosate
Description:	Dry radiolabelled material
Lot/Batch #:	XIX/5B
Purity:	95.88%
Stability of test compound:	Not specified

2. Vehicle and/ or positive control: water

3. Test animals:

Species:	Human skin in vitro
Source:	Human Tissue Bank (source not specified)
Age:	Not specified
Sex:	Not specified

B: STUDY DESIGN AND METHODS

Experiment dates: 18th April 2011 to 15th June 2011

Study Conduct:
Human in vitro membranes were prepared in static glass diffusion cells. Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of <10 kΩ (Davies et al, 2004) were regarded as having a lower integrity than normal and not used for exposure to the test materials. Cells were selected such that each application was represented by six intact membranes from three different subjects. The receptor chambers of the cells containing small magnetic stirrer bars were filled with a recorded volume of receptor fluid (physiological saline) and placed in a water bath maintained at a temperature of 32ºC ± 1ºC. Glyphosate is soluble in water at 10.5 g/L (Safety data sheet dated 26/2/2009) and this choice of receptor fluid ensures that the glyphosate can freely partition into the receptor fluid from the skin membrane and never reaches a concentration that would limit its diffusion. A pre-treatment sample (0.5 mL) was taken from each receptor chamber for analysis by LSC prior to dosing. An equal volume of fresh receptor fluid was added to each receptor chamber to replace the volume removed. The formulation was applied to the skin membranes as the product concentrate and as a 1/133 w/v aqueous spray strength dilution. The applications were left unoccluded for the duration of the experiment (24 hours). Samples of the receptor fluid (0.5 mL) were taken using an autosampler at pre-treatment, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours after application for analysis by LSC. After the 8 hour sample had been taken the skin was washed and allowed to dry naturally. Samples were taken during the procedure to determine mass balance. To assess penetration through human stratum corneum, successive layers of the skin surface were removed by the repeated application of adhesive tape (e.g. Scotch 3M Magic Tape, 1.9 cm wide), to a maximum of 5 strips (Trebilcock et al, 1994). A strip of adhesive tape was pressed onto the skin surface and then carefully peeled off to remove the stratum corneum. The adhesive strips were soaked individually in water to extract any test material. The extracts were sequentially numbered and analysed by LSC.
The penetrated (systemically available) dose is considered to be the amount of glyphosate detected in the receptor fluid. Material removed from the surface of the epidermis by the washing procedure is regarded as not bioavailable. Glyphosate recovered from the epidermis at the end of the experimental period is also

considered not to be bioavailable, although it is recognised that a proportion of this material may penetrate beyond the duration of the experimental period investigated in this study. In vivo, the majority of the dose in the epidermis, especially that recovered from the stratum corneum (i.e. that found on the tape strips), would eventually be lost by desquamation (Ramsey et al, 1992).

II. RESULTS AND DISCUSSION

Table 5.9-6: Summary of results for the concentrate Formulation

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Penetration Rate		Mean Amount and Percentage of Dose Penetrated		
	Time period (h)	Penetration rate (µg/cm^2/h ± SEM)	Time (h)	Amount (µg/cm^2)	Percentage
Formulation concentrate					
(366 g glyphosate /L)	0 - 2	0.914 ± 0.593	2	1.83	0.05
10 µL /cm^2 (3662 µg glyphosate /cm^2)	0 - 8	0.283 ± 0.167	8	2.7	0.074
Unoccluded	2 - 24	0.074 ± 0.035	24	3.51	0.096
Duration of exposure: 8h	0 - 24	0.109 ± 0.049			
n = 5			*LOQ*	*0.079*	*0.002*

1/133 w/v Aqueous Dilution

Table 5.9-7: Summary of results for the 1/133 aqueous dilution

Application of Test Materials and Actual Concentration of Dose Preparation	Mean Penetration Rate		Mean Amount and Percentage of Dose Penetrated		
	Time period (h)	Penetration rate (µg/cm^2/h ± SEM)	Time (h)	Amount (µg/cm^2)	Percentage
1/133 w/v aqueous dilution					
(2.72 g glyphosate /L)	0 - 1	0.009 ± 0.005	1	0.009	0.033
10 µL/cm^2 (27.2 µg glyphosate/cm^2)	0 - 8	0.003 ± 0.001	8	0.024	0.086
Unoccluded	1 - 24	0.002 ± 0.0009	24	0.050	0.183
Duration of exposure: 8h	0 - 24	0.002 ± 0.0009			
n = 5			*LOQ*	*0.0003*	*0.001*

III. CONCLUSION

The results obtained in this study demonstrate that the penetration of glyphosate from this glyphosate 360 IPA Salt (CA2273) formulation concentrate and its 1/133 w/v dilution, through human epidermis is at a very slow rate. The extent of glyphosate penetration through human skin from the concentrate was below 0.1% and amounted to less than 0.2% of the applied dose, for the aqueous dilution, after 24 hours. The vast majority of the applied dose could be removed by gentle skin washing after 8 hours. Only low proportions of the dose were associated with the skin at the end of the 24 hour experimental period.

Based on the EU and the OECD Globally Harmonized System (GHS) classification criteria, glyphosate is not to be classified for this endpoint

Annex point	Author(s)	Year	Study title
IIA, 5.9.9/04	Davies, D.J.	2003	Glyphosate SL (360g/l) Formulation (A12798Q): In Vitro Absorption Through Human Epidermis Syngenta Central Toxicology Laboratory, Alderley Park, Cheshire, UK Data owner: Syngenta Report No.: CTL/JV1732 Date: 2003-05-28 GLP: yes not published

Guideline:	OECD 428
Deviations:	None
Dates of experimental work:	2002-12-09 to 2003-05-28

Excecutive summary
The absorption and distribution of glyphosate from the A12798Q 360 g/L SL formulation was measured in vitro through human epidermis. The doses were applied as the concentrate formulation (360 g/L) and as a 3/200 v/v (5.4 g/L) spray strength dilution of the formulation in water. The absorption process was followed using [^{14}C]-labelled glyphosate, which was added prior to application. The doses were applied to the epidermal membranes at a rate of 5µl/cm^2 and left unoccluded for an exposure period of 6h and 24h. These applications were designed to simulate potential human dermal exposure to the 363g glyphosate/l SL formulation and its 3:200 v/v aqueous spray dilution during normal use.

The distribution of glyphosate within the test system and a 24 hour absorption profile (µg/cm^2/h) was determined. The results obtained in this study indicate that glyphosate is absorbed through human epidermis from the concentrate formulation at a very slow rate; the mean rate of absorption was 0.02µg/cm^2/h over a 24 h period. Absorption was also very slow for the 3/200 v/v aqueous dilution; the mean absorption rate over 24 h was 0.001µg/cm^2/h.
For the concentrate the majority of the applied dose, 100 and 103%, was removed by mild skin washing at 6 and 24 hours respectively, whilst 0.04 and 0.05% of the applied dose was left in the human epidermis at 6 and 24 hours respectively. For the spray strength dilution the majority of the applied dose, 90.8 and 87.9%, was removed by mild skin washing at 6 and 24 hours respectively, whilst 0.31 and 1.10% of the applied dose was left in the human epidermis at 6 and 24 hours respectively.

These data predict that the human dermal absorption of glyphosate from potential exposure to this formulation (A12798Q) either as the concentrate formulation or as a 3/200 v/v aqueous spray strength dilutions, would be minimal.

I. MATERIALS AND METHODS

A: MATERIALS:

1. Test material: **360g glyphosate/l SL formulation concentrate (A12798Q)**

Description:	brown liquid
Lot/Batch number:	FL020886
Purity:	28.3% (w/w) glyphosate
Stability of test compound:	Confirmed

Radiolabelled Test Material:	[^{14}C]-glyphosate
Radiochemical number:	6550
Radiochemical purity:	98.2%
Specific activity:	294.6 µCi/mg (11.0 MBq/mg)
Stability of test compound:	confirmed

B: STUDY DESIGN AND METHODS

In-life dates: Start: 12 February 2003 End: 18 March 2003

Diffusion cell: Diffusion of glyphosate into and across the skin to a receptor fluid was measured using glass diffusion cells in which the epidermis formed a horizontal membrane and provided an application area of 2.54cm^2.

Receptor fluid: The receptor fluid (physiological saline) was chosen to ensure that the glyphosate would freely partition into this from the skin membrane and never reach a concentration that would limit its diffusion. Glyphosate acid is highly soluble in water (11.6g/L; K_{ow} logP=<-3.2).

Skin preparations: Extraneous tissue was removed from human whole skin samples obtained from surgery or *post mortem*. The skin samples were immersed in water at 60°C for 40-45 seconds. The epidermis was carefully peeled from the dermis and stored frozen until required for use.

Skin preparation integrity: The integrity of the membranes was checked by measurement of the electrical resistance across the skin. Only those membranes with an acceptable resistance (>10kΩ), thereby showing that they were intact, were used on the study.

Test substance: The two doses were prepared to mimic the commercial 360g/L formulation and its aqueous spray dilution (3/200 v/v). An appropriate volume of [^{14}C]-labelled glyphosate (equivalent to 27.0 MBq) was blown down to dryness using a stream of nitrogen gas and added to 1 mL of the glyphosate formulation. To make the spray strength dilution an appropriate volume of [^{14}C]-labelled glyphosate (equivalent to 16.8 MBq) was blown to dryness and added to 15 µL of the unlabelled glyphosate formulation and 985 µL of deionised water. The doses were prepared as close to the time of application as was practicable and were analysed to confirm their suitability for use in the study.

Application to the skin: Each application was represented by six replicates from at least two different animals at a dose of 5µl/cm² and left unoccluded for the exposure period.

Temperature: Throughout the experiment the receptor fluid was stirred and the epidermal membranes were maintained at a normal skin temperature of 32 ± 1°C in a water bath.

Duration of exposure and sampling: For the cells exposed to the test preparations for 24 hours during which time samples of receptor fluid were taken at suitable intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hours) to allow adequate characterisation of the absorption profile. For cells assigned to the 6 hour exposure period, the receptor fluid samples were only taken 6 hours after application.

Terminal procedures: The donor chamber was carefully removed and washed with deionised water and the sample analysed by LSC. The epidermal surface of the skin was decontaminated by gently swabbing the application site with natural sponges pre-wetted with 3% Teepol® and with further sponges pre-wetted with water. Decontamination was shown to be complete following assessment of residual radioactivity levels on the skin surface with a Geiger counter. The sponges were digested in Soluene 350® and made up to a recorded volume. To assess penetration through the *stratum corneum*, the skin was allowed to dry and adhesive tape was repeated applied to the skin's surface and then carefully peeled off to remove the *stratum corneum*. The adhesive strips were soaked in methanol to extract test material. The extracts were

sequentially numbered and analysed by LSC. The remaining epidermal tissue was carefully removed from the receptor chamber and digested in Soluene 350® and analysed by LSC.

Data: 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/cm^2, 'percentage of dose absorbed' and rates of absorption (µg/cm^2/h). The results of the mass balance and distribution determinations are expressed in terms of amount (µg/cm^2) and 'percentage of applied dose' (see Tables below).

Definition of absorbed test material: The absorbed (systemically available) dose is considered to be the glyphosate detected in the receptor fluid. Material removed from the surface of the epidermis by the washing procedure is regarded as unabsorbed. The glyphosate recovered from the epidermis at the end of the exposure is also considered to be unabsorbed, although it is recognised that a proportion of this material may be absorbed beyond the duration of the exposure investigated in this study. *In vivo*, the majority of the dose in the epidermis, especially that recovered from the *stratum corneum*, would eventually be lost by desquamation.

II. RESULTS AND DISCUSSION

Recovery of radiolabelled test material in these experiments was very good (means of 107 and 95% of the applied dose for the concentrate and aqueous spray strength dilution respectively).

Concentrate formulation: Glyphosate absorption through human epidermis was fastest between 0-4 hours (0.07 µg/cm²/h) of application, after which it slowed to 0.02 µg/cm²/h (4-24 hours). Between 0-24 hours, the mean rate of absorption was 0.02 µg/cm²/h. The amount of glyphosate absorbed over time periods representing a range of typical 'working days' (6, 8 and 10 hours) were 0.20, 0.20 and 0.28µg/cm^2, respectively. In terms of percentages of applied dose, the respective amounts were 0.01, 0.01 and 0.02%.Over 24 hours, the amount absorbed was 0.50 µg/cm^2 (0.03% of the applied dose). Mild skin washing at 6 and 24 hours removed practically all (100 and 103%, respectively) of the applied dose from the surface of human epidermis. The percent of applied dose which was found to be associated with skin 6 and 24 hours following washing was 0.04 and 0.05%, respectively.

3/200 v/v dilution: The fastest rate of absorption through human epidermis occurred between 0-0.5 hours (0.011 µg/cm²/h), after which it slowed to less than 0.001µg/cm²/h (0.5-24 hours). Between 0-24 hours, the mean rate of absorption was 0.001 µg/cm²/h. The amount of glyphosate absorbed over the same time periods (6, 8 and 10 hours) were 0.01, 0.01 and 0.02 µg/cm^2, respectively. In terms of percentages of applied dose, the respective amounts was 0.04% for the 6,8 and 10 hour time points. Over 24 hours, the amount absorbed was 0.02 µg/cm^2 (0.07% of the applied dose). Washing at 6 and 24 hours removed 90.8 and 87.9% of the applied dose, respectively. For the 3/200 v/v spray dilution, the percent of applied dose which was found to be associated with skin 6 and 24 hours following application was 0.31 and 1.10%.

Table 5.9-8: Summary of glyphosate absorption through human epidermis

Application of Test Materials	Mean Absorption Rates		Mean Amount and Percentage of Dose Absorbed		
	Time period (h)	Absorption rate ($\mu g/cm^2/h \pm SEM$)	Time (h)	Amount ($\mu g/cm^2$)	Percentage absorbed
Concentrate Formulation					
(364g glyphosate/l)	1-4	0.07 ± 0.02	6	*0.20	0.01
5 µl/cm^2 (1821 µg ai/cm^2)	4-24	0.02 ± <0.01	8	*0.20	0.01
Unoccluded	0-24	0.02 ± <0.01	10	0.28	0.02
Duration of exposure: 24h			24	0.50	0.03
n = 4			LOQ	*0.25*	*0.01*
3/200 v/v aqueous spray diln					
(6.70g glyphosate/l)	0-0.5	0.011 ± 0.004	6	0.01	0.04
5 µl/cm^2 (33.5 µg ai/cm^2)	0.5-24	0.001 ± <0.001	8	0.01	0.04
Unoccluded	0-24	0.001 ± <0.001	10	0.02	0.04
Duration of exposure: 24h			24	0.02	0.07
n = 5			LOQ	*0.01*	*0.02*

* The LOQ values have been used as positive values in the calculation of the mean where values were <LOQ.

Table 5.9-9: Summary of glyphosate distribution from the concentrate formulation – 6 hour exposure

Test Compartment	Percentage of Dose Recovered (%)					Mean % Recovered	SD
	Cell 65	Cell 70	Cell 75	Cell 85	Cell 89		
Stratum Corneum	0.02	0.02	0.04	0.02	0.03	0.02	0.01
Donor Chamber	0.52	0.01	8.49	18.2	0.03	5.44	7.97
Skin Wash	110	102	93.6	98.9	96.4	100	6.13
Remaining Epidermis	0.01	<0.01	0.01	0.01	0.06	0.02	0.02
Absorbed	0.02	0.08	0.01	0.01	0.01	0.03	0.03
Total Recovered	110	102	102	117	96.5	106	8.06

Stratum corneum = *amount in tape strips; Remaining epidermis = epidermal tissue remaining after tape stripping; Absorbed = amount in receptor fluid*

Table 5.9-10: Summary of glyphosate distribution from the 3/ 200 aqueous spray dilution – 6 hour exposure

Test Compartment	Percentage of Dose Recovered (%)				Mean % Recovered	SD
	Cell 5	Cell 13	Cell 14	Cell 21		
Stratum Corneum	0.02	0.18	0.33	0.1	0.16	0.13
Donor Chamber	0.02	0.05	11.0	0.54	2.90	5.39
Skin Wash	94.6	94.0	80.7	94.0	90.8	6.75
Remaining Epidermis	0.04	0.09	0.38	0.08	0.15	0.16
Absorbed	0.02	0.02	0.03	0.04	0.03	0.01
Total Recovered	94.7	94.3	92.4	94.8	94.0	1.11

Table 5.9-11: Summary of glyphosate distribution from the concentrate formulation – 24 hour exposure

Test Compartment	Percentage of Dose Recovered (%)				Mean % Recovered	SD
	Cell 68	Cell 72	Cell 87	Cell 91		
Stratum Corneum	0.01	0.02	0.02	0.01	0.02	<0.01
Donor Chamber	0.01	0.01	0.01	18.1	4.53	9.04
Skin Wash	106	110	109	86.6	103	11.0
Remaining Epidermis	<0.01	<0.01	0.03	0.07	0.03	0.03
Absorbed	0.02	0.02	0.04	0.04	0.03	0.02
Total Recovered	106	110	109	105	107	2.37

Table 5.9-12: Summary of glyphosate distribution from the 3/200 aqueous spray dilution – 24 hour exposure

Test Compartment	Percentage of Dose Recovered (%)					Mean % Recovered	SD
	Cell 6	Cell 16	Cell 17	Cell 11	Cell 22		
Stratum Corneum	0.03	0.94	0.88	0.42	0.07	0.47	0.43
Donor Chamber	0.01	16.2	6.74	6.96	0.03	5.99	6.66
Skin Wash	95.0	77.0	87.0	85.9	94.6	87.9	7.39
Remaining Epidermis	0.02	0.56	1.93	0.59	0.06	0.63	0.78
Absorbed	0.08	0.09	0.07	0.05	0.03	0.07	0.02
Total Recovered	95.1	94.8	96.6	94.0	94.8	95.0	0.96

III. CONCLUSION

The results in this study demonstrated that the absorption of glyphosate from a 360g/L SL formulation or its aqueous dilution (3/200 v/v) is extremely slow through human epidermis when compared with the absorption rates of other penetrants using this in vitro technique (Dugard et al, 1984a; Dugard et al, 1984b).

The vast majority of glyphosate (greater than 87%) that may come into contact with human skin will be removed during normal washing procedures.

The small residual amounts of glyphosate found in human skin, especially that recovered from the stratum corneum, is most likely to be lost by desquamation in vivo. Over 24 hours, the amount absorbed for the concentrate was 0.50 µg/cm2 (0.03% of the applied dose) and was 0.02 µg/cm2 (0.07% of the applied dose) for the 3/200 spray dilution

IIA 5.10 Other/special studies

PART 1 : OTHER RELEVANT REGULATORY STUDIES

Three studies were conducted to further investigate effects of glyphosate which were previously observed in classical toxicological studies. A number of repeat dose studies in rodents have identified alterations of salivary glands, described as increased basophilic staining and enlargement of cytoplasm especially in the parotid salivary glands. The toxicological significance of this effect has been previously unexplained and because of this the 2004 JMPR review of glyphosate concluded that this treatment-related effect was of unknown toxicological significance. This lead the JMPR to established a group ADI for glyphosate and AMPA of 0–1.0 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100 (Atkinson et al., 1993). The JMPR evaluation hypothesised that the low pH of glyphosate technical acid in the diet caused local irritation in the oral cavity leading to the observed salivary gland effects. The objective of this study was to evaluate the potential effects of low pH diet on the parotid salivary glands. Citric acid was selected as an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Therefore, a study with citric acid was performed to evaluate the potential effects of a low pH on the parotid salivary glands. Citric acid was given to male rats in diet (14000 ppm) and via gavage (791-1316 mg/kg bw/day). Trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration)

was also given in a diet for eight weeks (minimum of 56 days). 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 was observed. These effects were noted as most severe in the low pH dietary test group. In the absence of cytotoxicity and hyperplasia the noted effects were considered as 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 (Haas 2002, IIA 5.10/01).

Since effects on salivary glands were previously not observed in every rat strain, a study was conducted (Allen, 1996; IIA 5.10/02) to investigate strain specific effects. Administration of diets containing 20000 ppm glyphosate acid to male rats for 4 weeks produced marked strain differences in the severity of effects in the parotid salivary gland. Salivary gland weights were increased after 4 weeks of treatment in the F344 and AP (Alpk:AP$_f$SD, Wistar-derived) strains. 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 (Alpk:AP$_f$SD, Wistar-derived) and CD (Sprague-Dawley; Charles River) strains involving small foci of cells only. Complete recovery of both salivary gland weights and histopathological changes was apparent in AP and CD strains following the 4-week recovery period. The salivary gland weight increased recovered in the F344 strain however there was evidence that the salivary glands had not fully recovered in all the F344 strain animals after a 13 week recovery period.

Based on the weight of evidence across the studies presented by the glyphosate taskforce it is proposed that the changes observed in the salivary gland (hypertrophy and basophilia of the parotid acinar cells) are a non-adverse adaptive response to treatment with a low pH diet for the following reasons:

- The effect is observed with another organic acid with a similar pH-dilution curve to glyphosate.
- The effect is only observed following treatment in the diet. The same effect has not been observed acoss an extensive database following other exposure routes. The ADME radiolabel studies indicate glyphosate does not accumulate in the salivary gland.
- The effect, seen primarily in the rat, is variable in severity and has not been observed consistently across sex, dose or strain.
- The salivary gland is not a significant target organ in other species.
- From a histopathological perspective across an extensive database, there is no accompanying evidence of cytotoxicity leading to necrosis or apoptosis, no evidence of inflammation or change in function and the cellular alterations do not progress with time to preneoplastic or neoplastic lesions (but in fact decrease in incidence and severity or disappearance all together with time).
- The effect is reversible upon cessation of treatment with a low pH diet.

Pharmacological activity of the test substance was investigated *in vivo* with rats, which were treated with a single oral dose of 5000 mg/kg glyphosate. One hour after dosing, no haematological, electrographic or behavioural/functional changes were observed when compared to control animals ([redacted] 1996, IIA 5.10/03). In the same study, *ex vivo* investigations with isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed. When administered to the isolated guinea pig ileum, glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents. This effect might be related to the gastrointestinal disturbances (stools and diarrhoea), that were seen in acute and short-term toxicity studies. Evaluation of innervated muscle response in the same study showed that the test substance, when administered at the maximum solubility concentration in physiological saline, did not cause any neuromuscular blocking activity.

Table 5.10-1: Summary of special studies

	Reference (Data owner)	Type of study Species, Strain	Application Route (Dose)	Test substance	Purity [%]	Results
Studies not reviewed in the 2001 evaluation	IIA 5.10/01 [redacted] 2010 (MON / GTF)	8-week oral toxicity; Sprague-Dawley Rat, ♂	Gavage (791-1316 mg/kg bw), Diet (14000 (Citric acid), 21400 ppm (Trisodium citrate dehydrate))	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
	IIA 5.10/02 [redacted] 1996 (SYN)	4-week oral toxicity; Sprague-Dawley (CD)/Fischer 344/Alpk:APSD (AP), Rat, ♂	Diet (20000 ppm)	Glyphosate acid	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 effects occurred in the AP and CD) strains involving small foci of cells only
	IIA 5.10/03 [redacted] 1996 (NUF)	Pharmacology Screening study; Sprague-Dawley Rat, ♂ + ♀	Gavage (5000 mg/kg bw, single dose), Injection	Glyphosate technical	95.3	No haematological, electrogra-phic 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 muscle
	IIA 5.10/04 [redacted] 2012 (MON)	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

Annex point	Author(s)	Year	Study title
IIA, 5.10/01	[redacted]	2010	An 8-Week Oral (Diet and Gavage) Toxicity Study of Citric Acid in Male Rats [redacted] Data owner: Monsanto/GTF Study No.: WIL-50361 Date: 2010-01-08 GLP: yes unpublished

Guideline: Guideline does not exist for this kind of study but data from the study report is similar to OECD 408.

Deviations: not applicable

Dates of experimental work: 2009-02-24 to 2009-05-15

Executive Summary
A number of repeat dose studies in rodents with glyphosate technical acid have identified alterations of the salivary glands, described as increased basophilic staining and enlargement of cytoplasm, especially in the parotid salivary glands. The toxicological significance of these observations were considered not relevant, by some reviewers and unknown by others. In the 2004 JMPR review of glyphosate, a hypothesis was proposed that the low pH of glyphosate technical acid in the diet caused local irritation in the oral cavity leading to the observed salivary gland effects. The objective of this study was to evaluate the potential effects of low pH diet on the parotid salivary glands. Citric acid was selected as an appropriate surrogate for glyphosate, having both a similar pH-dilution curve and low toxicity. Citric acid was presented in the diet (14000 ppm) and compared with a typical pH basal diet control group. A higher pH diet group fed basal diet with trisodium citrate dihydrate (21400 ppm, an equivalent citrate ion concentration) was also compared with the typical pH basal diet control group. In addition, low pH aqueous citric acid was administered by gavage and compared to a control deionised water gavage group to evaluate potential systemic effects of the citrate ion on the parotid salivary glands. These five test groups, each consisting of 10 male rats, were dosed for eight weeks (minimum of 56 days).
Clinical signs, bodyweight and food consumption were monitored during the study. All animals were subjected to a gross necropsy examination and a comprehensive histopathological evaluation of tissues was performed. The findings are summarised as follows:
There were no test substance-related clinical signs of toxicity, as well as no test substance-related effects on body weight, and food consumption.

Test substance-related effects on organ weights consisted of statistically significantly higher parotid salivary gland weights in the low pH diet group only (14000 ppm citric acid) when compared to the respective control group. Non-statistically significantly higher parotid salivary gland weights were noted in the gavage citric acid and high pH dietary (21400 ppm trisodium citrate dihydrate) groups when compared to their respective control group. There were no statistically significant test substance-related effects on the fused mandibular/sublingual salivary gland weights when the respective control and test substance-treated groups were compared; however, a non-statistically significantly higher fused mandibular/sublingual salivary gland weight was noted in the low pH diet group (14000 ppm citric acid).

Histological effects consisted of cytoplasmic alterations in the parotid salivary glands characterized by the presence of hypertrophied acinar cells with basophilic granular cytoplasm. Although the overall incidence of affected animals was similar in all control and citric acid or trisodium citrate dihydrate-treated groups, these effects were clearly most severe in the low pH diet group (14000 ppm citric acid in basal diet). With the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are considered to be an adaptive response to local irritation of the low pH diet in the oral cavity rather than an adverse effect.

Conclusion
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). The magnitude of change in parotid gland weight and severity of the cytoplasmic alteration in the parotid salivary glands was most severe in the low pH 14000 ppm diet citric acid group.

I. MATERIALS AND METHODS

A. MATERIALS

1. 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.

2. Vehicle and/ or positive control: Gavage: deionised water, Diet: plain diet

3. Test animals:

Species:	Rats
Strain:	Sprague-Dawley (CD)
Source:	Charles River Laboratories, Inc., Raleigh, North Carolina, US
Age:	approx. 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

B: STUDY DESIGN AND METHODS

In life dates: 2009-02-10 to 2009-04-21

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 5.10-2). 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 5.10-2: Study group assignment

Group Number	Test Substance application	Dose Level (mg/kg bw/day or ppm)	Dose Volume (mL/kg)	Number of animals
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, prostrate, 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.

II. RESULTS AND DISCUSSION

A. MORTALITY

No deaths occurred during the study.

B. 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.

C. BODY WEIGHT

There were no statistically significant differences when the respective control and test substance-treated groups were compared.

D. FOOD AND TEST SUBSTANCE 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.

E. PATHOLOGY

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 5.10-3).

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 5.10-3: Toxicologically relevant organ weight differences

	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 5.10-4). 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 5.10-4: 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.10/02	Allen, S.L.	1996	Glyphosate Acid: Comparison of Salivary Gland Effects in Three Strains of Rat. Central Toxicology Laboratory, Alderley Park Macclesfield, Cheshire, UK Data owner: Syngenta Study No.: CTL/P/5160 Date: 1996-08-19 GLP: yes unpublished

Guideline:	Guideline does not exist for this kind of study.
Deviations:	not applicable
Dates of experimental work:	1996-01-15 to 1996-05-14

The purpose of this study was to investigate the rat strain susceptibility of the effects of glyphosate acid on the salivary gland after 4 weeks administration in these strains of rat. In studies with F344 rats, glyphosate acid has been shown to cause effects on the salivary gland (NTP, 1992[8]). In contrast, there was no evidence of microscopic changes in the salivary gland in a previously conducted 28 day feeding study

[8] NTP (1992). Technical Report on Toxicity Studies of Glyphosate Administered in Dosed Feed to F344/N Rats and B6C3F1 Mice. United States Department of Health and Human Services, National Toxicology Program Toxicity Reports Series Number 16

with glyphosate acid (20000 ppm in the diet) in Alpk:AP$_f$SD rats, although there was an effect on gland weight (Milburn and Clapp, 1995)[9].

Study groups of 24 male Alpk:AP$_f$SD (Wistar-derived; AP), Sprague-Dawley (Charles River CD; CD) and Fischer 344 (F344) rats received 0 or 20,000 ppm glyphosate acid. Eight animals from each group were killed on Day 29 and the remaining animals were retained without treatment for a further four (8 rats/group) or 13 weeks (8 rats/group). Clinical observations, bodyweights and food consumption were measured and at the end of the scheduled periods, the animals were killed and subjected to a necropsy. Salivary glands were weighed and taken for subsequent histopathology examination.

Treatment with 20000 ppm glyphosate acid produced significant reductions in bodyweight and minor reductions in food consumption in AP and CD rats but no effects on bodyweight or food consumption were seen in the F344 rat. In contrast, salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the administration period. 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 slight effects involving small foci of cells only occurred in the AP and CD strains.
Recovery of effects was apparent in all strains during the recovery periods. Bodyweight and food consumption returned to control values in both AP and CD strains. After four weeks on control diet significant recovery of the salivary gland changes, in terms of both weight and micropathology, was evident in the F344 strain and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet slightly more treated F344 rats showed minor focal changes in the salivary gland compared to the contemporaneous controls and group mean salivary gland weights were increased slightly.

Conclusion
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 in the F344 strain. Similar but slighter effects occurred in the AP and CD strains.
Complete recovery of effects were 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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

Identification:	Glyphosate acid
Description:	White solid
Lot/Batch #:	P24
Purity:	95.6%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control: Plain diet

3. Test animals:

Species:	Rats
Strain 1:	Alpk:AP$_f$SD

[9] Milburn, G. & Clapp, MJL (1995) 28day dietary toxicity study in the rat. CTL/L/6624

Source:	Rodent Breeding Unit, Zeneca Pharmaceuticals, Alderley Park Macclesfield, Cheshire, UK
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

B: STUDY DESIGN AND METHODS

In life dates: 1996-01-15 to 1996-05-14

Animal assignment and treatment:
In a 28 days feeding study groups of 24 male Alpk:AP$_f$SD (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 and compound intake
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.

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

The mean achieved concentration of glyphosate acid in both batches of diet was within 2% of the target concentration (Table 5.10-5).

Table 5.10-5: Achieved concentrations of glyphosate acid in the diet

	Nominal concentration (ppm)	Mean analysed concentration (ppm)	% of nominal concentration
Batch 1	20,000	19,985	99.9
Batch 2	20,000	20,355	101.8

B. MORTALITY

There were no treatment-related deaths. One treated AP rat was killed in Week 7 following accidental damage to its snout.

C. CLINICAL OBSERVATIONS

There were no treatment-related findings in any of the groups noted during the study period.

D. 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 bodyweight 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 bodyweight was approximately 7% (after adjusting for initial bodyweight) at the end of the administration period. However, bodyweights quickly recovered and were 5% higher than controls (after adjusting for initial bodyweight) by the end of the 13-week recovery period.

F344 rats: No treatment related effects were observed.

E. 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.

F. NECROPSY

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 5.10-6: 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 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 5.10-7: 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
	Weight after 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
	Weight after 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

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.10/03	[redacted]	1996	Glyphosate Technical: Pharmacology Screening Study in the Rat [redacted] Data owner: Nufarm Study No.: 434/021 Date: 1996-06-28 GLP: yes unpublished
Guideline:			JMAFF, 59 Nohsan No. 4200 (1985)
Deviations:			not applicable
Dates of experimental work:			1996-02-06 to 1996-04-04

Executive Summary
The test material was evaluated for evidence of pharmacological activity using a series of *in vivo* and *ex vivo* screening methods. For *in vivo* studies five male and five female rats were dosed with glyphosate technical at a dose level of 5000 mg/kg with similar sized control groups receiving vehicle only. Approximately one hour after dosing control and treated animals were examined for either haematological changes, electrocardiographic changes or behavioural/functional changes. *Ex vivo* studies were evaluation of the isolated guinea pig ileum and isolated rat gastrocnemius muscle using saturated solutions of the test material.

***In vivo* studies**
There were no differences in response between treated and control animals.

***Ex vivo* studies**
Glyphosate Technical (12 mg/mL) caused a contractile response to isolated guinea pig ileum similar to that seen with acetylcholine. The effect seen was abolished when the ileum was pre-incubated with atropine sulphate.
Injection of tubocurarine resulted in a significant diminution of the contractile response of the rat gastrocnemus muscle when the sciatic nerve was stimulated. On the contrast there was no effect on muscle contraction when either glyphosate technical or physiological saline was injected.

Conclusion
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.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test materials:

Identification:	Glyphosate Technical
Description:	White powder
Lot/Batch #:	H95D161A
Purity:	95.3%
Stability of test compound:	No data given in the report.

2. Vehicle and/ or positive control:

in-vivo	1% carboxymethyl cellulose
ex-vivo (guinea pig)	distilled water, krebs physiological buffer solution,
ex-vivo (guinea pig)	physiological saline

3. Test animals:

in-vivo	Species:	Rats
	Strain:	Sprague-Dawley (CD)
	Source:	Charles River UK Ltd., Margate, Kent, UK
	Age:	no data
	Sex:	Males and females
	Weight at dosing:	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 dosing:	250 - 300 g
	Acclimation period:	no data
	Diet/Food:	Guinea Pig FDI Diet (Special Diets Services Ltd., Witham Essex, UK), *ad libitum*
	Water:	Tap water, *ad libitum*
	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
in-vivo	Species:	Rats
	Strain:	Sprague-Dawley (CD)
	Source:	Charles River UK Ltd., Margate, Kent, UK
	Age:	no data
	Sex:	Males and females
	Weight at dosing:	110 - 125 g
	Acclimation period:	no data
	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: 30 – 70%
		Air changes: at least 15/hour
		12 hours light/dark cycle

B: STUDY DESIGN AND METHODS

In life dates: 1996-03-25 to 1996-03-27

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 vain. 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) and acetylcholine (agonist), atropine (antagonist) and test material.

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 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 experiment was repeated on separate animals with doses of tubocurarine (positive control) injected into the dorsal aorta instead of sterile saline. The experiment was also repeated on a separate animal with the test material dissolved in sterile saline at a concentration of 12 mg/mL (maximum solubility).

The following parameters were evaluated: Response to injection of sterile saline, tubocurarine and test material.

II. RESULTS AND DISCUSSION

A. BLOOD PARAMETERS
There were no biologically significant differences, among the parameters measured, between treated and control animals.

B. CARDIOVASCULAR SYSTEM
There were no biologically significant differences, among the parameters measured, between treated and control animals.

C. NERVOUS SYSTEM
There were no biologically significant differences, among the parameters measured, between treated and control animals.

D. 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.

E. 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 gastrocnemus 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.

III. CONCLUSION

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.

Annex point	Author(s)	Year	Study title
IIA, 5.10/04	[redacted]	2012	Glyphosate – A 28-Day Oral (Dietary) Immunotoxicity Study in Female B6C3F1 Mice [redacted] ImmunoTox®, Inc., Virginia Bio Technology Research Park, Richmond, VA 23219, USA Project No.: WI-10-460 (Study No.: WIL-50393) Data owner: Monsanto Date: 2012-03-21 GLP: yes not published

Guideline:	US-EPA OPPTS 870.7800 (1998)
Deviations:	None
Dates of experimental work:	2010-10-05 - 2010-11-17

Executive Summary

The potential immunotoxicity of glyphosate was evaluated after repeated dietary administration to B6C3F1 mice. Four groups of 10 female mice were offered diets containing glyphosate concentrations of 0, 500, 1500 or 5000 ppm (equivalent to 0, 150, 449, and 1448 mg/kg bw/day) and for 28 consecutive days. 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).

The animals were checked twice daily for mortality and once daily for clinical signs. Detailed clinical examinations were performed once per week. Body weights were recorded twice weekly. Food consumption was recorded in weekly intervals, and food intake was calculated for the corresponding body weight intervals. Blood samples for IgM antibody analysis were collected from all mice at scheduled necropsy. At termination, the animals were sacrificed and subjected to a full macroscopic post-mortem examination. Spleens and thymus were weighed and specified tissues preserved.

There were no test substance-related effects on survival, clinical observations, body weight, food consumption, as well as any gross pathological changes. There were no test substance-related effects on spleen or thymus weights (absolute or relative to final body weight), spleen cellularity, or the T-cell dependent antibody response (TDAR), as measured by the AFC IgM Specific Activity (AFC/10^6 spleen cells) and Total Spleen Activity (AFC/spleen), at any dosage level tested.

Conclusion

Treatment of female B6C3F1 mice for 28 days with diets containing glyphosate was well tolerated and did not suppress the humoral component of the immune system when evaluated using the AFC assay. 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 at 500, 1500, and 5000 ppm was considered to be 5000 ppm (equivalent to 1448 mg/kg of body weight/day).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Identification:	Glyphosate
Description:	White powder
Lot/Batch #:	GLP-0807-19475-T
Purity:	95.11 % (dried)
Stability of test compound:	Expiry date: 2011-06-10

2. Vehicle and/ or positive control: Basal diet
Cyclophosphamid monohydrate

3. Test animals:

Species:	Mouse
Strain:	B6C3F1/Crl
Source:	Charles River Laboratories, Inc., Ralaigh, NC, USA
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

B: STUDY DESIGN AND METHODS

In life dates: 2010-10-05 to 2010-11-16

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 (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1955, 1964) 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 (Sokal and Rohlf, 1981) 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 (Bartlett, 1937). Homogeneous data were evaluated using a parametric one-way ANOVA (Kruskal and Wallis, 1952). When significant differences occurred, the treatment groups were compared to the basal diet control group using Dunnett's test (Dunnett, 1955, 1964). Non-homogeneous data were evaluated using a non-parametric ANOVA (Wilson, 1956). When significant differences occurred, the treatment groups were compared to the basal diet control group using the Gehan-Wilcoxon test when appropriate (Gross and Clark, 1975). The Jonckheere's test (Hollander and Wolfe, 1973) 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 (Sokal and Rohlf, 1981) and compared to the basal diet control group. The criteria for accepting the results of the positive control group included a statistically significant ($p\leq0.05$) 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).

II. RESULTS AND DISCUSSION

A. ANALYSIS OF DOSE FORMULATIONS

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.

B. MORTALITY AND CLINICAL SIGNS

There were no mortalities observed during the study period.

C. CLINICAL OBSERVATIONS

There were no test substance-related clinical findings.

D. BODY WEIGHT

There were no test substance related

E. FOOD CONSUMPTION AND COMPOUND INTAKE

There were no test substance-related effects on food consumption noted.

The group mean achieved doses are summarised below.

Table 5.10-8: Group mean achieved dose levels of glyphosate

Dose group	Dietary concentration (ppm)	Mean achieved dose level (mg/kg bw/day)
1 (negative control)*	0	0.0
2 (low)	500	150.1
3 (mid)	1500	449.1
4 (high)	5000	1447.5
5 (positive control)	50 mg/kg CPS**	0.0

* basal diet group
** CPS = cyclophosphamid

F. NECROPSY

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 5.10-9 below.

Table 5.10-9: Final body weight and organ weight data

Dose group	Body weight	Spleen		Thymus	
	(g)[#]	weight (mg)[#]	% body weight (%)[#]	weight (mg)[#]	% body weight (%)[#]
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 $p \leq 0.01$

G. AFC ASSAY RESULTS

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 5.10-10 below.

Table 5.10-10: Results of AFC assay

Dose group	Spleen cells (x 10^7)#	IgM AFC / 10^6 spleen cells #	IgM AFC/spleen (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 ± 1.24	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 $p \leq 0.01$

III. CONCLUSION

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.

Part 2. LITERATURE REVIEW

Monsanto Company has been conducting routine surveillance of technical literature for glyphosate-related publications in a structured fashion since early 1997. During the period from 1997 to the present time, the search process and the literature databases used have been modified as new resources and technology became readily available. The technical databases that are used for the search include: Web of Science[SM], BIOSIS Previews®, CAB Abstracts® (CABI), MEDLINE®, and CA Plus (Chemical Abstracts Plus). The searches are done 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 will also identify publications that consider glyphosate and surfactants, (such as polyoxyethylenealkylamines, or POEA), in the context of glyphosate formulations.

Starting from the ongoing Monsanto literature database, all the peer-reviewed publications covering the time period from 2001 through 2011 that relate to the four key disciplines addressing exposure and hazard (toxicology, ecotoxicology, residues and environmental fate) were assessed within the appropriate discipline for inclusion in the literature review for the submission. Some publications address more than one discipline, and are included in each relevant discipline. More recent publications have continued to be reviewed up to shortly before submission, and selected publications have been included.

At the request of the Bundesambt für Verbraucherschutz und Lebensmittelsicherheit (BVL), additional publications cited in a recent document prepared by Earth Open Source10 have also been included in the literature review. Many of the cited peer-reviewed publications were already included, but others were not within the scope of this literature review, primarily because the publication date was prior to 2001. The additional peer-reviewed publications have been included and are discussed within the appropriate discipline.

The peer-reviewed publications identified for inclusion during the literature search were reviewed within each discipline 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); 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

[10] Earth Open Source report. 2011. Roundup and birth defects: Is the public being kept in the dark? Authored by Antoniou M, Habib MEEM, Howard CV, Jennings RC, Leifert C, Nodari RO, C Robinson, Fagan J. Available from: http://www.earthopensource.org/files/pdfs/Roundup-and-birth-defects/RoundupandBirthDefectsv5.pdf

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.

Approximately 2000 peer-reviewed publications from the Monsanto technical literature database were assessed, and of those about 1000 were assigned a Category 1, 2 or 3 and selected for inclusion in the submission.

A full description of the literature search methodology is provided in a separate document (Carr and Bleeke, 2012).

The publications selected for inclusion are listed in Document L for each respective section, under the Annex point for 'Other/Special Studies': Point IIA 5.10 (Toxicology), Point IIA 6.10 (Metabolism and Residue), Point IIA 7.13 (Environmental Fate), and Point IIA 8.16 (Ecotoxicology). Under each point, the list of Other/Special Studies is presented in three tables:

- Table 1 lists other relevant studies conducted by the Glyphosate Task Force or member companies in support of the submission, that do not fit within any other dossier points .
- Table 2 lists all the relevant peer-reviewed publications from the literature that were selected for inclusion in the submission.
- Table 3 lists the publications and other documents that are cited within the discussion of the literature. These include documents such as government or company reports; publications that are included in the literature review under another section of the dossier; and publications that are outside the scope of the literature review.

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

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

Publications are presented in Tier II style summaries followed by Klimisch ratings then responses/comments on the paper. Results reported and discussed in the peer reviewed open literature review do not affect the conclusions drawn in the core glyphosate dossier.

1. Literature Review of Developmental and Reproductive Toxicity (DART) and Endocrine Disruption (ED) Publications

Publications suggesting glyphosate or glyphosate based formulations are developmental toxicants, reproductive toxicants or endocrine disruptors include *in vitro* studies, *in vivo* studies and epidemiological studies with weak, statistically non-significant associations. Some epidemiological studies evaluate associations with pesticides in general or classes of pesticides, with no mention of glyphosate or glyphosate based products, and thus warrant no further discussion (e.g. Benítez-Leite, 2009) other than the OECD Tier II like summary and Klimisch rating (Klimisch, 1997). Many of these published since 2000 are specifically discussed in a comprehensive glyphosate DART review publication by three internationally recognized experts (Williams et al., 2012), referenced in Doc L Table 2 and included in Doc K. Further discussions of some 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 clearly published the criteria for inclusion on List 1 was strictly based on exposure potential, not hazard, specifically stating in the Federal Register (2009);

> "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 successfully completed 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 expected to be reviewed this year. However, the Agency gas 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. Therefore, in an effort to disclose the findings of the glyphopsate EDSP data to the scientific community, the JGTF is considering publishing a Weight of Evidence review of glyphosate with respect to endocrine disruption.

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)

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.

In Vitro Glyphosate DART/ED Publications

Many *in vitro* research publications have characterized pesticide formulations, including glyphosate based formulations, as toxic and endocrine disrupting products. Researchers and editorial boards have frequently overlooked 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. For example, Walsh et al. (2000) 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) demonstrated (i) 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; (ii) comparable cytotoxicity dose-response curves for several common household detergents or surfactants; and (iii) a variety of surfactants demonstrate cytotoxic effects that are not specific to biochemical pathways within intact cells. Levine (2007) concludes by emphasizing the importance of considering the biological plausibility of observed *in vitro* effects for in-tact animals.

Subsequent research addressing the steroidogenesis pathway confirmed glyphosate lacked endocrine disruption potential specific to this pathway. Quassinti et al. (2009) 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) 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., 2010). 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.

The Seralini 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; Benachour et al, 2007; Benachour and Seralini, 2009; Gasnier et al, 2009; Gasnier et al, 2010; Gasnier et al., 2011; Clair et al., 2012; Mesnage et al., 2012), 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 often 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, simply 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) 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 mircosomes (Richard et al, 2005; Benachour et al, 2007; Gasnier et al, 2009), the data are confounded and thus uninterpretable where surfactants are introduced to such *in vitro* systems. This is noted in the US EPA Aromatase Inbibition 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."

Research from the Seralini laboratory has repeatedly gained general public and media attention, including dissemination on "you-tube" and public lecture tours in various countries, in which allegations against glyphosate based products and biotechnology in agriculture are made. The selective use of literature, with absence of contradicting research (e.g., Kojima et al. (2004) demonstrated glyphosate lacked affinity for estrogen-α, estrogen-β and androgen receptors) demonstrates consistent and undeterred bias in the authors'

publication record. Numerous authoritative reviews have discounted the relevance of the Seralini team's research to human health risk assessment; some of these are referred to in specific publication reviews below. Several more recent publications from this group investigate homeopathic plant extract remedies for effects they attribute to glyphosate exposures in formulated products *in vitro* (Gasnier et al.(2010); Gasnier et al.(2011)).

Another *in vitro* publication claiming a specific developmental toxicity pathway has gained significant public traction, media attention and widespread international public lecture tours by the lead investigator. Paganelli et al. (2010) from the Carrasco research laboratory in Argentina conducted three *in vitro* assays, (i) frog embryos exposed to glyphosate formulation; (ii) frog embryos directly injected without injection blank negative controls; and (iii) fertilized 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.

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) and Beuret et al. (2005) (two authors are common to each paper and from the same university department) 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).

Dallegrave et al. (2003; 2007) published results of two non-guidelines rat developmental toxicity studies, in which a glyphosate based formation containing POEA was evaluated. Numerous reporting deficiencies and inconsistencies pose difficulties in data interpretation

Romano et al. (2010) 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 very detailed review was undertaken by experts in the fields of reproductive and developmental toxicology and endocrinology; William R. Kelce, M.S., Ph.D, Fellow ATS; James C. Lamb, IV, Ph.D, DABT and Fellow ATS; John M. DeSesso, Ph.D, Fellow ATS. Their critique is referenced in Doc L and included in Appendix K. Most recently, Romano et al. (2012) 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) base their hypothesis on selectively discussed literature implicating glyphosate as an endocrine disruptor, predominantly with citations to research from the Seralini laboratory.

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 et al., 2012) 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.

POEA DART Studies in Williams et al. (2012)

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 previously submitted by Monsanto to BfR (Bleeke et al. 2010) is referenced in Doc L and included in Doc K. 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) recently evaluated and detailed the results of DART studies with two different POEA surfactants, summarized below.

Pregnant female rats were administered MON 0818, a POEA surfactant, at 0, 15 100 and 300 mg/kg/day. The NOAEL for maternal toxicity was 15 mg/kg/day and the NOAEL for rat developmental toxicity was the highest dose tested, 300 mg/kg/day (Holson, 2001).

A reproductive and developmental multigenerational screening study dosed MON 0818 in diets at 0, 100, 300 and 1000 ppm. The majority of endpoints evaluated were unaffected by treatment, including testis morphology, sperm parameters and testosterone and thyroid hormone levels. The mid-dose of 300 ppm (approximately 20 mg/kg/day) was considered the NOAEL for reproductive and developmental toxicity based on the following results in F0 at the high dose, 1000 ppm: increases in unaccounted for implantation sites with reduced mean number of pups and litter size in the high dose group; three high dose dams delivered litters of two-four pups each, with total litter loss by post natal day (PND) 4 in two of these litters. Upon breeding of F1 generation none of the findings noted in F0 were reproducible, and given some were not statistically significant, they were considered equivocal. However, a clear NOAEL for reproductive/developmental toxicity was considered to be the mid dose of 20 mg/kg/day (Knapp, 2007).

Another reproductive/developmental study of a different POEA surfactant, MON 8109 evaluated doses of 0, 30, 100, 300 and 2000 ppm in diet. A single dose group of MON 0818 at 1000 ppm in diet was also included to determine whether litter effects previously noted at this dose were treatment related (Knapp, 2008).

- MON 0818 dosed at 1000 ppm (76 and 86 mg/kg/day premating in males and females respectively) did not reveal the litter effects noted in the previous study at this dose. Two maternal incidents were not considered related to treatment; one female with dystocia died on PND 1 (this was also noted in one female of the control group F1 in the previous study at the same facility) and a second female was euthanized due to a ruptured uterus on gestation day 30. No test substance-related effects were noted for systemic toxicity, reproductive endpoints, pup survival or mortality. Therefore the overall DART NOAEL for MON 0818 was considered 1000 ppm, approximately 81 mg/kg/day.
- The MON 8109 systemic toxicity NOAEL in males and females was 300 ppm, based on mean body weight loss, reduced mean body weight gain and decreased food consumption at 2000 ppm. Developmental/reproductive effects at 2000 ppm included reduced mean number of implantation sites, increased number of unaccounted for implantation sites, decreased mean litter size at PND 0, reduced mean number of births, reduced survival at PND 4 and reduced mean pup weight at PND 1. The MON 0818 reproductive/developmental NOAEL was also 300 ppm (approximately 23 mg/kg/day).

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 by experts in the field of epidemiology, Pam Mink, Jack Mandel, Jessica Lundin and Bonnielin Sceurman (Mink et al., 2011). In evaluating ADD/ADHD a positive association with glyphosate use was reported by Garry et al (2002), but cases were parent reported 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) 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), human *in utero* exposures would be very limited.

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

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 (P450scc) 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 P450scc 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 dibutyryl [$(Bu)_2$]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

MATERIALS AND METHODS

1. Test material:

Test item: Ammo, Ambush, Fusilade, Cyclone, Roundup, Banvel, Cotoran, Dual, glyphosate. Surfactants not identified or quantified in formulations.

Active substance(s):
- Ammo: **cypermethrin**: (*R,S*)-α-cyano-3-phenoxybenzyl(1*R,S*)-*cis*,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
- Ambush: **permethrin**: 3-phenoxybenzyl(1*R,S*)-*c i s* ,*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
- Fusilade: **fluazifop-*p*-butyl**: (*R*)-2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]propionic acid
- Cyclone: **paraquat**: 1,1´-dimethyl-4,4´-bipyridinium
- Roundup: **glyphosate**: *N*-(phosphonomethyl) glycine
- Banvel: **dicamba**: 3,6-dichloro-*o*anisic acid
- Cotoran: **fluometuron**: 1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl) urea

Purity:	• Dual: **metolachlor**: 2-chloro-6´ethyl-*N*-(2-methoxy-1-methylethyl)aceto-toluidine. • Ammo (300 g/L cypermethrin) • Ambush (240 g/L permethrin) • Fusilade (120 g/L fluazifop-*p*-butyl) • Cyclone (240 g/L paraquat) • Roundup (180 g/L glyphosate) • Banvel (480 g/L dicamba) • Cotoran (480 g/L fluometuron) • Dual (958 g/L metolachlor)
Source:	Glyphosate – Sigma Other pesticides – unknown source

2. Vehicle and/or positive control:

Vehicle control:	Yes (DMSO, ethanol < 0.4 %)
Positive control:	No data

3. Test system / cells / animals:

Cell culture:	Mouse MA-10 Leydig tumor cell line
Species:	Mouse
Source:	M. Ascoli, University of Iowa College of Medicine (Iowa City, IA)
Maintenance conditions:	Waymouth's MB 752/1 medium + 15% horse serum Temperature: 37° C, Atmosphere: 5% CO_2
Plate cultures #1:	75,000 cells/well in a 96-well plate. For dose–response, time–course, steroidogenic enzyme activity, reversibility, and mixture studies.
Plate cultures #2:	50 x 10^6 cells onto 25 x 25 cm tissue culture dishes. For nuclear run-on analysis.
Plate cultures #3:	1.5 x 10^6 cells into 100-mm culture dishes, Grown until 80% confluence. For the remaining studies.

4. Test methods:

Study type:	Inhibition of steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	2 or 4 h
Dose/concentration levels:	Ambush, Ammo: 5, 10, 50 µg/mL Banvel, Cotoran, Dual, Fusilade: 1, 5, 10 µg/mL Cyclone: 0.5, 1, 5 µg/mL Roundup: 12.5, 25, 50, 100 µg/mL
Treatment:	MA-10 cells were stimulated using a maximal stimulatory dose of $(Bu)_2$cAMP (1 mM). In some tests (P450scc and 3β-HSD

enzyme activity), steroidogenic substrates (22R-HC, 25 µM or pregnenolone, 10 µM) were provided.

All treatments were performed in serum-free media.

Final concentrations of the solvents DMSO and ethanol were < 0.4 %.

5. Observations/analyses:

Dose–response and time-course studies:

Measurement: Steroid levels and total protein synthesis.

Calculation: IC_{50} values (concentration that leads to an inhibition of 50%) were calculated as the slope of the linear regression line obtained from Eadie/Hofstee plots of steroidogenesis dose–response data.

Analysis: For steroid determination in Roundup-treated cells, each data point was the average ± SE of the means from at least three separate experiments in which treatments were performed in quadruplicate.

For progesterone production in cells treated with other pesticides, each data point is the mean ± SE of four replicates in a single experiment that was repeated once.

Progesterone production and total cellular protein synthesis

Radioimmunoassay (RIA).

Measurement: Quantification of progesterone

Preparation of samples: Standard curves were prepared in serum-free Waymouth's medium.

Analysis: Analysis of RIA data was performed using a computer program specifically designed for this purpose (not further specified).

Data are expressed as ng/mL media.

Determination of total cellular protein synthesis:

Measurement: Total protein content was determined using a modification of the Bradford method (no treatment with Expre^{35}S^{35}S).

Preparation of samples: After treatment, cells were solubilized in 0.25 M NaOH at 37°C. Protein was precipitated overnight at 4°C using cold 20% trichloroacetic acid (TCA). TCA-precipitable material was transferred onto glass fiber filters, rinsed with 5% TCA, dried, and counted in a liquid scintillation counter.

Analysis: Results were reported as counts per minute per mg protein (2 or 4 h).

Each data point is the mean ± SE of four replicates in a single experiment, which was performed three times.

Determination of P450scc and 3β-HSD activity and reversibility:

Measurement: <u>P450scc enzyme activity:</u> Pregnenolone in medium
<u>3β-HSD enzyme activity:</u> Progesterone in medium

Preparation: <u>Evaluation of P450scc enzyme activity</u>:
22*R*-HC was provided as substrate to MA-10 cells in the presence and absence of the xenobiotic as well as cyanoketone

	and SU 10603 (inhibitors of 3β-HSD and P450c17, respectively). Evaluation of 3β-HSD enzyme activity: pregnenolone was provided as substrate, and MA-10 cells were treated in the presence and absence of the xenobiotic
Analysis:	Each data point represents the average ± SE of the means from at least three separate experiments in which treatments were performed in quadruplicate.
Effects on enzyme and StAR expression:	Protein levels, mRNA levels, gene transcription
Isolation of mitochondria and Western blot analysis:	
Measurement:	Protein levels of P450scc, β-HSD, StAR
Preparation:	Western blot analysis of mitochondrial protein was performed. Mitochondria were isolated by homogenization of the cells followed by differential centrifugation. After detection of StAR, membranes were stripped and then successively probed with P450scc or 3β-HSD antisera.
Analysis:	The bands of interest were quantitated using a BioImage Visage 2000 imaging system. Values obtained were expressed as integrated optical density units. Each data point represents the average ± SE of the means from three separate experiments in which treatments were performed in triplicate.
Isolation of RNA and Northern blot analysis:	
Measurement:	mRNA levels of of P450scc, β-HSD, StAR
Preparation:	Total RNA was isolated using Trizol Reagent and quantitated. For Northern blot analysis 20 µg total RNA was loaded into each well. Labeling of cDNA probes for mouse StAR, P450scc, 3β-HSD, and 18S rRNA was achieved by random priming (Prime-It II; Stratagene, La Jolla, CA) using [α-32 P] dCTP (SA 3,000 Ci/mmol; New England Nuclear) according to the manufacturer's protocol. After Northern blot analysis with StAR cDNA, blots were stripped and then successively probed with P450scc, 3β-HSD, and 18S rRNA cDNA.
Analysis:	The bands of interest (RNA) were quantified. Each data point represents the average ± SE of the means from three separate experiments in which treatments were performed in triplicate.
Gene expression:	
Measurement:	StAR, P450scc
Isolation of nuclei:	
Preparation:	After treatment, cells were harvested with a rubber policeman and centrifuged. The cell pellet was resuspended and homogenized. The homogenate was layered and centrifuged. The supernatant was discarded and the pellet containing nuclei

was resuspended, frozen on dry ice, and stored in liquid nitrogen.

Nuclear run-on analysis:

Measurement: Radioactivity was detected using a Phosphorimager 445 SI.

Analysis: Signals were quantitated using ImageQuant version 4.1 software in volume mode, which integrates the intensity of each pixel within the defined area.

Values were obtained as arbitrary units. Each data point represents the average ± SE of five separate experiments.

Protein kinase A (PKA) activity determination:

Measurement: PKA activity was measured with the SignaTECT cAMP-dependent protein kinase assay system.

Analysis: Three separate experiments were performed in which treatments were performed in triplicate.

Mixture studies:

Measurement: Progesterone was measured.

Analysis: Each data point represents the average ± SE of the means from three separate experiments in which treatments were performed in triplicate.

Statistics: Statistically significant differences were determined by one-way analysis of variance and Fisher–protected least-square difference multiple comparison using the software program Statview SE + Graphics.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable with restrictions – Not reliable for Roundup**

Comment: Non-standard test systems, but publication meets basic scientific principles. However, surfactant blend in Roundup confounds results.

2. 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. Roundup data unreliable for endpoints measured, due to mitochondrial membrane damage.

3. Klimisch code: **2 for glyphosate data, 3 for Roundup data**

Response - GTF

- 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).
    - 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) 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 mitrchondrial 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).

- Given the significant differences in physico-chemical properties between glyphosate and formulation surfactants, environmental fate and transport of these compounds are likely to be different. Likewise, absorption, distribution, metabolism and excretion (ADME) differences between glyphosate and formulation surfactants at low concentration exposures in the field, environment or food residues will very likely result in insignificant concomitant physiological exposures.

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

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 lae*V*is* 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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Classic ®; Glyphosate
Active substance(s):	Glyphosate
Source:	Roundup Classic ®: Monsanto Glyphosate: Sigma Aldrich
Purity:	Roundup Classic ®: 48% (w/v) glyphosate salt Glyphosate: not reported

2. Positive control: Specified under the respective test

3. Test organisms and systems:

Species:	*Xenopus laevis*
Embryo culture:	*Xenopus laevis* embryos obtained by in vitro fertilisation
Source:	Not specified
Culture conditions:	Embyos were incubated in 0.1 x modified Barth´s saline (MBS)
Species:	Chicken

Strain:	White Leghorn
Source:	Not specified
Stage:	Egg (fertilized)
Guideline:	Non-guideline tests
GLP:	No
Guideline deviations:	Not applicable
***Xenopus* embryo Culture and Treatments:**	
Stage of embryos:	2 cell
Dose levels:	1/3000, 1/4000, and 1/5000-dilutions of Roundup Classic® prepared in 0.1× MBS (modified Barth's saline)
Treatment:	Treatments were performed from the 2-cell stage.
Rescue experiments:	0.5 or 1 µM Ro-415253 was added at the 9-cell stage
Culture conditions:	Embryos were incubated in 0.1 x MBS. Cyclopamine was used at 100 µM concentration in 0.1 x MBS and was applied from the 2-cell stage until fixation. Embryos were fixed in MEMFA when sibling controls reached the desired stage.
Negative control:	Not adequately described
Positive control:	None
Xenopus Embryo Injections, Whole Mount in Situ Hybridization and Cartilage Staining:	
Dose levels:	360 or 500 pg of glyphosate (N-(phosphonomethyl) glycine (Sigma 337757).
Exposure route:	injection
Stage of embryos:	2 cell
Treatment:	Embryos were injected with 360 or 500 pg of glyphosate (N-(phosphonomethyl) glycine (Sigma 337757) per cell into one or both cells at the 2-cell stage. Glyphosate was coinjected with 10 ng of Dextran Oregon Green (DOG, Molecular Probes) to identify the injected side.
Culture condition:	Embyos were incubated in 0.1 x MBS. And fixed in MEMFA when sibling controls reached the desired stage.
In situ hybridisation:	Wholemount in situ hybridisation (WMISH) was performed with digoxigenin-labeled antisense RNA probes, but without the proteinase K step. Embryos were fixed in MEMFA at stages 45-47, washed with PBS, stained overnight in 0.04 %Alcian Blue, 20% acetic acid, and 80 % ethanol. Afterwards embryos were washed.
Detection of RA Activity:	
Dose levels:	1/3000, 1/4000, and 1/5000 Roundup Classic® dilutions
Exposure route:	injection
Stage of embryos:	1-2 cell
Treatment:	Embryos were injected with 320 pg of the plasmid RAREhplacZ (RAREZ) per cell into one cell at the 2-cell stage and placed immediately in the test substance dilutions
Negative control:	Negative control was not evaluated with vehicle injection.

	Therefore effects of decreased pH or vehicle coformulant (Dextran Orange Green) were not assessed.
Positive control:	Xenopus embryos were injected with the RAREZ plasmid and incubated at late blastula stage with 0.5 or 5 μM all-transretinoic acid (RA, Sigma R2625).
Rescue experiment:	Embryos injected with the reporter plasmid were incubated in a 1/4000 test substance dilution from the 2-cell stage, and when they reached the blastula stage, 1 μM of Ro 41-5253 was added.

Treatments of Chicken Embryos:

Stage:	Egg
Dose levels:	20 μL of 1/3500 or 1/4500 dilutions of Roundup Classic®.
Treatment:	Injection after opening a small window in the shell of fertilized chicken eggs, above the air chamber in the inner membrane. After injection the window was sealed with transparent adhesive tape
Negative control:	Injected with 20 μL of H2O without pH or osmolality adjustment
Positive Control	None
Pre-incubation conditions:	Placement: eggs were placed with their blunt end up; Temperature: room temperature; Duration: 30 minutes.
Incubation conditions:	Light: Darkness; Temperature: 38 C; Humidity: 56-58% Rotation: regular

Whole-Mount Inmunofluorescence and WMISH of Chicken Embryos:

Treatment:	Embryos were fixed 2-4 hin freshly prepared 4% paraformaldehyde, rinsed and processed for analysis.
	Wholemount in situ hybridization (WMISH) was performed as described for Xenopus embryos, using a c-shh probe.

4. Measurements/analyses:

Measurements:	Basal luminiscence was detected in uninjected and untreated embryos.
	The endogenous RA activity was measured in embryos injected with RAREZ (plasmid RAREhplacZ).
	When sibling controls reached the neurula stages, all embryos were processed for chemiluminiscent quantitation of the reporter activity by using the β-gal reporter gene assay (Roche).
	Luminiscence was measured on duplicate samples in FlexStation 3 equipment (Molecular Devices), and values were normalized by protein content.
Statistics:	A two-tailed t-test was employed to analyze the significance in the difference of the means. The experiment was repeated three times.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Non-guideline study that is not sufficiently described for assessment. Inadequate positive and negative control experiments.

2. Relevance of study: **Not relevant:** Irrelevant routes of exposure and inappropriately high doses. Test system not adequate for human risk assessment.

3. Klimisch code: **3**

Response 1 – summarized from Williams et al. (2012)

- 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. (2012) 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 (2012) 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.) and birth malformations. In the cited study (Benitez et al.), 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)

- 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)

- 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."

Summaries of the follow up published letters to the Editor by Mulet, Palmer follow

Author(s)	Year	Study title
Mulet, J.M.	2011	Letter to the Editor Regarding the Article by Paganelli et al. (2010) Chemical Research in Toxicology Volume: 24 Number: 5 Pages: 609

Abstract

No abstract.

[The author of the letter states that the study of Paganelli et al., 2010, about teratogenic effect of glyphosate when injected invertebrate embryos, is based on misused citations or non-peer reviewed data]

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Classic
Active substance(s):	Glyphosate
Description:	Not reported
Source of test medium:	Not reported
Lot/Batch #:	Not reported
Concentration:	480 g/glyphosate IPA salt/L

2. Studies addressed:

Paganelli et al. (Chem. Res. Toxicol. (2010), 23, 1586-1595)

In vitro teratology studies:

Xenopus embryo culture and treatments with glyphosate

Xenopus embryo treatment with glyphosate and whole-mount in *situ* hybridization and cartilage staining

Detection of RA (retinoic acid) activity

Treatment of chicken embryos with glyphosate and whole-mount immunofluorescence

KLIMISCH EVALUATION

1. Reliability of study: **Not applicable**

Comment: In this publication the author expresses some major concern about the article by Paganelli et al. (Chem. Res. Toxicol. (2010), 23, 1586-1595) in terms of over interpretation of results

2. Relevance of study: **Relevant** (no original publication but letter to the editor regarding the article by Paganelli et al., 2010)

3. Klimisch code: **Not applicable**

Author(s)	Year	Study title
Palma, G.	2011	Letter to the Editor Regarding the Article by Paganelli et al. (2010) Chemical Research in Toxicology Volume: 24 Number: 6 Pages: 775-776

Abstract

No abstract.

[The author of the letter claims that the study by Paganelli et al., 2010, 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]

MATERIALS AND METHODS

1. Test material:

Test item: Roundup Classic

Active substance(s): Glyphosate (isopropylamine salt)

Description: Not reported

Source of test medium: Not reported

Lot/Batch #: Not reported

Concentration: 480 g/glyphosate IPA salt/L

2. Studies addressed: Paganelli et al.(Chem. Res. Toxicol. (2010), 23, 1586-1595)

In vitro teratology studies: *Xenopus* embryo culture and treatments with glyphosate

Xenopus embryo treatment with glyphosate and whole-mount in *situ* hybridization and cartilage staining

Detection of RA (retinoic acid) Activity

Treatment of chicken embryos with glyphosate and whole-

mount immunofluorescence

KLIMISCH EVALUATION

1. Reliability of study: **Not applicable**

Comment: In this publication the article by Paganelli et al. (Chem. Res. Toxicol. (2010), 23, 1586-1595) is discussed in detail. The author of the letter claims that the study by Paganelli et al. contains major deficiencies and errors in terms of experimental design, descriptions of the methods used, and the interpretation of results

2. Relevance of study: **Relevant** (No original publication but letter to the editor regarding the article by Paganelli et al., 2010)

3. Klimisch code: **Not applicable**

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
Source of test item:	Glyphosate: Sigma-Aldrich, Saint Quentin Fallavier, France
Lot / Batch #:	Not specified
Purity:	not reported
Test item:	Roundup ®
Active substance(s):	Glyphosate
Source of test item:	Roundup®, (produced by Monsanto, obtained from a commercial source)
Lot / Batch #:	Not specified
Purity:	Roundup ®: 360 g/L acid

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells / animals:

Cell line:	Human choriocarcinoma derived placental cell line (ref JEG3, ECACC 92120308)
Species:	Human
Source:	CERDIC (Sophia-Antipolis, France)

Maintenance medium:	Phenol red–free EMEM containing 2 mM glutamine, 1% nonessential amino acids, 100 U/mL antibiotics (mix of penicillin, streptomycin, and fungizone), 1 mM sodium pyruvate, and 10% fetal calf serum
Cells:	Human placental microsomes
	Equine testicular microsomes
Source:	Human:
	Full-term placentas of young healthy and non-smoking women (Centre Hospitalier Régional de Caen, France) and equine testis by differential centrifugations.
	Equus:
	Equine testis
Microsome preparation:	Microsomal fractions (endoplasmatic reticulum) were obtained using differential centrifugations.
	Tissues were washed with 0.5 M KCl, homogenised in 50 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose and 1 mM DTT, and centrifuged at 20,000 g. The supernant was ultracentrifuged at 100,000 g, and the pellet was washed twice, disoolved in the same buffer containing 20% glyceol and stored at -70°C until use. All preparations steps were carried out at 4°C.

4. Test methods:

GLP:	No (for all tests)
MTT assay:	Assessment of cell viability
	Cleavage of MTT into a blue colored product (formazan) by mitochondrial enzyme succinate dehydrogenase, to evaluate JEG3 cell viability exposed to Roundup or glyphosate during various times.
Guideline:	Non-guideline assays
Guideline deviations:	Not applicable
Test substance preparations:	2% solution of Roundup and an equivalent solution of glyphosate were prepared in Eagle's modified minimum essential medium (EMEM; Abcys, Paris, France), and the pH of glyphosate solution was adjusted to the pH of the 2% Roundup solution (~ pH 5.8). Successive dilutions were then obtained with serum-free EMEM.
Dose concentrations:	In serum-containing medium (18, 24, 48 h): Roundup: 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0 % Glyphosate: 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0 % In serum-free medium: Roundup (1 h): 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 % Glyphosate (1 h): 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 % Glyphosate + Roundup 0.02% (18 h): 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 % Glyphosate + Roundup 0.1% (18 h): 0.02, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 %
Treatment:	Fifty thousand cells per well in 24-well plates were grown to 80% confluence, washed with serum-free EMEM and exposed to various concentrations of Roundup or equivalent glyphosate concentrations

Incubation conditions:	Cells were washed with serum-free EMEM and incubated with 250 µL MTT per well for 3 h at 37°C. 250 µL of 0.04 N-hydrochloric acid–containing isopropanol solution was added to each well.
Positive control:	None
Negative control:	None
Replicates per dose level:	3 x 3
Radioimmunoassay (RIA):	Measurement of aromatase activity *in vitro*
Guideline:	Non-guideline assays
Guideline deviations:	Not applicable
Dose concentrations:	In serum free medium: Roundup (1 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2 % Glyphosate (1 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8 % Roundup (18 h): 0.01, 0.02, 0.04, 0.08 % Glyphosate (18 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6 %
Positive control:	None
Negative control:	None
Incubation conditions:	Duration: 90 min Temperature: 37 C Atmosphere: 5% CO_2 200 nM androstenedione
Replicates per dose level:	3 x 3
RT-PCR:	Quantification of cytochrom P450 aromatase mRNA levels in JEG3 cells
Guideline:	Non-guideline assays
Guideline deviations:	Not applicable
Dose concentrations:	In serum free medium: Roundup (1 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2 % Glyphosate (1 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6, 0.8 % Roundup (18 h): 0.01, 0.02, 0.04, 0.08 % Glyphosate (18 h): 0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.6 %
Positive control:	None
Negative control:	None
Incubation conditions:	Duration: 90 min Temperature: 37 C Atmosphere: 5% CO_2 200 nM androstenedione
Sample preparation:	Total RNA was isolated from JEG3 cells using the guanidium/phenol/chloroform method. RNA samples were treated with DNase I at 37 C for 30 min to remove genomic DNA. Then DNase I was inactivated at 65°C for 10 min.
Tritiated water release assay:	Assessment of aromatase activity in human placental microsomes *in vitro*
Guideline:	Non-guideline assays

Guideline deviations:	Not applicable
Dose concentrations:	Roundup: 0.01, 0.06, 0.1, 0.5, 0.7, 1.0 , 3.0, 6.0 % Glyphosate: 0.01, 0.06, 0.1, 0.7, 1.0 , 3.0 %
Positive control:	None
Negative control:	None
Treatment of human microsomal fractions:	50 µg of human placental microsomes were incubated with radiolabeled androstenedione (100 pmol/tube) at 37°C for 15 min in the presence or absence of various concentrations of Roundup or glyphosate in 1 mL total volume of 50 mM Tris-maleate buffer (pH 7.4).The reaction was started by adding 100 µL of 0.6 mM H±NADPH and stopped with 1.5 mL chloroform and then centrifuged at 2,700 g at 4°C for 5 min. After adding 0.5 mL 7% charcoal/1.5% dextran T-70 solution into the preparation, the centrifugation was repeated fro 10 min.
Treatment of equine microsomal fractions:	2 µg of equine testicular microsomes were incubated for 3 min at 25°C with various concentrations of radiolabeled androstenedione (in the presence or absence of various concentrations of Roundup in 0.5 mL of H^+-NADPH containing Tris-maleate buffer (pH 7.4).
Spectral studies:	Assessment of reductase and aromatase activities
Guideline:	Non-guideline assays
Guideline deviations:	Not applicable
Dose concentrations:	Roundup: 0.1 % Glyphosate: 0.0046 %
Positive control:	None
Negative control:	None
Purification of reductase / aromatase:	Equine reductase was obtained after chromatographic separation, by ω-aminohexyl-Sepharose 4B and adenosine 2´, 5´-diphosphate agarose, respectively, hydrophobic interaction and affinity columns. Equine cytochrom P450 aromatase was purified from equine microsomes, after its separation from reductase, by successive chromatographic steps.

5. Observations/analyses:

MTT assay	
Measurements:	The optical density was measured using a spectrophotometer at 560 nm for test and 640 nm for reference.
Radioimmuno assay (RIA)	
Measurements:	The conversion of androstenedione to E1 by the aromatase complex was measured in cell supernatants by radioimmunoassay (RIA). The aromatase activity was expressed in relation to the protein concentration that was evaluated in cell extracts using bovine serum albumin as standard
RT-PCR	
Measurements:	Quantitation of mRNA by RT-PCR using M-MLV-RT (Moloney murine leukemia viruse reverse transcriptase). The absence of DNA contamination in RNA samples was

checked in controls without M-MLV-RT.

All PCR reactions were performed using an ABI Prism 7000 Sequence Detection System.

Tritiated water release assay

Measurements: Microsomal aromatase activity was evaluated by tritiated water release from radiolabeled substrate [1β-3H]-androstenedione. This method based on the stereo specific release of 1β-hydrogen from the androstenedione substrate, which froms tritiated water during aromatisation.

Aromatase activity was determined by measuring the radioactivity of the 0.5 mL aqueous phase.

Spectral studies:

Measurements: Reductase activity was determined by the measurement of the increasing absorbance of the preparation, corresponding to the reduction of the cytochrome C in the presence of H+-NADPH at 550 nm for 2 min at 37 C using a Kontron-Uvikon 860 spectrophotometer.
The absorbance of purified equine aromatase in the presence or absence of glyphosate or Roundup was recorded from 375 to 475 nm with a spectrophotometer.

The spectra of aromatase with glyphosate or Roundup alone were subtracted from the incubation spectrum.

Statistics for all tests: All data are presented as the mean ± SE. The experiments were repeated three times in triplicate unless otherwise indicated. Statistically significant differences were determined by a Student *t*-test using significance levels of 0.01 and 0.05.

KLIMISCH EVALUATION

1. 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; cytoxic 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.

2. Relevance of study: **Not relevant**: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.

3. Klimisch code: **3**

Response 1 – summarized from Williams et al. (2012)

- 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 ≤ 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)

- 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 as azole 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

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 (LD_{50}) 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

MATERIALS AND METHODS

Cytotoxicity assay

1. Test material:

Test item:	Roundup Bioforce® and glyphosate
Active substance(s):	Glyphosate
Source:	Glyphosate: Sigma-Aldrich (Saint Quentin Fallavier, France) Roundup Bioforce®: Monsanto,(Antwerp, Belgium)
Purity:	Glyphosate: not reported Roundup Bioforce® : 360 g/L acid glyphosate (equivalent to 480 g/L of isopropylamine salt of glyphosate
Lot/Batch #:	not reported
Homologation:	Roundup Bioforce® 9800036

2. Vehicle: Eagle's modified minimum essential medium (EMEM; Abcys, Paris, France)

3. Test system / cells:

Cell cultures:	Human embryonic kidney (HEK) 293 cell line (ECACC 85120602) choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308)
Species:	Human
Source:	CERDIC (Sophia-Antipolis, France)
Cell line maintenance:	phenol red-free EMEM containing 2 mM glutamine, 1% non-essential amino acid, 100 U/mL of antibiotics (mix of penicillin, streptomycin, and fungizone), and 10% fetal calf serum (Biowhittaker, Gagny, France). The JEG3 cell line was supplemented with 1 mM sodium pyruvate.
Culture conditions:	Temperature: 37°C Atmosphere: 5% CO_2, 95% air 48 h

4. Test method:

MTT assay	Assessment of cell viability
Guideline:	None guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	24-well plates, washed with serum-free EMEM
Test conditions:	A 2% solution of Roundup and an equivalent solution of glyphosate were prepared in EMEM and the pH was adjusted to about 5.8. From these stock solutions successive solutions were prepared in serum-free EMEM or serum-containing EMEM. The assays were conducted in 24-well plates. HEK 293 cells or JEG3 cells were grown to 80 % confluence, washed with serum-free EMEM and then exposed to various concentrations of Roundup Bioforce ® or the equivalent concentrations of glyphosate, in serum-free or serum-containing EMEM for 1, 24, 48 or 72 h. Afterwards cells were washed with serum-free EMEM and incubated with 250 µL MTT for 3 h at 37°C. per well. Then 250 µL of 0.04 N-hydrochloric acid containing isopropanol were added to each well, the plates were shaken. Measurements were done at 560 nm for test substance wells and at 720 nm for reference wells.
Dose levels:	0.01, 0.05, 0.1, 0.5, 0.8, 1, 2% of Roundup or equivalent concentrations of glyphosate in serum-free EMEM or serum-containing EMEM
Cells per well:	50000
Exposure duration:	1, 24, 48, and 72 h
Replicates per dose level:	9

5. Observations/analyses:

Measurements:	Cell viability
Statistics:	All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of $p < 0.01$ or $p < 0.05$.

Aromatase activity inhibition

1. Test material:

Test item:	Roundup Bioforce® and glyphosate
Active substance(s):	Glyphosate
Source:	Glyphosate: Sigma-Aldrich (Saint Quentin Fallavier, France) Roundup Bioforce®: Monsanto,(Anvers, Belgium)
Purity:	Glyphosate: not reported Roundup Bioforce® : 360 g/L acid glyphosate (equivalent to 480 g/L of isopropylamine salt of glyphosate
Lot/Batch #:	not reported
Homologation:	Roundup Bioforce® 9800036

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells:

Cell culture:	HEK 293 cell line (ECACC 85120602)
Species:	Human
Source:	CERDIC (Sophia-Antipolis, France)
Tissue for microsome preparation #1:	full-term placentas of young healthy and non-smoking women
Species:	Human
Source:	Centre Hospitalier Régional de Caen (France)
Tissue for microsome preparation #2:	Equine testis
Species:	Horse
Source:	Not reported
Microsome preparation:	Human placental and equine testicular microsomes: Tissue preparation was done by differential centrifugations. All steps were conducted at 4°C. Tissues were washed with 0.5 M KC1, homogenized in 50 mM phosphate buffer (pH 7.4) containing 0.25 M sucrose and 1 mM Dithiothreiol DTT, and centrifuged at 20,000*g*. The supernatant was then ultracentrifuged at 100,000*g*, and the final pellet was washed twice, dissolved in the same buffer containing 20% glycerol, and stored at -70 C.

4. Test methods:

Study type:	Measurement of aromatase activity by tritiated water release assay Measurement of reductase a1ctivity in purified reductasee Moieties from equine testicular microsomes
Guideline:	Non-guideline assays
GLP:	No
Guideline deviations:	Not applicable
Test conditions:	Tritiated water release assay: 293 cells were transfected with human aromatase cDNA and exposed to nontoxic concentrations of glyphosate alone or Roundup. Human placental microsomes were incubated with various concentrations of glyphosate alone or Roundup. Reductase activity: Equine testis microsomes or the purified

reductase moieties were incubated with or without Roundup

Aromatase inhibition:

Equine testicular microsomes were pre-incubated with a saturating concentration (i.e. 11.6%) or without Roundup.

Dose levels: For aromatase activity:

Glyphosate: < 0.2%
Roundup Bioforce®: 1% of product

Test substance solutions were prepared in EMEM (for 293 cells) and in 50 mM Tris-maleate buffer, pH 7.4 or without pH adjustment (microsomes)

In addition for aromatase and reductase activity:

Roundup at IC_{50} (=)

Exposure duration: Tritiated water release assay:

293 cells: 24 h
human placental microsomes: 15 min

Reductase activity:

Equine testicular microsomes: 15 min

Aromatase inhibition (pre-incubation):

Equine testicular microsomes: 30 min

Replicates per dose level: 9

5. Observations/analyses:

Measurements: Aromatase and residual aromatase activity was determined with the tritiated water release assay. Radioactivity of released tritiated water was assessed by liquid scintillation counting.

Reductase activity was determined by the measurement of the increasing absorbance of the preparation, corresponding to the reduction of the cytochrome C in the presence of H^+-NADPH at 550 nm for 2 min at 20 C using a Kontron-Uvikon 860 spectrophotometer.

Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.

KLIMISCH EVALUATION

1. 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; cytoxic 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.

2. Relevance of study: **Not relevant**: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.

3. Klimisch code: **3**

Response 1 – GTF

- 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), 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. Therfore, 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., 2010).

Response 2 – summarized from Williams et al. (2012)

- 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

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 R formulation-treated crops.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®; AMPA
Active substance(s):	Glyphosate
Source of test items:	Glyphosate: Sigma-Aldrich, France Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® (produced by Monsanto, all available on the market)
Lot/Batch #:	Not specified
Purity:	Glyphosate: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450)

Homologation:	Roundup Express®: 2010321 Bioforce® or Extra 360: 9800036 Grands Travaux®: 8800425 Grands Travaux plus®: 2020448
Test item:	AMPA (aminomethylphosphonic acid)
Source:	Sigma-Aldrich (Saint Quentin Fallavier, France)
Lot / Batch #:	Not reported
Purity:	Not reported
Test item:	Polyethoxylated tallowamine (POEA)
Source:	Pr. R. Bellé (UMR 7150 CNRS/UPMC, Station Biologique de Roscoff, France)
Lot / Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells:

Primary cell culture:	HUVEC (human primary cells of the umbilical vein cord endothelial cells)
Source:	Lonza
Culture conditions:	Specific endothelial growth medium EGM-2 SingleQuots (CC-4176) containing hEGF, hydrocortisone, GA-1000 (Gentamicin, Amphotericin-B), FBS (fetal bovine serum), VEGF, hFGF-B, R3-IGF-1, ascorbic acid, and heparin. Cells were grown in 48-well plates over a period of 24 h at 37 °C (5% CO_2, 95% air) to a confluence of 80%. Afterwards they were washed with serum-free EGM-2.
Cell lines:	Human embryonic kidney 293 cell line (ECACC 85120602) Human choriocarcinoma-derived placental JEG3 cell line (ECACC 92120308)
Source:	CERDIC (Sophia-Antipolis, France)
Culture conditions:	Phenol red-free Eagle's modified minimum essential medium (EMEM; Abcys, Paris, France) containing 2 mM glutamine, 1% nonessential amino acid, 100 U/mL antibiotics (a mix of penicillin, streptomycin, and fungizone; Lonza), 10 mg/mL of liquid kanamycin (Dominique Dutscher, Brumath, France), and 10% FBS (PAA, les Mureaux, France). The JEG3 cell line was supplemented with 1 mM sodium pyruvate. 50000 cells were grown at 37°C (5% CO_2, 95% air) over a 48 h period to 80% confluence and were washed with serum-free EMEM.

4. Test methods:

MTT assay:	Assessment of cell viability
ToxiLight® assay:	Bioluminescent assay for quantitative measurement of cell membrane damage
Caspase-Glo® 3/7 assay	Assessment of caspase activity or apoptosis induction
Microscopy:	Assessment of cell viability due to cell morphology
Guideline:	Non-guideline assays
GLP:	No

Guideline deviations:	Not applicable
Cell treatments for all tests:	Cells were exposed to various dilutions of the four Roundup formulations, glyphosate, AMPA and POEA in serum-free medium for 24 hours. In another case, cells were incubated with glyphosate, AMPA, and POEA mixtures by pairs at the final nontoxic dilution on SD (succinate dehydrogenase) of 0.5% on the human cell lines (293 or JEG3) and 0.05% on the human primary cells (HUVEC) in comparison to Roundup Bioforce or Extra 360.
Dose levels:	Roundup formulations, glyphosate, AMPA and POEA: 14 concentrations ranging from 10 ppm to 2 % Additional AMPA concentrations: 4, 6, 8 and 10% POEA concentrations. 1 and 5 ppm Combined exposures of G, AMPA and POEA mixtures: For the two cell lines, the first mixture was the combination of glyphosate (0.4999%) with POEA (0.0001%); the second was the combination of glyphosate (0.4%) with AMPA (0.1%), and the third was AMPA (0.4999%) plus POEA (0.0001%). Combined exposures of G, AMPA and POEA mixtures: For the primary HUVEC cells, the first mixture was glyphosate (0.04999%) with POEA (0.0001%); the second was glyphosate (0.04%) with AMPA (0.01%), and the third was AMPA (0.04999%) plus POEA (0.0001%).
Test conditions:	MTT assay: After treatment for 24 h the supernants were recovered for the ToxiLight bioassay, and adherent cells were washed with serum-free medium and incubated with 200 µL MTT per well. The plates were incubated for 3 h at 37°C. Afterwards 200 µL of 0.04 N-hydrochloric acid containing isopropanol were added, the plates were shaked. Optical density was measured at 570 nm. ToxiLight assay: After 24 h exposure the 50 µL of the above mentioned supernants were added to a 96-well plate and incubated under agitation with 50 µL AK detection reagent (AKDR) for 15 minutes protected from light. The luminescence was measured using a luminometer at 565 nm. Serum-free medium served as negative control. Serum-free medium served as negative control. The positive control was the active reagent AKDR mixed with cells treated in serum-free medium. Caspase-Glo® 3/7 assay: This assay was used for caspase activity or measurement of apoptosis induction. After treatment of 50 µL cell cultures to various dilutions of test items as described above, 50 µL/well of Caspase-Glo® 3/7 reagent was added and plates were incubated for 15 minutes at room temperature protected from light before luminescence was measured. Serum-free medium served as negative control. The positive control consisted of the active reagent mixed with cells treated in serum-free medium. The luminescence was measured using a luminometer at 565 nm. Cell Microscopy: At the end of the 24 h treatments, the serum-free medium was removed, and cells were fixed in absolute ethanol –chloroform – acetic acid (6:3:1, v/v/v) for 1 day at -

20°C. Each well was washed with PBS (pH 7.4) and incubated with 1 µg/mL DAPI solution. Staining of DNA with DAPI was examined using a fluorescence microscope.

Replicates per dose level: 3

5. Observations/analyses:

Measurements: Cell viability, membrane damage, apoptosis induction, cell morphology

Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01.

KLIMISCH EVALUATION

1. Reliability of study: **Not Reliable**

Comment: Exceedingly high doses above the limit dose for this study type. Inappropriate test system for formulations containing surfactant; cytoxic 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. No positive controls were included.

2. Relevance of study: **Not relevant** (Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants)

3. Klimisch code: **3**

Response – summarized from the French Agency for Food Safety (AFSSA, 2009)

- 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.
- Cytoxicity and induction of apoptosis may 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

Abstract*

Glyphosate-based herbicides are the most widely used across the world; they are commercialized 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 ERα, ERβ) 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

MATERIALS AND METHODS

Cytotoxicity assays

1. Test material:

Test item:	Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®
Active substance(s):	Glyphosate
Source of test items:	Glyphosate: Sigma-Aldrich, France Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® (available on the market)
Lot/Batch #:	Not specified
Purity:	Glyphosate: not reported Roundup Express®: 7.2 g/L (R7.2) Bioforce® or Extra 360: 360 g/L (R360) Grands Travaux®: 400 g/L (R400) Grands Travaux plus®: 450 g/L (R450)

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells:

Cell cultures:	Hepatoma cell line HepG2, breast cancer cell line MDA-

MB453-kb2

Species:	Human
Source:	HepG2: ECACC, Salisbury, UK MDA-MB453-kb2: ATCC, Molsheim, France
Culture conditions HepG2:	Phenol red-free EMEM containing 2 mM L-glutamin, 1% non-essential amino acid, 100 U/mL antibiotics (mix of penicillin, streptomycin, fungizone), 10 mg/mL liquid kanamycin, 10% fetal bovine serum
Culture conditions MDA-MB453-kb2:	Leibovitz-15 (L15) medium supplemented with 10% foetal calf serum. Cells were incubated at 37°C and the medium was removed every 48 h.

4. Test methods:

MTT assay:	Assessment of cell viability of HepG2 cells
ToxiLight® assay:	Bioluminescent assay for measurement of cell membrane damage of HepG2-cells
Alamar Blue® assay:	Assessment of cell viability of HepG2 cells
Caspase-Glo® 3/7 assay	Assessment of caspase activity or apoptose induction
Neutral red assay:	Assessment of cell viability of MDA-MB453-kb2 cells
Guideline:	Non-guideline assays
GLP:	No
Guideline deviations:	Not applicable
Test conditions:	MTT assay: 2% Roundup Bioforce® and an equivalent solution of glyphosate to Roundup Bioforce were prepared in serum-free medium and adjusted to pH 5.8. From these stock solutions consecutive dilutions up to 10^{-7} were used for measurement. Assays were conducted in 48-well plates. After treatment for 24 h the supernants were recovered for the ToxiLight bioassay, and adherent cells were washed with serum free medium and incubated with 120 µL MTT per well. The plates were incubated for 3 h at 37°C. Afterwards 120 µL of 0.04 N-hydrochloric acid containing isopropanol were added, the plates were shaked. Measurements were done at 570 nm. ToxiLight assay: After 24 h exposure the 50 µL of the above mentioned supernants were added to a 96-well plate and incubated with 50 µL AK detection reagent (AKDR) for 15 minutes protected from light. The luminescence was measured using a luminometer at 565 nm. Serum-free medium served as negative control. The positive control was the active reagent AKDR mixed with cells treated in serum-free medium. Alamar Blue assay: About 30000 HepG2 cells per well were grown for 24 h in 96-well plates and then exposed to 250 µL of test substance solutions for 24 h (at pH 7.4). Afterwards 100 µL of Alamar Blue solution was added to each well and incubated for 2 h at 37°C. The optical density was measured at 540 and 620 nM. The viability was expressed as percentage of the control results (medium only). Caspase-Glo® 3/7 assay: This assay was used for caspase activity or measurement of apoptose induction. Cells were exposed to R450 for 24 or 48 h in 96-well plates. Afterwards

	50 µL/well of Caspase-Glo® 3/7 reagent was added and plates were incubated for 45 minutes at room temperature protected from light before luminescence was measured. Serum-free medium served as negative control. The positive control consisted of the active reagent mixed with cells treated in serum-free medium. Neutral red assay: about 50000 MDA-MB453-kb2 cells were seeded in 24-weel plates and grown for 24 h at 37°C. Afterwards cells were exposed to test substance solutions for 24 h. Cells were washed and incubated with neutral red solution for 3 h at 37°C. After a further washing the viability was assessed by fluorescence measurement.
Dose levels:	Glyphosate: not reported Roundup Express®: 7.2 g/L Bioforce® or Extra 360: 360 g/L Grands Travaux®: 400 g/L Grands Travaux plus®: 450 g/L
Replicates per dose level:	4 x 3 replicates

5. Observations/analyses:

Measurements:	Cell viability, membrane damage, apoptose induction
Statistics:	All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.

Genotoxicity test

1. Test material:

Test item:	Grands Travaux®
Active substance(s):	Glyphosate
Source of test items:	Grands Travaux® (available on the market)
Lot/Batch #:	Not specified
Purity:	400 g/L

2. Vehicle and/or positive control: medium / Benzo[a]pyrene 50 µM

3. Test system / cells:

Cell cultures:	Hepatoma cell line HepG2
Species:	Human
Source:	HepG2: ECACC, Salisbury, UK
Culture conditions HepG2:	Phenol red-free EMEM containing 2 mM L-glutamin, 1% non-essential amino acid, 100 U/mL antibiotics (mix of penicillin, streptomycin, fungizone), 10 mg/mL liquid kanamycin, 10% fetal bovine serum

4. Test methods:

Study type:	Single-cell gel electophoresis assay (Comet assay)
Guideline:	Non-guideline assay The assay was conducted according to the method developed by Singh et al., 1988, with some modifications for cell preparation (Valentin-Severin et al., 2003).

	(Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 84–191. Valentin-Severin, I., Le Hegarat, L., Lebon, A.M., Lhuguenot, J.C., Chagnon, M.C., 2003. Use of hepG2 cell line for direct or indirect mutagens screening: comparative investigations between comet and micronucleus assay. Mut. Res. 536, 79-90)
GLP:	No
Guideline deviations:	Not applicable
Dose levels:	1, 2.5, 5, 7.5, 10 ppm
Exposure duration:	24 h
Replicates per dose level:	3 x 2 replicates
Analysed cells per replicate:	100

5. Observations/analyses:

Measurements:	Observed nuclei were classified into 4 classes: 0 (undamaged), 1 (minimum damage), 2 (medium damage) and 3 (maximum damage)
Statistics:	All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.

<u>Aromatase disruption</u>

1. Test material:

Test item:	Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®
Active substance(s):	Glyphosate
Source of test items:	Glyphosate: Sigma-Aldrich, France Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® (available on the market)
Lot/Batch #:	Not specified
Purity:	Glyphosate: not reported Roundup Express®: 7.2 g/L Bioforce® or Extra 360: 360 g/L Grands Travaux®: 400 g/L Grands Travaux plus®: 450 g/L

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells:

Cell cultures:	Hepatoma cell line HepG2
Species:	Human
Source:	HepG2: ECACC, Salisbury, UK
Culture conditions HepG2:	Phenol red-free EMEM containing 2 mM L-glutamin, 1% non-essential amino acid, 100 U/mL antibiotics (mix of penicillin, streptomycin, fungizone), 10 mg/mL liquid kanamycin, 10% fetal bovine serum

4. Test methods:

Study type:	Measurement of aromatase activity by tritiated water release assay, semi-quantitative RT-PCR
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Test conditions:	Tritiated water release assay: HepG2 cells were exposed to non-toxic concentrations of glyphosate alone or Roundup. RT-PCR: HepG2 cells were exposed to non-toxic concentrations of glyphosate alone or Roundup. RNA was extracted and reverse transcribed (using 200 U MMLV-RT at 42°C for 60 min). The resulting cDNA was subjected to RT-PCR.
Dose levels:	Glyphosate: 0.06, 0.2, 0.3% Roundup Express®: 0.3, 0.5, 0.8% of product Bioforce® or Extra 360: 0.08, 0.1, 0.3% of product Grands Travaux®: 0.001, 0.003, 0.005 % of product Grands Travaux plus®: 0.005, 0.007 % of product
Exposure duration:	24 h
Replicates per dose level:	4 x 3 replicates

5. Observations/analyses:

Measurements:	Tritiated water release assay: radioactivity of released tritiated water was assessed by liquid scintillation counting. RT-PCR: Aromatase mRNA levels were normalised with control gene GAPDH and analysed photographically.
Statistics:	All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01 or 0.05.

<u>Anti-estrogenic and anti-androgenic effects</u>

1. Test material:

Test item:	Glyphosate, Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus®
Active substance(s):	Glyphosate
Description:	
Source of test items:	Glyphosate: Sigma-Aldrich, France Roundup Express®, Bioforce® or Extra 360, Grands Travaux®, Grands Travaux plus® (available on the market)
Lot/Batch #:	Not specified
Purity:	Glyphosate: Roundup Express®: 7.2 g/L Bioforce® or Extra 360: 360 g/L Grands Travaux®: 400 g/L Grands Travaux plus®: 450 g/L

2. Vehicle and/or positive control: Medium / ICI 182 x 780 (10^{-8}M) and Nilutamide (10^{-6} M)

3. Test system / cells:

Cell cultures:	Hepatoma cell line HepG2, breast cancer cell line MDA-

MB453-kb2

Species: Human

Source: HepG2: ECACC, Salisbury, UK
MDA-MB453-kb2: ATCC, Molsheim, France

Culture conditions HepG2: Phenol red-free EMEM containing 2 mM L-glutamin, 1% non-essential amino acid, 100 U/mL antibiotics (mix of penicillin, streptomycin, fungizone), 10 mg/mL liquid kanamycin, 10% fetal bovine serum
For anti-estrogenic activity, HepG2 cells were grown in phenol red-free MEM

Culture conditions MDA-MB453-kb2: Leibovitz-15 (L15) medium supplemented with 10% foetal calf serum. Cells were incubated at 37°C and the medium was removed every 48 h.

4. Test methods:

Gene-receptor tests with luciferase activity measurement

Guideline: Non-guideline assays

GLP: No

Guideline deviations: Not applicable

Test conditions: Anti-estrogenic activity test: 120000 HepG2-cells per well were grown at 37°C (5% CO_2, 95% air) in MEM supplemented with 2 mM glutamine, 1% non-essential amino-acis and 10% of dextran-coated charcoal foetal calf serum in 24-well plates. After 24 h the cells were transfected with a mixture of 5 different plasmids (ERE-TK, hERα, hERβ, pCMVβGal and psG5) and incubated for 1 h at 37°C (5% CO_2, 95% air). Afterwards the medium was removed and replaced by1 mL of medium without foetal calf serum and incubated for further 24 h. Cells were co-treated with the test substance solutions and β-estradiol (10^{-8} M). ICI 182 x 780 (10^{-8} M) served as positive control. At the end of treatment cells were lysed with Reporter lysis buffer and frozen at -80°C for at least 30 min, and prepared for activity measurements.

Anti-androgenic activity test: 50000 MDA-MB-453-kb2 cells per well were grown in 24-well plates in L-15 medium without phenol-red supplemented with 5% dextran-charcoal fetal calf serum at 37°C without CO_2. After 24 h the medium was removed and cells were washed with PBS and exposed to Roundup solutions in co-treatment with DHT (4 x 10^{-10} M). Nilutamide (10^{-6} M) was used as positive control. After 24 h cells were lysed and luciferase activity was measured.

Dose levels: Anti-estrogenic activity test:
Glyphosate: 0.1, 0.2, 0.3%
Roundup Express®: 0.1, 0.2, 0.3% of product
Bioforce® or Extra 360: 0.05, 0.1, 0.15, 0.2% of product
Grands Travaux®: 0.00025, 0.0005, 0.00075, 0.001 % of product
Grands Travaux plus®: 0.001, 0.002, 0.003 % of product

Anti-androgenic activity test:
Glyphosate: 0.05, 0.1, 0.15%
Roundup Express®: 0.05, 0.1, 0.15, 0.2% of product
Bioforce® or Extra 360: 0.01, 0.02, 0.03, 0.04, 0.05% of

product
Grands Travaux®: 0.00005, 0.0001, 0.00015, 0.0002 % of product
Grands Travaux plus®: 0.001, 0.002, 0.003, 0.004 % of product

Replicates per dose level: 3 x 3 replicates

5. Observations/analyses:

Measurements: Anti-estrogenic activity test: Luciferase and β-galactosidase activities and protein level.

Luciferase activity for each treatment group was normalised to β-galactosidase activity and protein level (Luc x Prot/Gal) and compared to the control (17 β-estradiol) set at 100%.

Anti-androgenic activity test: Luciferase activity were measured and reported as a percentage of the data obtained with the androgen DHT

Statistics: All data were reported as mean ± standard error. Statistical differences were determined by Student t-test using significant levels of 0.01.

KLIMISCH EVALUATION

1. 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; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.

2. Relevance of study: **Not relevant**: Excessive doses exceed typical *in vitro* limit doses. *In vitro* test system is inappropriate with surfactants.

3. Klimisch code: **3**

Response 1 – summarized from Williams et al. (2012)

- 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)

- 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, in cluding β-galactosirase activity, cototoxicity for select assays.
  - Positive control data not reported.
  - Confusion between maximum residue levels verses systemic concentrations in humans.

Author(s)	Year	Study title
Clair, E., Mesnage, R., Travert, C., Seralini, G.E.	2012a	A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells *in vitro*, and testosterone decrease at lower levels Toxicology in Vitro Volume: 26 Number: 2 Pages: 269-279

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Bioforce® and glyphosate
Active substance(s):	Glyphosate
Description:	Not reported
Source:	Glyphosate: Sigma-Aldrich (Saint Quentin Fallavier, France) Roundup Bioforce®: not reported
Lot/Batch #:	Not reported
Purity:	Glyphosate: not reported Roundup Bioforce®: 360 g/L acid glyphosate (corresponding to 100%)
Homologation:	Roundup Bioforce® 9800036

2. Vehicle and/or positive control:

Dulbecco Modified Eagle's Medium/Ham F12 Medium (DMEM; Biotech GmbH, Dutscher, Brumath, France)

3. Test system / cells / animals:

Species:	Rat
Strain:	Sprague-Dawley

Source:	Janvier, Le Genest-Saint-Isle, France or University Centre of Biological Resources, Caen, France
Age of test animals at study initiation:	70 days ± 5
Sex:	male
Body weight:	Not reported
Acclimation period::	Not reported
Diet/Food:	Standard food, *ad libitum*
Water:	Water, *ad libitum*
Housing::	Not reported
Environmental conditions:	Temperature: 20 ± 22°C Humidity: not reported Air changes: not reported 12-hour light/dark cycle
Cell Culture:	Leydig, Sertoli and germ cells
Species:	Rat
Source:	Sprague-Dawley rats
Cell line maintenance:	DMEM/Ham F12 nutrient medium (1:1, v/v) supplemented with or without hGC (human homolog of LH physiologically involved in endocrine regulation of Leydig cells) for Leydig cells culture and with serum replacement 3 for Sertoli and germ cells.
Culture conditions: :	Temperature: 32°C Atmosphere: 5% CO_2, 95% air

4. Test methods:

Bioluminescent ToxiLight ™ bioassay:	Cytotoxicity assessment
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	96 or 24-well plates
Test conditions:	Before the assay, cells were treated with different dilutions of Roundup Bioforce ® or glyphosate ± 1 UI/mL of hCG during different exposure time points. The adenylate kinase detection reagent (AKDR) was prepared in a buffer (5 g/10 mL). Subsequently 50 mL of supernatant were transferred to an opaque black 96-well plate. 50 µL of AKDR reagent were put into each well. The plates were then left under agitation for 15 min in the dark, and light was measured using a luminometer.
Dose levels:	Not exactly specified; several concentrations from 0 – 1.0% dilutions of Roundup Bioforce® or equivalent concentrations of glyphosate in DMEM/Ham F12 medium
Cells per well:	10^5 per well in 96-well plates and 3 x 10^5 per well in 24-well plates
Exposure duration:	3, 6, 9, 12, 18, 24 or 48 h
Replicates per dose level:	9

Caspase –Glo™ 3/7 assay:	Cytotoxicity assessment, apoptose assessment
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	96 -well plates
Test conditions:	Before the assay, cells were treated with different dilutions of Roundup Bioforce® or glyphosate ± 1 UI/mL of hCG during different exposure time points. The Caspase-Glo® 3/7 reagent was prepared in a buffer. After 30 min at room temperature, 50 µL of Caspase-Glo® 3/7 reagent was added to 50 µL of culture medium containing the cells previously treated. After shaking the plate 15 min, an incubation period of 45 min at ambient temperature in the dark was required to stabilize the signal before luminescence measurement with a luminometer was performed.
Dose levels:	Not exactly specified; several concentrations from 0 – 1.0% dilutions of Roundup Bioforce® or equivalent concentrations of glyphosate in DMEM/Ham F12 medium
Cells per well:	10^5 per well in 96-well plates
Exposure duration:	3, 6, 9, 12, 18, 24 or 48 h
Replicates per dose level:	9
DAPI-labelling:	Apoptose assessment
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	24 -well plates
Test conditions:	After 24 h incubation with various dilutions of the test substances, 24-well plates were centrifuged and the medium was removed slowly. Leydig cells were fixed for a day in ab absolute ethanol-chloroform–acetic acid (6:3:1, v/v/v) at -20 °C. The wells were rinsed with PBS (pH7.4) and incubated with 1µg/mL of a solution containing DAPI during 30 min. Each well was washed with water and then observed with a microscope using a fluorescent mode.
Dose levels:	0.05, and 1 % of Roundup Bioforce® and 1% of glyphosate in DMEM/Ham F12 medium
Cells per well:	30000 per well in 24-well plates
Exposure duration:	24 h
Replicates per dose level:	9
3β-hydroxysteroid dehydrogenase (3β-HSD) activity:	Assessment of testosterone production
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	96-well plates

Test conditions:	Leydig cells were exposed to different concentrations of the test substances. Afterwards the wells containing the pretreated cells and 3β-HSD reagent containing DHEA (substrate), NAD (cofactor), NBT and nicotinamide were incubated at 37 °C for 45-60 min**.** Subsequently, as soon as the cells were stained, a solution of 10% acetic acid was added to solubilise the previously formed formazan crystals. The 3β-HSD activity was then measured by reading the optical density of each well at 560 nm (formazan) through a plate reader.
Dose levels:	Not exactly specified; several concentrations from 0 – 0.1% dilutions of Roundup Bioforce® or equivalent concentrations of glyphosate in DMEM/Ham F12 medium
Cells per well:	Not reported
Exposure duration:	24 h
Replicates per dose level:	9
Radioimmunoassay (RIA) of testosterone:	Assessment of testosterone production
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	Not reported
Test conditions:	The RIA was carried out on Leydig cells by competition and stopped using the method of activated charcoal. 200 µL of unlabeled testosterone standard solution, phosphate buffer or culture supernatant were incubated with 100 µL of radioactive testosterone and 100 µL of rabbit anti-testosterone antibody. After 30 min at ambient temperature the mixture was placed at 4 °C until the next day. Afterwards 500 µL of charcoal/dextran (50%/5%) was added and the mix incubated at 4 °C. Finally, the tubes were centrifuged (10 min at 2400 rpm at 4 °C) and the radioactivity counted.
Dose levels:	0, 0.0001, 0.0005, 0.001, 0.0025, 0.005, 0.0075 and 0.01 % dilutions of Roundup Bioforce® or glyphosate in DMEM/Ham F12 medium
Cells per well:	Not reported
Exposure duration:	24 h
Replicates per dose level:	9
Real time PCR:	Measurement of mRNA expression of aromatase, androgen receptor and estrogen receptor α- and β.
Guideline:	Non-guideline assay
GLP:	No
Guideline deviations:	Not applicable
Plate culture:	6-well plates
Test conditions:	After exposure of Leydig cells with the test substances cell pellets were treated with Trizol for the cell degradation. The chloroform was added to recover the aqueous phase containing the RNA. RNA precipitation was done by adding isopropanol and washing by adding 70% ethanol.

	250 ng of RNA , 200 U of MMLV-RT (Moloney murine leukemia virus reverse transcriptase), 0.2 g of random primers, 500 mM of each dNTP and 20 U of recombinant RNasin® were incubate 90min at 37°C to obtain cDNA, The reaction was stopped by 5 min at 75 °C. The polymerase chain reaction was performed on cDNA using the method GoTaq® qPCR Master Mix (Promega). The PCR conditions were an initial step at 95 °C for 3 min, then 40 cycles of 30 s at 95°C abd 60°C for 60 s. mRNA levels of aromatase, estrogen receptor α and β and androgen receptor were normalized using the L19 control gene.
Dose levels:	0, 0.001, 0.005 and 0.01 % dilutions of Roundup Bioforce® or glyphosate in DMEM/Ham F12 medium
Cells per well:	Not reported
Exposure duration:	24 h
Replicates per dose level:	9

6. Observations/analyses:

Measurements:	Citotoxicity of Roundup Bioforce® or glyphosate measured through adenylate kinase activities; measurements of caspases 3 and 7 (key-caspases of apoptosis) in cell cultures by means of bioluminescence-based method; study of chromatin condensation by DAPI-labelling; measurement of 3β-HSD activity; changes in testosterone production secreted from Leydig cells in medium
Statistics:	All data are present as means ± SEM. Statistically significant differences from controls were determined by an ANOVA test followed by Bonferroni post-test with $p<0.001$ (****), $p<0.005$ (***), $p<0.01$ (**) and $p<0.05$ (*).

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Non-guideline *in vitro* test with methodological (i.e. no positive controls included) and reporting deficiencies (e.g. dose levels not always specified).

2. Relevance of study: **Not relevant** (Due to reliability. In addition, *in vitro* data, do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment purposes.)

3. Klimisch code: **3**

Response - GTF

This publication presents no new findings relevant to the current discussions of glyphosate safety. It is clear from the previous work of Seralini and others that surfactants can injure or kill cells when applied to exposed cells living in a Petri-dish environment. It also is not surprising that injured cells demonstrate activation of injury-response systems or suffer from a general decline in a wide variety of cellular functions, including hormone production in cells which normally serve that function. 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) following worst case dietary exposure at the ADI.

The experiments reported in this publication involve two additional cell types; Leydig and Sertoli cells from rat testes. However, Petri dish experiments in a laboratory are not representative of exposures to a living animal and are not informative about real-world risks to humans. Instead, these experiments demonstrate what we already know – substances, soaps, detergents of surfactants, can injure unprotected cells *in vitro*.

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate formulation
Source:	Unknown retail supplier
Purity:	Not reported
Concentration:	15% home use preparation

2. Vehicle and/or positive control:

SFBS medium / no positive control

3. Test system/cells:

Cell line:	MCF-7
Source:	American Type Culture Collection (Rockville, MD, USA)
Growing medium:	MEM (minimal essential medium), phenol red-free MEM
Source:	Gibco (Gaithersburg, MD, USA)
Culture conditions:	Not reported
Further materials:	17β-estradiol (E2) (Sigma, St. Louis, USA), fetal bovine serum (FBS) (Summit Biotechnology, USA) RZPD microarray chips (Deutsches Ressourcencentrum für Genomforschung GmbH, Berlin, Germany) Roche´s cDNA synthesis kit (Roche) Real time PCR kit (ABI, NJ, USA); ABI 7500 Real-Time PCR

system thermocycler (ABI, USA)

4. Test method:

Study type:	*In vitro* DNA microarray analysis, quantitative real-time PCR (qrtPCR)
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Dose levels:	0.1, 0.01, 0.001 or 0.0001% dilutions of the glyphosate stock solution containing 15% glyphosate.
Duration of exposure:	18 h
Exposure:	MCF-7 cells were grown in MEM in T-150 vented culture flasks. Upon reaching 60% confluency, the medium was removed and replaced with phenol red-free MEM containing 10% stripped fetal bovine serum (SFBS), to reduce the E2 availability to the cells. After a growing period of 24 hours the cells were treated with glyposate concentrations at 0.1, 0.01, 0.001 or 0.0001% dilutions of the stock solution (i.e. 15% glyphosate) with or without 3 x 10^{-10} M E2 for 18 hours.
DNA micro array:	Microarray analysis was performed in commercially available microarray slides. After 18 h exposure cells were harvested and RNA was purified. Closed DNA (cDNA) was generated from the isolated RNA using Roche´s cDNA synthesis kit. Cyanine-5 and cyanine-3-labeled anti-sense RNA was generated and hybridized using Wellmer´s protocol. The labelled RNA was loaded with a labelled control sample onto the array slides. Array slides were scanned in an Axon Genepix 4000B. Details of the hybridisation and scanning procedures were not reported.
qrtPCR:	Test was conducted in semi-skirted 96 well PCR plate using a commercially available PCR system
Replicates per gene of interest:	3

5. Observations/analyses:

Measurements:	Scan of microarray slides, quantitative rt-PCR
Statistics:	Statistical analysis utilized one-way ANOVA followed by Dunnett's test to analyse differences between control and chemically treated samples, with $P < 0.05$ considered to be statistically significant.

KLIMISCH EVALUATION

1. Reliability of study: **Not Reliable**

Comment: Not acceptable *in vitro* methods for test mixtures containing surfactant. Well documented study publication which meets basic scientific principles, but surfactants are inappropriate test substance in cell lines.

2. 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. *In vitro* cytotoxicity of surfactants, however, is a significant confounder in data interpretation. Data do not reflect real *in vivo* exposure situations, and therefore not relevant for human risk assessment

purposes.)

3. Klimisch code: **3**

Response - GTF

- 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.
- The authors describe a “bewildering array” of possible human health endpoints, which are conspicuously absent in the vast glyphosate toxicology data base.
- The concluding sentence, with implications of both adult and foetal cell damage, lack biological plausibility when considering glyphosate *in vivo* ADME, kinetics and toxicology data.

IN VIVO DART/ED PUBLICATIONS

Author(s)	Year	Study title
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. Volume: 30 Number: 4 Pages: 513-534

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 hypothalami-pituitary-testis axis which control the reproductive efficiency.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate (N-(phosphonomethyl) glycine)-containing pesticide Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate)-containing pesticide
Active substance(s):	Glyphosate Carbofuran
Source:	Glyphosate: Monsanto Company, USA Carbofuran: Brichima S.P.A., Italy, Brifur
Purity:	Not reported
Lot/Batch #:	Not reported

2. Vehicle: Gelatine capsule

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white
Source:	Not reported
Age of test animals at study initiation:	8 months
Sex:	Male
No. of rats:	20
Body weight:	2863 ± 59.8 g

Acclimation period:	Not reported
Diet/Food:	Ration pellets consisting of 48% berseem hay (Trifolium alexandrinum), 18% wheat bran, 16% ground corn, 14% soybean meal, 3% molasses, 0.5% salt, and 0.5% vitamins. Feed provided *ad libitum*
Water:	Water provided *ad libitum*
Housing:	Individually in cages
Environmental conditions:	Temperature: Not reported Humidity: Not reported Air changes: Not reported Light/dark cycle: Not reported

4. Test system:

Study type:	Toxic Effects of Carbofuran and Glyphosate on Semen Characteristics in Rabbits.
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	18 weeks
Pre-exposure period:	6 weeks
Duration of exposure:	6 weeks
Recovery period:	6 weeks
Dose levels:	1st group – control group; 2nd group – 1/100 LD_{50} carbofuran; 3rd group – 1/10 LD_{50} carboruran; 4th group – 1/100 LD_{50} glyphosate; 5th group – 1/10 LD_{50} glyphosate The doses of the pesticides were calculated according to the animals' body weight on the day before dosing. (The LD_{50} values of both pesticides were not reported. Dose levels were not reported as mg/kg bw/day.
Animals per dose group:	4 animals per group
Administration:	Given orally into a gelatine capsule

5. Observations/analyses:

Test substance preparations:	Stability, achieved concentrations, homogeneity not reported
Mortality:	Not reported
Clinical signs:	Not reported
Body weight:	Measured weekly in the morning before access to feed and water
Collection of test material:	Semen was collected once a week from all animals and continued throughout the 18-week experimental period
Measurement:	Volume of each ejaculate; Determination of seminal initial fructose was carried out directly after collection; Methylene blue reduction time (MBRT) was measured using

	methylene blue semen mixture in a capillary tube; Assessment of live, dead and abnormal spermatozoa were performed using an eosin-nigrosine blue staining mixture; Evaluation of sperm concentration by the improved Neubauer hemocytometer slide using weak eosin solution; Semen osmolality was determined by measuring the freezing point depression by using Osmete A (Precision Systems Inc., Sudbury, Mass., USA).
Food- and water consumptions:	Not reported
Haematology:	Not done
Clinical chemistry:	Not done
Urine analysis:	Not done
Sacrifice/pathology:	Not reported
Organ weights:	Not reported
Histology and morphometry:	Not reported
Statistics:	Data were analysed by generalized linear model procedure, Statistical Analysis System (SAS, 1984). The level of significance was reported as $P < 0.05$.

KLIMISCH EVALUATION

1. 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 LD_{50}. 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.
2. Relevance of study:	**Not relevant** (Due to very low confidence in study conduct and the inadequacy of reporting)
3. Klimisch code:	**3**

Response – summarized from Williams et al. (2000)

- 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Herbycigon
Active substance(s):	Glyphosate
Source:	Herbycigon: M.F. L. S.R.L., Argentinia
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle: Tap water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	National University of San Luis, Argentina
Age of test animals at study initiation:	Not reported
Sex:	Females
Body weight:	210-230 g
Acclimation period:	1 week
Diet/Food:	20 g of stock laboratory diet (elaborated at Cargill) per day; ingredients: meat flour, bone and meat flour, fish meal, blood flour, soybean meal, toasted soybean, soy expeller, sunflower flour, cotton flour, peanut meal, animal fat, corn, wheat, sorghum, oat, barley, wheat bran, rice bran, gluten meal, vitamins A, E, B, D3, K3, and B12, niacin, pantothenic acid, choline, ascorbic acid, bone ash, salt, calcium carbonate, oyster, manganese oxide, zinc oxide, ferrous sulfate, copper oxide, sodium selenite, iodine, and cobalt.

Water:	35 ml of potable water per day
Food and water for control group:	Low water and food (10 ml and 10 g, respectively)
Housing::	After mating, individually in cages
Environmental conditions:	Temperature: 22-25°C Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

4. Test system:

Study type:	Enzymatic activity of cytosolic enzymes in pregnant rats and fetuses
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	21 days during pregnancy
Dose levels:	0 (tap water), glyphosate solution 0.5% w/v in tap water (dose: 0.2 ml glyphosate/ml water), glyphosate solution 1% w/v in tap water (dose: 0.4 ml glyphosate/ml water)
Animals per test substance group:	8
Animals per control group:	Tap water control group: 8 Low water and low food control group: 6 The latter group received only 10 g food and 10 mL tap water per day. This treatment began in the second week after the high-dose group exhibited a decreased water- and foodintake.
Administration:	The test substance was prepared as solution in tap water. 35 mL of the test substance preparations were provided in water bottles per day and animal
Mating:	Female rats at the proeatrus stage were housed for one night with fertile males. Fertilisation was assumed by the presence of spermatozoa in the vaginal smear. That day was designated as gestation day 1.

5. Observations/analyses:

Analyses of test material preparations:	Not reported
Measurements:	Enzymatic activity of isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, malic dehydrogenase
Mortality:	Not reported
Clinical signs:	Not reported
Maternal body weight:	Measured daily
Food- and water consumptions:	Measured daily
Test substance intake:	Not reported
Haematology:	Not reported
Clinical chemistry:	Not reported
Urine analysis:	Not reported
Sacrifice/pathology:	On day 21 of gestation, rats were anesthetisied with

	diethylether. Each foetus was delivered by rapid hysterectomy, identified, weighed and then killed by decapitation. Maternal and foetal livers, hearts, and brains were immediately removed, washed in a cold saline solution, and stored at -20°C until analysis. Foetal organs were pooled.
Tissue sample processing:	Livers, hearts, and brains (0.5g/1 ml buffer) were homogenised in an Ultra Turrax with 0.5 M Tris-HClbuffer, pH 7.4 containing 1 mM dithiothreitol. Cytosolic fractions were obtained by ultra centrifugation.
Measurements (enzymatic assays):	Enzymatic activities of isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, and maic dehydrogenase were measured in the supernant by the determination of the rate of NADPH formation at 340 nm in a spectrometer. The results were expressed as µmol NADP/min/mg protein. Protein concentration was measured by Biuret reaction.
Organ weights:	Liver, hearts and brains of maternal females
Histopathology:	Not done.
Statistics:	Signifficant differences among means were considered at a level of $P < 0.05$ and identified by one-way ANOVA, Kolmogorov-Smirnov, and Newman-Keul procedures. In all the cases the variances were homogeneous.

KLIMISCH EVALUATION

1. 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).
2. Relevance of study:	**Not relevant** (Due to reliability. In addition, study was performed with a glyphosate formulation (commercialised in Argentina) and not with glyphosate)
3. Klimisch code:	**3**

Response 1 – GTF

- 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.
    - For the low dose group, based on 360 g/L glyphosate solution containing 18% surfactant, 0.1 mL glyphosate (conservatively assumed to be the formulation)/mL water = 0.018 mL surfactant/mL water. Assuming water consumption of 10 mL/day surfactant intake = 0.18 mL per rat per day. Assuming surfactant density of 1 g/mL and 250 gram rat, surfactant low dose = 720 mg/kg/day.
    - Conservative high surfactant dose estimate = 1440 mg/kg/day
    - Conservative estimate of surfactant intake is at least one order of magnitude greater than parental and DART NOAEL values reported in Williams et al. (2012).

Response 2 – summarized from Williams et al. (2012)

- 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.
- Same comments apply to Bueret et al. (2005; on-line version 2004), in which test group dams showed a 23% reduction in food consumption, 21% reduction in water consumption and 42% reduction in body weight gain versus controls.

Author(s)	Year	Study title
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® in Wistar rats Toxicology letters Volume: 142 Pages: 45-52

Abstract*

The aim of this study was to assess the teratogenicity of the herbicide glyphosate-Roundup(R) (as commercialized in Brazil) to Wistar rats. Dams were treated orally with water or 500, 750 or 1000 mg/kg glyphosate from day 6 to 15 of pregnancy. Cesarean sections were performed on day 21 of pregnancy, and number of corpora lutea, implantation sites, living and dead fetuses, and resorptions were recorded. Weight and gender of the fetuses were determined, and fetuses were examined for external malformations and skeletal alterations. The organs of the dams were removed and weighed. Results showed a 50% mortality rate for dams treated with 1000 mg/kg glyphosate. Skeletal alterations were observed in 15.4, 33.1, 42.0 and 57.3% of fetuses from the control, 500, 750 and 1000 mg/kg glyphosate groups, respectively. We may conclude that glyphosate-Roundup(R) is toxic to the dams and induces developmental retardation of the fetal skeleton.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup ®
Active substance:	Glyphosate
Source:	Monsanto of Brasil
Lot/Batch #:	BS 1096/98
Concentration:	360 g/L
Surfactant Class:	Polyoxyethyleneamine (POEA)
Concentration:	18% (w/v) (POEA)

2. Vehicle: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Department of Pharmacology, Instituto de Ciencias Basicas da Saude, Brazil
Age of test animals at study initiation:	90 days
Sex:	Male and virgin female
Body weight:	200-280 g
Acclimation period:	Not reported
Diet/Food:	Laboratory rat chow, *ad libitum*
Water:	Water , *ad libitum*

Housing:	Polyethylene (65 x 25 x 15 cm) home cages, with sawdust-covered floors
Environmental conditions:	Temperature: 22 ± 2°C Humidity: not reported Air changes: not reported 12-hour light/dark cycle

4. Test system:

Study type:	Developmental toxicity study
Guideline:	Refers to the EPA (Environmental Protection Agency), 1996. Guidelines for Reproductive Toxicity Risk Assessment-EPA/630/R-96/009, Washington, USA, pp. 1-163. (reproductive toxicity protocols; segment II).
GLP:	no
Guideline deviations:	Reduced allowed mating time
Duration of study:	From day 6 up to 15 of gestation
Dose levels:	0 (water), 500, 750 or 1000 mg/kg glyphosate-Roundup® diluted in water
Animals per dose group	Sixty pregnant rats were divided into four groups (n=15±1 per group).
Administration:	Test substance preparations were prepared by diluting the Roundup-formulation with appropriate volumes of distilled water. Applications were done once daily by oral gavage Dosing volume: 10 mL/kg bw
Mating:	3 females were placed in a cage with one male during the dark period. Females showing sperm in the vaginal sperm on the following morning were housed individually. The other females were returned to the cage of the same male, each dark period for 15 consecutive days.

5. Observations/analyses:

Test substance preparations:	Not reported
Mortality:	Assessed, but details (e.g. time points, etc) not specified.
Clinical signs:	Not reported
Body weight:	Maternal body weights were determined daily during pregnancy and lactation periods. Offspring body weights were determined in weekly intervals from lactation to puberty
Body weight gain:	The body weight noted at day 0 (sperm positive smear) in parent females was considered as 100 %. The differences observed during the study with regard to this parameter were expressed as relative weight gain.
Food- and water consumptions:	In three day intervals during pregnancy. Data presented as relative intakes without reference to how data were normalized.
Test substance intake:	Not applicable
Sacrifice/pathology:	On day 21 of gestation dams were anesthetized with a combination of 5 mg/kg bw xylazine and 90 ,g/kg bw ketamine injected intramusculary and subjected to caesarean section. The uterus was removed and weighed with its contents.

Organ weights:	The weights of the following organs were determined and relative organ-to-body weights were calculated. Maternal: heart, lungs, liver, spleen and kidney
Developmental parameters:	Number of living and death foetuses, number of implantation sites, corpora lutea, resorptionssex of pups, sex-ratio, external malformations and skeletal alterations. Reported errors include more foetuses than implantation sites in one dose group. Note artifacts from atypical fixing and staining of foetal skeletons may have caused skeletal damage.
Statistics:	Parametric data, expressed as mean ± S.E.M., were analyzed by repeated measure ANOVA or one-way ANOVA, followed by the Duncan test when appropriate. The non-parametric data, expressed as proportion or percentage, were analyzed by the x^2-test. Differences were considered to be statistically significant when $P<0.05$.

KLIMISCH EVALUATION

1. Reliability of study:	**Not reliable**
Comment:	Study design similar to US-EPA and OECD 414, with deviations (e.g. group size, inadequate dosing period) and reporting deficiencies. In addition, some methodological deficiencies (e.g. histopathological methods)
2. Relevance of study:	Relevant study type for investigating developmental endpoints, but questionable relevance of this specific study based on low reliability of data and interpretation. Test material was a formulated product, not glyphosate.
3. Klimisch code:	**3**

Response 1 – GTF

- This non-guideline prenatal developmental toxicity study with a POEA containing formulation may be compared directly with the test guideline and GLP compliant POEA rat prenatal developmental toxicity study, in which the same POEA surfactant maternal NOAEL was 15 mg/kg/day, and developmental NOAEL was considered the highest dose tested, 300 mg/kg/day.
- Approximate calculated exposures to the either glyphosate or POEA surfactant in the formulation can not be verified because the publication is unclear whether doses are based on the glyphosate content or actual formulation.
    - If based on dose levels of 500, 750 or 1000 mg/kg formulation, surfactant doses are 90, 135 and 180 mg/kg/day, well in excess of systemic maternal NOAEL value of 15 mg/kg/day reported by Williams et al. (2012).
    - If based on dose levels of 500, 750 or 1000 mg/kg glyphosate technical acid (versus the salt form in the formulated product), surfactant doses are even more extreme, approximately 250, 375 and 5000 mg/kg/day, well in excess of systemic maternal NOAEL value of 15 mg/kg/day reported by Williams et al. (2012).
- This publication reports excessively high and unrealistic exposures to the POEA surfactant in the tested formulation.
- While reporting weight gain in an atypical manner as relative %, actual reported mean body weight gains for mid and high dose groups align with the control group, while the low dose group body weight gain is approximately 20% less than the control group, indicating significant maternal toxicity in the low dose group. This significant non-dose related toxicity brings the quality and accuracy of this study into question.

Response 2 – summarized from Williams et al. (2012)

- Non-guideline prenatal developmental toxicity study design.
- Test material an unspecified commercial formulation "Roundup," which was reported to consist of 360 g/L glyphosate and 18% (w/v) POEA.
- Treatment doses unclear as to whether glyphosate or formulation concentrations.
- 15 rats per group, significantly lower than the recommended minimum of 20 litters per group in OECD 414.
- High dose group was further reduced to 7 pregnant dams due to maternal deaths.
- Few data presented in the publication.
- Unusual data presentation for body weight, food intake and water consumption, all a relative numbers without any reference to normal values.
- Fetal findings are presented as percentages or unsubstantiated mean values throughout the article, which complicates interpretation.
- Further investigation data presented notes a number of reporting errors (see Williams et al., 2012, Table 3). For example, in the 750-mg/kg/d treatment group, more fetuses than implantation sites were reported.
- Reports a dose-related increased incidence of skeletal alterations.
- Unusual methods described to fix and stain the fetal skeletons for evaluation, which may have led to artifacts that were falsely categorized as alterations (use of a a proteolytic enzyme which may have digested peptide bonds in the bone matrix). The reported skeletal alterations showed an extremely high prevalence of incomplete ossification of various bone structures, which are signs of a developmental delay that correct themselves within a brief period.
- treatment during gestation days 6-15 rather that to full term as per current test OECD 414 guidelines
- "Based on the use of these questionable methods, and the obviously flawed reporting of data, it is not possible to draw any conclusions regarding the developmental effects of "Roundup" treatment from this article. Furthermore, because a commercial formulation was used, it is not possible to attribute any observed effects to glyphosate specifically."

Author(s)	Year	Study title
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 Volume: 81 Pages: 665-673

Abstract*

Glyphosate is the active ingredient and polyoxyethyleneamine is the surfactant present in the herbicide Roundup (R) formulation commercialized in Brazil. The aim of this study was to assess the reproductive effects of glyphosate-Roundup (R) on male and female offspring of Wistar rats exposed during pregnancy and lactation. Dams were treated orally with water or 50, 150 or 450 mg/kg glyphosate during pregnancy (21-23 days) and lactation (21 days). These doses do not correspond to human exposure levels. The results showed that glyphosate-Roundup (R) did not induce maternal toxicity but induced adverse reproductive effects on male offspring rats: a decrease in sperm number per epididymis tail and in daily sperm production during adulthood, an increase in the percentage of abnormal sperms and a dose-related decrease in the serum testosterone level at puberty, and signs of individual spermatid degeneration during both periods. There was only a vaginal canal-opening delay in the exposed female offspring. These findings suggest that in utero and lactational exposure to glyphosate-Roundup (R) may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup ®
Active substance(s):	Glyphosate
Source:	Monsanto of Brazil
Lot/Batch #:	Not reported
Concentration:	360 g/L
Surfactant:	Polyoxyethyleneamine (POEA)
Concentration:	18% (w/v) POEA

2. Vehicle: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Department of Pharmacology, Federal University of Rio Grande do Sul, Brazil
Age of test animals at study initiation:	90 days
Sex:	Male and female
Body weight:	250-350 g
Acclimation period:	Not reported
Diet/Food:	standard lab rat chow (Nuvital®, Curitiba/PR, Brazil), *ad*

	libitum
Water:	Water, *ad libitum*
Housing::	Polyethylene (65 x 25 x 15 cm) home cages with sawdust-covered floors
Environmental conditions:	Temperature: 22 ± 2°C
	Humidity: not reported
	Air changes: not reported
	12-hour light/dark cycle

4. Test system:

Study type:	Reproductive toxicity
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	21-23 days during pregnancy;
	21 days during lactation
Dose levels:	0 (water), 50, 150, 450 mg/kg glyphosate-Roundup®
Mating:	3 females were placed in a cage with one male during the dark period. Females showing sperm in the vaginal sperm on the following morning were housed individually. The other females were returned to the cage of the same male, each dark period for 15 consecutive days.
Animals per dose group:	Sixty primigravid female rats were randomly divided into 4 groups of 15 animals each
Administration:	Test substance preparations were prepared by diluting the Roundup-formulation with appropriate volumes of distilled water.
	Applications were done once daily by oral gavage
	Dosing volume: 10 mL/kg bw

5. Observations/analyses:

Test substance preparations:	Not reported
Mortality:	Assessed, but details (e.g. timepoints, etc) not specified.
Clinical signs:	Assessed, but details (e.g. timepoints, etc) not specified.
Body weight:	Maternal body weights were determined daily during pregnancy and lactation periods.
	Offspring body weights were determined in weekly intervals from lactation to puberty.
Body weight gain:	The body weight noted at day 0 (first period day) in parent females was considered as 100 %, for each period. The differences observed during the study with regard to this parameter were expressed as relative weight gain.
Food- and water consumptions:	Not done
Test substance intake:	Not applicable
Haematology:	Not done
Clinical chemistry:	Not done
Hormone levels:	For determination of testosterone levels, blood was collected at termination, and the serum was removed. The samples were

	analysed in duplicate using a double-antibody according to the standard protocol for the radioimmunoassay (RIA) with Diagnostic Products Corporation testosterone kits.
Urine analysis:	Not done
Litter data:	Litter size, number of living and dead pups, viable pups, sex ratio (male/female)
Offspring development:	The development of offspring was assessed daily from lactation until puberty. The following characteristics were assessed: ears unstuck, fur emergence, incisor eruption, eye opening, testis descent (by scrotum palpation starting after the 15th postnatal day), preputial separation (by manually retracting the prepuce with gentle pressure after the 30th postnatal day) and opening of the vaginal canal (after the 30th postnatal day)
Sacrifice/pathology:	Males: One male from each litter (n = 15/group) was randomly selected for assessment of treatment-related systemic and reproductive effects at puberty (age: 65 days) and adulthood (age: 140 days). Selected males were sacrificed by thiopental anaesthesia followed by diaphragm incision. Females: One female from each litter (n = 15/group) was randomly selected for assessment of treatment-related systemic and reproductive effects at puberty (age: 65-70 days) and adulthood (age: 140 days).
Organ weights:	The weights of the following organs were determined and relative organ-to-body weights were calculated. Males: heart, lungs, liver, spleen, kidneys, adrenal glands and brain; testis, epididymis, seminal vesicle with coagulating glands (without fluid) and prostate Females: heart, lungs, liver, spleen, kidneys, adrenal glands and brain; uterus, oviducts and ovaries
Histopathology:	Five testes per dose group were fixed in Bouin´s solution immediately after removal, embedded in paraffin, sectioned at 3 µm and stained with hematoxylin/eosin. 20 essentially round seminiferus tubules per testis were analysed microscopically. The following parameters were assessed: tubule diameter, percentage of seminiferus tubules with complete spermatogenesis, presence of degenerating, sloughed and/or infiltrating cells, and absence of tubular lumen and of elongated spermatids.
Reproductive toxicity assessment:	Relative weight of the reproductive organs expressed as percentage of body weight and of reproductive indices, including sperm number per epididymis tail, daily sperm production, sperm transit, sperm morphology, testis morphology and serum testosterone level. Spermatid and sperm counts were determined.
Statistics:	Parametric data, expressed as mean ± standard error (SEM), were analyzed by repeated measure ANOVA or one-way ANOVA, followed by the Bonferroni test when appropriate. The nonparametric data, expressed as proportion or percentage, were analyzed by the chi-square test. Differences were

considered statistically significant when $P < 0.05$.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable with restrictions**

Comment: Study that does not comply with any test guideline. Reporting deficiencies. Conflicting results include decreased testes weights but increased testosterone levels in high dose. Questionable micrograph quality and interpretation may be artifacts of processing techniques. Conclusions not consistent with findings when viewed in light of dose-response or historical data for this strain of rat.

2. Relevance of study: **Not relevant** based on lack of dose-response, contradicting findings and unreliable data quality)

3. Klimisch code: **3**

Response 1 – GTF

- This non-guideline reproductive toxicity study with a POEA containing formulation may be compared to the DART NOAEL values for POEA surfactants reported in Williams et al. (2012).
- Approximate calculated exposures to the either glyphosate or POEA surfactant in the formulation can not be verified because the publication is unclear whether doses are based on the glyphosate content or actual formulation.
- If based on dose levels of 50, 150 or 450 mg/kg formulation (18% POEA), surfactant doses are 9, 27 and 81 mg/kg/day. In this case, doses are in the range of NOAEL values reported by Williams et al. (2012).
- Based on dose levels of 50, 150 or 450 mg/kg glyphosate technical acid (versus the salt form in the formulated product), POEA surfactant doses would be approximately 25, 75 and 225 mg/kg/day. In this case the low/mid doses are in the range of NOAEL values and the high dose exceeds NOAEL values reported by Williams et al. (2012).
- The findings reported by Dallegrave et al. (2007) are contrary to the GLP and guideline compliant studies reviewed by Williams et al. (2012), in which no effects on testis morphology, sperm parameters or testosterone levels were evident.

Response 2 - summarized from Williams et al. (2012)

- Non-guideline prenatal developmental-reproductive toxicity study design.
- Test material an unspecified commercial formulation "Roundup," which was reported to consist of 360 g/L glyphosate and 18% (w/v) POEA.
- Treatment doses unclear as to whether glyphosate or formulation concentrations.
- Maternal toxicity was not observed.
- Reproductive outcomes (number of pups, sex ratio, etc.) and pup weights unaffected.
- Statistical increased percentage of abnormal sperm in male offspring at the low but not medium or high dose offspring, suggesting a random finding
- Non-dose-related delay in vaginal opening in females within the normal physiological range for the species and in line with historical control data.
- Non-dose-related early preputial separation in the high dose males within the normal physiological range for the species and in line with historical control data.
- Contrary to expected outcome of early preputial separation, a statistical decrease in blood testosterone levels was also observed at puberty for high dose males.
- Decreased testosterone level was no longer evident at adulthood
- No dose-related findings in adult sperm production parameters

- Investigators fail to mention enlarged interstitial cells in the micrographs, suggesting limited experience conducting such histological examinations.
- The other reported histological interpretations, reduction in elongated spermatids and the presence of vacuolization at puberty and degeneration of the tubular lumen at adulthood, may be attributable to an artifact of tissue processing rather than exposure related effects.
- Multiple guideline study types and a subchrionic National Toxicology Program study do not report the testicular anomalies described by Dallegrave et al. (2007).

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

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 semineferous tubules' morphology, decreased significantly the epithelium height ($P < 0.001$; control = 85.8 ± 2.8 µm; 5 mg/kg = 71.9 ± 5.3 µm; 50 mg/kg = 69.1 ± 1.7 µm; 250 mg/kg = 65.2 ± 1.3 µm) and increased the luminal diameter ($P < 0.01$; control = 94.0 ± 5.7 µm; 5 mg/kg = 116.6 ± 6.6 µm; 50 mg/kg = 114.3 ± 3.1 µm; 250 mg kg = 130.3 ± 4.8 µm); (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 ± 12.9 ng/dL; 5 mg/kg = 108.6 ± 19.6 ng/dL; 50 mg/dL = 84.5 ± 12.2 ng/dL; 250 mg/kg = 76.9 ± 14.2 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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Transorb
Active substance(s):	Glyphosate
Source:	Monsanto Co., St. Louis, MO; Monsanto of Brazil Ltda, São Paulo, Brazil
Purity:	480 g/L of glyphosate (648 g/L as isopropylamine salt)
Lot/Batch #:	Not reported

2. Vehicle: Water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Not reported
Age of test animals at study initiation:	21 days
Sex:	Male
No. of rats:	68

Body weight:	Not reported
Acclimation period:	Not reported
Diet/Food:	Commercial balanced mixture for rats
Water:	Mineral water available *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 23 ± 1°C Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

4. Test system:

Study type:	Evaluation of endocrine disruption potential of glyphosate formulation by assessment of rats prepubertal reproductive development.
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	From postnatal day (PND) 23 until PND53
Dose levels:	Control group – deionized water; 5, 50 or 250 mg/kg of body weight of glyphosate-Roundup Transorb
Animals per dose group:	4 treatment groups, 17 animals per group
Animal selection	No mention of avoiding selection of siblings within the same group to control for possible litter effects
Administration:	The glyphosate-Roundup Transorb was diluted in a watery suspension and administered once a day, by gavage; Dosing volume: 0.25 mL/100 g of body weight, Application time: between 7 and 8 a.m. each day

5. Observations/analyses:

Test substance preparations:	Stability, achieved concentrations, homogeneity not reported
Mortality:	Not reported
Clinical signs:	Not reported
Body weight:	The experimental design was composed of random blocks, with the formation factor of these blocks as the body weight at the PND23. All the animals were weighed, and the average and standard deviation were calculated. The animals having body weights lower or higher than two standard deviations from the average were removed from the experiment.
Determination of puberty age:	Evaluation of the balanopreputial separation was made, which consists of the separation of the preputial membrane and the externalization from the glands of the penis. The assessment, which included gentle tissue manipulation, was performed once per day from the PND33 and was completed at the time of the balanopreputial separation. No discussion on whether this was a blinded procedure to avoid bias.
Hormone measurements:	Hormone concentrations of testosterone, estradiol and

	corticosterone in the serum were measured by radioimmuno-assay (RIA) from commercial kits (Testosterone Total Coat-A-Count, Estradiol Coat-A-Count and Coat-A-Count Corticosterone in rats, DPC, Los Angeles, CA, USA).
Food- and water consumptions:	Not reported
Haematology:	Not done
Clinical chemistry:	Not done
Urine analysis:	Not done
Sacrifice/pathology:	On PND 53. No details reported.
Organ weights:	The testes (right and left) and the adrenal glands (right and left) were weighed in absolute values and then transformed to relative weights as mg/100 g of body weight at PND53.
Histology and morphometry:	The testes and adrenal glands of all 68 animals werefixed in Bouin´s solution for 8 h, treated with alcohol, embedded in paraffin and prepared as stained laminas with hematoxylin and eosin. Laminas were analysed by light microscopy (40x and 100 x magnification). The linear morphometry from the seminiferous tubules were analysed by determining the tubular diameter (measured from the basal lamina to the basal lamina in the opposite direction), seminiferous epithelium (from the basal lamina to the neck of the elongated spermatids) and luminal diameter. Micrographs presented are of poor quality with artifacts such as shrinkage. Considered together with the natural variably in spermatogenesis of pubescent rats, the accuracy of morphometric data comes into question. For each tubule, the averages were calculated for the measurements indicated and, then, the average of each Weld was also calculated. The measurement for each animal was obtained through measure of all the analyzed Welds.
Statistics:	The variables under study were first submitted to tests of normality from Kolmogorov–Smirnov and homocedasticity by the test of Bartlet. When some of the premises of parametric testing were not obtained, non-parametric tests were chosen for subsequent averages and tests. Statistical differences were considered significant when the value of P was lower than 0.05. The values were expressed in mean (x) and standard error of the mean (±SEM). Data analysis of daily weights was performed through the two-way analysis of variance for repeated measures (MANOVA) by a general linear model (GLM). The weights were compared between diVerent groups and diVerent ages, considering the evolution expected by the body growth. The day and the weight of the complete balanoprepucial separation were compared among the groups using non-parametric analyses by the Kruskal–Wallis method followed by the post hoc Dun test. The testis and the adrenal weights were analyzed by the Kruskal–Wallis followed by the post hoc Dunn test, or by using a one-way analysis of variance (ANOVA) followed by the post hoc Tukey test. The testis measures of tubular diameter and

epithelium depth, as well as the serum concentrations of testosterone, estradiol and corticosterone, were analyzed by the ANOVA followed by the Tukey test.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Study with methodological and reporting deficiencies or conflicting findings. Eg, increased relative testicular weights, but decreased testosterone measurements.

2. 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. Test material was a formulated product, not glyphosate.

3. Klimisch code: **3**

A comprehensive review, pointing out a significant number of issues with this publication, was undertaken by experts in reproductive and developmental toxicology and endocrinology; William R. Kelce, M.S., Ph.D, Fellow ATS; James C. Lamb, IV, Ph.D, DABT and Fellow ATS; John M. DeSesso, Ph.D, Fellow ATS. Their critique is referenced in Doc L and included in Appendix K and their summary is quoted below.

> "To the uninformed reader, this manuscript by Romano et al. appears to demonstrate that exposure to Roundup Transorb alters testosterone levels and testis morphology. In this respect, the importance of these data to the scientific literature can be grossly over-interpreted by the uninformed reader. Upon closer examination, the authors have failed to provide robust data to support their conclusion that the "commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disturbances in the reproductive development of rats". The authors failed to measure many of the key parameters in the validated pubertal male assay protocol by Stoker et al., (2000a) and hence generated data that were internally inconsistent, incomplete or in error. The results lack the scientific rigor necessary to support a definitive scientific conclusion and certainly do not equal or offset previous large, definitive and GLP-compliant studies concluding that Roundup and glyphosate do not affect reproductive development."

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 Oliviera, C.A.	2012	Glyphosate impairs male offspring reproductive development by disrupting gonadotropin expression Archives of Toxicology Volume: 86 Number: 4 Pages: 663-673

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Transorb
Active substance(s):	Glyphosate (isopropylamine salt)
Source:	Monsanto Co., St. Louis, MO; Monsanto of Brazil Ltda, São Paulo, Brazil
Purity:	480 g/L of glyphosate (648 g/L isopropylamine salt)
Lot/Batch #:	Not reported

2. Vehicle: Water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Not reported

Age of test animals at study initiation:	90 days
Sex:	Female
No. of rats:	12
Body weight:	Not reported
Acclimation period:	Not reported
Diet/Food:	Commercial balanced mixture for rats, *ad libitum*
Water:	Water available *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 23 ± 1°C Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

4. Test system:

Study type:	Glyphosate effects on the reproductive development of male offspring
Guideline:	Non-guideline study
GLP:	No
Guideline deviations:	Not applicable
Duration of exposure:	From gestational day 18 to postnatal day (PND) 5
Dose levels:	Control group – deionised water; 50 mg/kg bw of glyphosate
Animals per dose group:	2 treatment groups, animals per group – not reported
Administration:	Roundup Transorb was diluted in a watery suspension and administered once a day by gavage from Gestation Day 18 to Post Natal day 5; Dosing volume: 0.25 mL/100 g bw, Application time: between 7 and 8 a.m. each day

5. Observations/analyses:

Test substance preparations:	Stability, achieved concentrations, homogeneity not reported
Mortality:	Not reported
Clinical signs:	Not reported
Body weight:	The pups were weighted at PND21 (weaning), PND30, PND40 and PND60 to compare the body growth between the groups.
Sexual partner preference:	The sexual partner preference was assessed at PND 60 by exposing male offspring from treated and non-treated mothers to female stimulus rats (i.e. ovariectomised female rats that were treated with estradiol (50 µg/kg bw s.a. 54 h before the test) and progesterone (2 mg/kg bw s.c., 6 h before test)).
Sexual behaviour:	Sexual behaviour was assessed at PND 60 by exposing the male rats to an oestrus-induced female for 40 min. Several parameters were assessed incl. Number of mounts, intromission, ejaculatory intervals, number of attempted mounts).
Determination of puberty age:	Evaluation of the balanopreputial separation (separation of the preputial membrane and externalization from the glands of the

	penis). The assessment (including gentle tissue manipulation) was performed once per day from the PND33 and was completed at the time of the balanopreputial separation.
Hormone measurements:	Hormone serum concentrations of testosterone, estradiol in the serum were measured by radioimmunoassay-assay (RIA) from commercial kits (Coat-A-Count, DPC, Los Angeles, CA, USA). The serum FSH and LH measurements were determined by chemiluminescence immunoassay using Luminex xMAP technology (Milliplex MAP rat pituitary panel, Billerica, MA, USA).
Pituitary hormone levels:	mRNA-levels of LH, FSH and GH were assessed by real-time PCR in homogenised pituitary tissues. Protein expression of LH, FSH and GH was assessed in homogenised pituitary tissues using Western-blot analysis.
Food- and water consumptions:	Not reported
Haematology:	Not reported
Clinical chemistry:	Not reported
Urine analysis:	Not reported
Sacrifice/pathology:	Not reported
Organ weights:	The testes, epididymides and seminal vesicle were weighed, and the values were converted to relative weights of mg/100 g bw at PND60. The epididymis was previously divided into three segments: caput, corpus and cauda. The seminal vesicle was weighted with fluid (undrained) and after fluid removal (drained).
Sperm evaluation:	At PND 60, the sperm counts were determined. Testes and epididymes (capus, corpus, cauda) were weighed. The tunica albuginea was removed from the testes, and the parenchyma was homogenized. The samples were then diluted 10 times in saline, and the mature spermatids resistant to homogenization were counted using a hemocytometer. Daily sperm production was calculated. The segments of the epididymis were cut with a scissor, homogenized, diluted and counted. The number of spermatozoa in each homogenate was determined and the total number of spermatozoa for the parts of the epididymis were calculated. The mean time for sperm transit through the epididymis was calculated.
Histology and morphometry:	The testes were fixed in Bouin's solution for 8 h, treated with alcohol and embedded in paraffin, and were prepared as stained laminas with hematoxylin and eosin. Laminas were analysed by light microscopy (40x and 100 x magnification). Linear morphometry of the seminiferous tubules were analyzed by determining the tubular diameter, seminiferous epithelium length and luminal diameter. For each tubule, the averages were calculated for the measurements indicated, and the average of each field was also calculated. The measurement for each animal was obtained by measuring all the analyzed fields.
Statistics:	First the Kolmogorov–Smirnov tests for normality and the

Bartlett test for homoscedasticity. For analysis of body growth the multi-way analysis of variance for repeated measures (MANOVA) by a general linear model (GLM) was used. Weights were compared between different groups and ages, considering the expected changes with age. The sexual behavior and day of PPS were compared among the groups using the Mann–Whitney *U* test. Weights of seminal vesicle (drained and undrained) were compared by paired Student's *t-test.* All other parameters were analyzed by Student's *t-test*. Statistical differences were considered significant when the value of P was < 0.05. Values were expressed as means and the standard error of the mean (±SEM) for parametric and interquartile ranges of nonparametric analysis.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: Not relevant (due to questionable dosing regimen and atypical array of endpoints measured

3. Klimisch code: **3**

This quality and value of this follow up study to Romano et al. (2010) is consistent with their previous publication. Selective literature citations in the introduction frame the basis for this research as endocrine disruption potential, referring mostly to the publications from Seralini laboratory, previously discussed. The concluding sentences inaccurately cite published *in vitro* research (Richard et al., 2005) as evidence that "women occupationally exposed to this herbicide have reproductive disorders".

From the outset, the study design and endpoint selection are not consistent with other research in the field of developmental and reproductive toxicology, suggesting a lack of experience in this well published and studied discipline. Dosing was very limited to dams, starting on gestation day 18, well after organogenisis, through post natal day 5. No controls for litter effects appear to be reported, confounding interpretation of results.

Any glyphosate exposure to offspring either before or after parturition is questionable. ADME studies with glyphosate clearly demonstrate poor absorbtion via the gastrointestinal tract, rapid excretion of systemic doses via urine and a lack of bioaccumulation. Restricted placental transfer for glyphosate is documented in an *ex vivo* human perfusion system, in which the three other compounds tested (caffeine, benzoic acid and antipyrine) demonstrated much greater transfer kinetics across the placenta (Mose et al., 2008). Given the physico-chemical properties and *in vivo* kinetics of glyphosate, exposure to offspring during lactation should be considered negligible, if any.

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) compared to Romano et al. (2012); these include increased day of preputional sepratarion (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 μmin 2012) and luminal height (94 μm in 2010; 257μm in 2012). Therefore, results are difficult to interpret, particularly for relevance to human health risk assessment. The merits of this publication should be placed in context with the quality of the authors' previous published research (Romano et al., 2010), as critiqued by experts in DART and ED above.

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various pesticides (herbicides, insecticides, fungicides, miscellaneous)
Active substance(s):	Dicamba, glyphosate, 2,4-DB, 2,4-D, MCPA, atrazine, cyanazine, carbaryl, captan
Chemical families:	Phenoxy acetic acid (phenoxy herbicides), triazine, organophosphates, thiocarbamate

2. Vehicle and/or positive control: Not applicable

3. Test group:

Number of test persons:	2110
Age:	≤ 44 years
Sex:	Females
Inclusion criteria:	The couple had to be living year round on the study farm; The wife had to be 44 years of age or younger; At least one member of the couple had to be working on the farm

4. Test system:

Type:	Retrospective epidemiological study
Collection of data:	Questionnaire
Guideline:	Non-guideline study
GLP / GCP:	no

5. Observations / analyses:

Information in questionnaires:	Demographic and lifestyle information; Pesticides currently and historically used on the farm and around the home; Medical history; Complete reproductive history farm activities
Outcome of interest in analysis:	Self reported spontaneous abortion of less than 20 weeks' gestation
Subgroups created:	Spontaneous abortions of less than 12 weeks' and 12–19 weeks' gestation
Pesticide use:	Information from the farm operator husband, and wife to construct a history of monthly agricultural and residential pesticide use
Identification of pesticides:	Using a database of registered pesticide products in Canada
Dose levels:	Not reported
Grouping of pesticides:	Major classes of use: herbicides, insecticides, fungicides, and miscellaneous others (including those that could not be classified)
Number of reported pregnancies:	3936
Number of reported spontaneous abortions:	395
Number of reported early abortions:	226
Number of reported late abortions:	169
Analysed exposure to pesticides:	For two windows: - pre-conception, the 4-month period from 3 months before conception to the calendar month of conception (consistent with potential sperm-mediated effects); - post-conception, the 3-month period from the first calendar month after conception to the end of the first trimester (consistent with a fetotoxic effect)
Number of pesticide variables:	17
Number of possible risk factors for spontaneous abortions:	21
Statistics:	Crude odds ratios (ORs) using logistic regression for each combination of pesticide unit, exposure window, and gestational age at abortion category. To explore statistical interactions between the various pesticide units and other risk factors for spontaneous abortion, we used the Classification and Regression Tree (CART) method.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: **Not relevant** (Study design is not suitable for assessment of glyphosate exposure).

3. Klimisch code: **3**

Response 1 – GTF

- This publication reports an "exploratory analysis" of pesticide exposure timing as a possible risk factor for spontaneous abortion.
- 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 spontantous abortin (OR = 1.1).
- Authors note multiple limitations of the study relating to exposure
    - likely misclassification of pesticides
    - correct assignment of exposure window to pre- or/and post-conception
- This is one of several publications arising from the Ontario Farm Family Health Study (OFFHS), in which farm couples were asked to recall on-farm activities and pesticide usage over the last 5 years. Participants were also asked to recall all pregnancy outcomes (38% of which occurred more than 10 years earlier). This information was gathered via mail questionnaires with telephone follow-up for non-respondents.
- OFFHS information gathering methodology has high potential recall bias. Blair and Zahm (1993; referenced in Doc L, available in Doc K) 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.
- Lack of control for putative pesticide effect(s) and consideration use of multiple pesticides further compromise the utility of the data set.
- Arbuckle et al. (2001) Reported findings linked preconception use of phenosyacetic acids, triazines, glyphosate and thiocarbamates with weak but stastically significant spontaneous abortions.
- Authors considered the findings reported "hypothesis generating", and cautioned that "results should be interpreted with care and tested in other studies".
-

Response 2 – Summarized from Williams et al. (2012)

- 395 spontaneous abortions were reported out of 3936 pregnancies; rate of spontaneous aborting in Arbuckle et al. (2001) 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various pesticides (herbicides, insecticides, fungicides, livestock chemicals)
Active substance(s):	Glyphosate, atrazine, 2,4-DB, 2,4-D, MCPA, dicamba, carbaryl, and other pesticides
Chemical families:	Phenoxy herbicides, thiocarbamates, organophosphates, triazines

2. Vehicle and/or positive control: Not applicable

3. Test group:

Number of test persons:	2964 couples (initial inclusion) 1898 couples (complete response)
Age:	Females: ≤ 44 years
Sex:	Males/females
Inclusion criteria:	The couple had to be living year round on the study farm; The wife had to be 44 years of age or younger; At least one member of the couple had to be working on the farm
No. of pregnancies analyzed:	3984
Miscarriage cases due to glyphosate:	17

4. Test system:

Type:	Retrospective epidemiological study
Collection of data:	Questionnaire, telephone interview, interview
Guideline:	Non-guideline study
GLP / GCP:	no

5. Observations/analyses:

Information in questionnaires:	Mother's and father's age, education, jobs outside the farm (classified as potentially hazardous or nonhazardous), tobacco use, alcohol use, caffeine use, mother's language, ethnicity, religion, parity, per capita income, child's sex, interval between conception and the survey, and the month of conception.
Classification of pregnancies:	Singleton live births, multiple gestations (twins, triplets), miscarriage (recognized pregnancy loss before 20 weeks of completed gestation), stillbirth (pregnancy loss at 20 or more weeks of completed gestation), medically induced abortion, currently pregnant, or other (ectopic pregnancy, hydatidiform mole, unknown).
Criteria for classification of pregnancies:	Singleton live births were classified as preterm if they occurred before the completion of 37 weeks of gestation and small for gestational age (SGA) if they fell below the 10^{th} percentile of birth weight for gestational age based on Canadian percentiles. Sex ratio was defined as the proportion of males among singleton live births.
Analyzed outcomes of pregnancies:	Risk of miscarriage (pregnancies ending in miscarriages, singleton live births, induced abortions, and stillbirths, as well as current pregnancies of 20 or more weeks of gestation), preterm delivery (all live births and current pregnancies of 37 or more weeks of gestational age), and SGA births (all live births of known weight and gestational age), as well as sex ratio (all live births of known sex), not addressing stillbirths and other more rare outcomes due to insufficient numbers for analysis.
Farm activities:	Over the past 5 years; for each reported activity, months of the year were asked
Activities that involve direct pesticide exposure:	Mixing or applying crop herbicides, crop insecticides and fungicides, livestock chemicals, yard herbicides, and building pesticides.
Man's exposure classification:	Based on man's experiences in the time window of 3 months before conception to the time of conception. During that time window, specific to each pregnancy, we first determined whether he had engaged in any activities associated with direct pesticide exposure for 1 or more months. Defined 2 groups of activities: - chemical activity; - nonchemical activity + no activity.
Use of protective equipment:	Information gathered on date of use, but not specified to each of the chemical activities.
Pesticide use:	Information from farm operator (who may or may not have been the male partner) regarding the application of specific pesticides on the farm in the time period of interest.

Data analysis: Unadjusted risk ratios between the potential confounders and each of the four outcomes (miscarriage, preterm delivery, SGA, and sex ratio) were calculated, starting with finely stratified exposure variables. Based on the pattern of crude results, variables were eliminated and categories of variables were collapsed to retain only those variables and strata that yielded risk ratios of less than 0.8 or greater than 1.2. For each of the pregnancy outcomes, a logistic regression model was constructed that used the reduced set of variables and category levels. Additional variables were eliminated from the logistic regression models, and categories were collapsed or converted to continuous variables as appropriate.
For each of the four outcomes, risks and relative risks were generated, contrasting exposed to unexposed groups. Men with no activity or no chemical activity served as the referent, with various subsets of men defined by activity, use of protective equipment, and farm chemical use constituting the exposed groups. Adjusted odds ratios were calculated using logistic regression models with all the predictors of each outcome described above along with the exposure of interest.
Because multiple pregnancies per woman were included, the variance estimates from the logistic regression are expected to be slightly underestimated on average. Several logistic regression analyses were conducted based on generalized estimating equations, which account for the within-woman correlation across pregnancies.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: **Not Relevant** (Study design is not suitable for assessment of glyphosate exposure).

3. Klimisch code: **3**

Response to Savitz, Arbuckle and the Onratio Farm Family Health Study (OFFHS) taken from monsanto.com
http://www.monsanto.com/products/Documents/glyphosate-background-materials/gly_reprooutcomes_bkg.pdf

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) [category 'E' in this literature review], Curtis et *al.* (1999) [outside the scope of this literature review as per the introduction describing literature review categories], and Arbuckle et *al.* (2001) [previously reviewed publication] - use data from the Ontario Farm Family Health Study (OFFHS) (Arbuckle 1994). Savitz *et al.* (1997) 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) studied whether reported pesticide use by males or females was associated with delayed pregnancy,

while Arbuckle *et al.* (2001) looked for associations between reported pesticide use and spontaneous abortion.

The OFFHS was a questionnaire-type study in which farm couples were asked to recall on-farm activities and pesticide usage on the farm during the previous 5 years. They were also asked to recall all pregnancy outcomes, 38% of which occurred more than 10 years before the survey. The farm couples lived year-round on a farm and the OFFHS investigators employed mail questionnaires to collect information about pregnancy outcomes from the mothers. Telephone follow-up was employed for non-respondents.

In the study by Savitz et *al.,* 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.,* 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) 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:

1. Uncertainty about exposure
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). 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.

2. Low biological plausibility
Biologic plausibility is an important criterion for deciding whether a reported statistical association between a pesticide and a disease is likely to be valid. Glyphosate, even at very high doses in chronic feeding studies, does not cause adverse reproductive outcomes in laboratory animals (USEPA 1993, WHO 1994). This makes statistical associations from epidemiologic studies less plausible.

3. Inaccuracy of reported pregnancy outcomes
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.

4. Confounding
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, Curtis *et al.* 1999). Glyphosate and other pesticides were weakly associated with spontaneous abortion in the study by Arbuckle (2001). 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.,* 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). 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 (USEPA 1993, WHO 1994) 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.

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Author(s)	Year	Study title
Garry, V. F. Harkins, M. E. Erickson, L. L. Long-Simpson, L. K. Holland, S. E. Burroughs, B. L.	2002b	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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Herbicides, insecticides, fumigants, fungicides
Active substance(s):	At least 15 different substances that were not further specified. (Only pesticide classes were assessed)
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	Not reported
Sex:	Males and females

4. Test system:

Study type:	Epidemological study for the assessment of birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA.
Collection of data:	Interview and questionnaire
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Inclusion criteria:	Farm families with live births fathered by a pesticide applicator
No. of live births with birth defects:	1532
No. of family participants:	695
No. of family with children:	536
No. of control persons:	None

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Confounding variables such as maternal smoking, drinking, age, and chronic diseases such as diabetes and hypertension were examined. In this retrospective study, where possible, familial genetic history (pedigree), pregnancy medication use, and nonmedicinal drug use (including vitamins) were assessed in families with birth defects. Each certified pesticide applicator was initially interviewed by phone regarding current and past pesticide use in agriculture with specific attention to product name, years used, and the number of days per year applied. Approximately 6 months later, where possible, the subject was re-interviewed by written questionnaire to document common pesticide use by pesticide class, acreage treated, type of crop, and use of personal protective gear. Overlap between the two questionnaires was intentional to validate use of pesticides by class (herbicides, insecticides, fumigants, fungicides).
Statistics:	Regression analysis, two-sided *t*-tests, and analysis of variance methods were employed. Variables considered for regression analysis included mother's age, smoking status, alcohol use, and season of conception. Chronic diseases such as diabetes, pharmacologically treated hypertension, and arthritis and occupations other than agriculture were considered separately. Specific medication use during pregnancy and dietary information were not considered in our survey. Residence at a rural site (towns with populations <3,000) or on a farm during childhood (<18 years of age) was considered a factor in some

of these statistical analyses.

Conditional logistic regression analysis for matched studies was performed with SAS statistical program. Odds ratios and 95% confidence intervals were obtained. Both univariate and multivariate analyses were done. In the pooled analysis an adjustment was made for study, study area and vital status. When risk estimates for different pesticide exposures were analysed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

KLIMISCH EVALUATION

1. 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).

2. Relevance of study: **Not relevant** (Glyphosate not mentioned.)

3. Klimisch code: **3**

Response 1 – summary from Mink et al. (2011)

- 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/AHDH 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)

- 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 that 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, fungicides, and insecticides
Active substance(s):	Various active substances that were not specified in detail
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: None

3. Test group:

Species:	Human
Age of test persons:	Exposed group to herbicides: 43.5 y; non-exposed (no pesticide use during the relevant application season): 43.0 y; non-exposed (control): 41.8 y
Sex:	Exposed group: 144 males non-exposed group: 49 males

4. Test system:

Study type:	Epidemiological study to determine male reproductive hormones and thyroid function in pesticide applicators
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Data collection:	Interview and questionnaire
Duration of study:	Not specified
Application rate:	Not reported
Persons per group:	144 exposed; 49 non-exposed (control)
Application technique:	Ground, aerial, manual, and custom ground spraying and seed treatment
Test conditions:	The test group consisted of 144 randomly selected applicators residing in the Red River Valley (RRV). Exposure occurred during the applications. The non-exposed group consisted of 49 individuals selected as the volunteers from the community blood bank. Non-exposed controls were matched by age, health, and smoking status with the pesticide applicators. Control samples taken in summer and in fall were from different subjects!
Inclusion criteria:	No chronic disease, no chronic medication, herbicide use frequency >10 day/year, no use of fungicides, or fungicide use of < 5 days/year, no or < 5 agricultural pesticide application during the last year
Blood sampling:	Exposed group: blood samples were collected in summer and fall. Non-exposed group: Blood samples were collected and processed as for the exposed group.

5. Observations/analyses:

Clinical history:	All subjects
Exposure assessment:	Past pesticide use (incl. use frequency, application technique); During and at the end of the application season , detailed assessment of used pesticides, application rates, use frequency, application techniques etc., as well as a reproductive health history of the family.
Clinical signs:	Not performed
Body weight:	Not performed
Haematology:	Not performed.
Clinical chemistry:	LH and FSH levels; Total and free testosterone levels; TSH, total and free T4 levels
Urine analysis:	Not performed.
Other:	Offspring gender ratio
Statistics:	Urban control and pesticide applicator subject comparison groups were matched by age (within 5 yr) and smoking status. Within-group hormonal measurements from summer and fall were compared using paired t-tests. Between-group

comparisons were conducted using two-tailed t-tests for significance. The criterion for significance was set at $p<0.05$. Applicators and control subjects whose values exceeded the established normal clinical range (outliers) for these hormones were treated separately in our analysis.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: Not relevant (Due to reliability. In addition, no direct assessment of glyphosate exposure was made).

3. Klimisch code: **3**

Response – GTF

- The publication brings little if any 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various chemical groups – carbamates, halogenated hydrocarbons, phosphates, pyrethroids, and endocrine disruptors (total of 327 pesticides)
Active substance(s):	Various active substances incl. glyphosate
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: None

3. Test group:

Species:	Human
Age of test persons:	18 - >35y
Sex:	Exposed group: 73 females non-exposed group: 611 females

4. Test system:

Study type:	A Case-Control Study of Pesticides and Fetal Death Due to Congenital Anomalies
Guideline:	None

GLP / GCP:	No
Guideline deviations:	Not applicable
Duration of study:	Not applicable
Application rate:	Not reported
Persons per group:	73 exposed; 611 non-exposed (control)
Application technique:	Ground and aerial spraying
Test conditions:	The exposed group consisted of 73 selected cases which were located in the same square mile or surrounding square miles from an area where the pesticides were applied. Exposure occurred during 1-20, 1-13, and 3-8 weeks of pregnancy by ground or aerial spraying. The non-exposed group consisted of 611 healthy females not exposed to the specific pesticide during the relevant time period. None of the persons (exposed, non-exposed) were involved in application of pesticides.
Case identification:	Exposed group: identified congenital anomalies in foetuses from the death certificates. Late foetal deaths after week 20 were considered. Non-exposed group: normal births defined as livebirths with no congenital malformations.

5. Observations/analyses:

Clinical history:	Exposed and non-exposed persons
Clinical signs:	Exposed persons only
Body weight:	Not performed.
Haematology:	Not performed.
Clinical chemistry:	Not performed.
Urine analysis:	Not performed.
Statistics:	Stratified analyses were used to determine which covariates had potential to be confounders. The exposure prevalence among controls and the distribution of covariates by case-control status were assessed. Stratified odds ratios (ORs) were examined to screen for potential effect modifiers. Inclusion criteria for potential effect modifiers required that stratum-specific ORs differ by 100% or more. On the basis of the results of these stratified analyses, we included no interaction term in the model. Adjusted ORs and 95% confidence intervals (CIs) were calculated using logistic regression for those exposed according to the nine-TRS definition, and again for those exposed in the one-TRS definition, separately for each of the five pesticide classes. Separate analyses for ground and aerial modes of application were also completed for those exposed in the nine TRSs. These analyses were limited to those exposed to the specific pesticide class and mode of interest. For those who returned questionnaires, an analysis that adjusted for variables not available from the birth and death certificates was conducted.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Epidemological study with methodological deficiencies (e.g. glyphosate was included in the pesticide class of phosphates, thiophosphates, phosphonates, no differentiation between single and multiple exposures, correlation, if any, only to pesticide classes and not to specific active substances)

2. Relevance of study: **Not relevant** (No glyphosate-specific results.)

3. Klimisch code: **3**

Response – summary from Williams et al. (2012)

- Classes of pesticides were evaluated in this study, with glyphosate included as one of 47 active ingredients in the broad category of "phosphates/triphosphotates/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) 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) 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

Abstract*

Pesticides associated to genetically modified foods (PAGMF), are engineered to tolerate herbicides such as glyphosate (GLYP) and gluphosinate (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

MATERIALS AND METHODS

1. Test material:

Test items / active substances:	Glyphosate;	
	Gluphosinate;	
	Bacillus thuringiensis;	
	AMPA (aminomethyl phosphoric acid)	
	3-MPPA (3-methylphosphinicopropionic acid)	
	Cry1Ab protein (Bt toxin)	
Purity:	Not reported	

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human	
Sex:	Female	
Age:	Pregnant woman:	32.4 ± 4.2 yr (mean)
	Non-pregnant women:	33.9 ± 4.0 yr (mean)
Number of test persons (pregnant):	30	
Number of control persons (non-pregnant):	39	

4. Test system:

Study type:	Maternal and foetal exposure to pesticides associated to genetically modified foods
Guideline:	Non-guideline study
GLP:	No

Guideline deviations:	Not applicable
Duration of study:	Not reported
Collection of data:	
Inclusion criteria:	Subjects were pregnant and non-pregnant women living in Sherbrooke, an urban area of Eastern Townships of Quebec, Canada. No subject had worked or lived with a spouse working in contact with pesticides. Eligible groups were matched for age and biody mass index (BMI)
BMI:	Pregnant woman: 24.9 ± 3.1 kg/m^2 (mean) Non-pregnant women: 24.8 ± 3.4 kg/m^2 (mean)
Exposure conditions:	It was assumed that the subjects were exposed due to the diet of herbicide-tolerant genetically modified crops.
Diet:	The diet taken is typical of a middle class population of Western industrialized countries. A food market-basket, representative for the general Sherbrooke population.
Additional factors:	Participants were not known for cigarette or illicit drug use or for medical condition (i.e. diabetes, hypertension or metabolic disease).

5. Observations/analyses:

Sampling:	Blood sampling was done before delivery for pregnant women or at tubal ligation for nonpregnant women and was most commonly obtained from the median cubital vein, on the anterior forearm.
Measurements:	Levels of GLYP, AMPA, GLUF and 3-MPPA were measured using gas chromatography–mass spectrometry (GC–MS). Cry1Ab protein levels were determined in blood using a commercially available double antibody sandwich (DAS) enzyme-linked immunosorbent assay.
Statistics:	PAGMP (pesticides associated to genetically modified plants) exposure was expressed as number, range and mean ± SD for each group. Characteristics of cases and controls and PAGMP exposure were compared using the Mann–Whitney U-test for continuous data and by Fisher's exact test for categorical data. Wilcoxon matched pairs test compared two dependent groups. Other statistical analyses were performed using Spearman correlations. Analyses were realized with the software SPSS version 17.0. A value of $P < 0.05$ was considered as significant for every statistical analysis.

KLIMISCH EVALUATION

1. 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.

2. 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.)

3. Klimisch code: 3

Response – Monsanto Letter to the Editor

Comment: Aris and Leblanc "Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada"

To the Editor,

We have reviewed the publication of Aris and Leblanc entitled "Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada", and wish to provide comment. The study has also been the subject of a regulatory review (FSANZ) which reached conclusions similar to our own. Findings for glyphosate and AMPA are consistent with previous publications (cquavella et al., 2003; Curwin et al., 2007), and levels detected are consistent with intakes far below any level of concern (Curwin et al., 2007). Glyphosate has not demonstrated reproductive or developmental toxicity in repeated mammalian studies. The recent inclusion of glyphosate in Tier-1 endocrine disrupter screening is the result of exposure potential, not evidence of endocrine disruption as implied by Aris and Leblanc.

Attempts to detect Cry proteins in the blood of GM-fed animals have been limited by methodological challenges and commercial immunoassay kits (as used in this study) did not produce valid results in porcine blood. An assay system validated for use in bovine blood failed to detect Cry1Ab (LOD 1 ng/mL) despite very much higher intake (as % diet or per kg body weight) than humans, making assay validation essential. The authors did not provide validation information for the Cry1Ab assay in human blood. A standard curve was said to span a range of 0.1–10 ng/mL, but no statistical limit of detection is reported. It appears that the authors have reported all signals above baseline as confirmed "detects", despite the fact that many samples have concentrations below the likely detection limit of this assay system based on our own experience. Thus, the number of Cry1Ab detects is likely overstated, probably significantly.

The antibody in the Agdia immunoassay kit is known to bind to other cry proteins, and can also bind to fragments derived from the intact protein. While protein digestion and absorption primarily takes place as mono to tri-peptides, small quantities of proteins or larger protein fragments are absorbed as a part of normal human physiology.

Cry1Ab and related proteins (which may interact in this assay system) are widely used in organic agriculture on foods intended for direct human consumption. Cry1Ab is present in GM maize intended primarily for animal feed and processing to food ingredients (corn syrup, starch, etc.), and human consumption is expected to be quite low. Further, very little corn is consumed by humans in a raw state, and cooking denatures Cry1Ab protein eliminating its biological (insecticidal) activity.

Although we believe that the reported rate of detection is elevated, it is possible that Cry1Ab (or fragments) can be found in some individuals with a sufficiently high intake and sensitive assay system. This must be put in proper perspective. Cry proteins as a class are exempt from tolerance (i.e. no maximal intake levels were set), indicating that any potentially achievable exposure raises no safety concern. The no-effect level for purified Cry1Ab in acute animal testing is 4000 mg/kg (highest level tested). For a theoretical 50 kg female, this is the equivalent of 200,000,000 _g of Cry1Ab protein. Detection of 1 ng/mL of Cry1Ab in the blood of a 50 kg female (assuming 20% extracellular fluid volume, as proteins generally do not distribute intracellularly) is crudely equivalent to 10 _g of total Cry1Ab – 20-million times less than a dose which has no discernable effect.

In short, results for glyphosate are unsurprising and raise no health concerns. Detections of Cry1Ab appear to be over-reported. Based upon the limited intake of Cry1Ab and the fact that little protein is absorbed intact, reported detections may be technical artifacts and at best represent protein fragments in addition to intact protein – the vast majority of which are expected to be biologically inactive after processing.

Cry1Ab has been subjected to extensive safety assessment accounting for human exposure with a large margin of safety. Contrary to Aris and Leblanc, available traits are approved for human consumption, even if not the primary intent of cultivation. Mammalian toxicity has not been demonstrated with Cry1Ab or related Cry proteins, and all of the women and infants were normal. The reported findings, even if they should prove to be correct, raise no safety concerns.
The authors are full-time employees of Monsanto company, a manufacturer of products incorporating glyphosate and Cry1Ab.

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Author(s)	Year	Study title
Benítez-Leite, S. Macchi, ML and Acosta, M.	2009	Malformaciones congénitas asociadas a agrotóxicos. Arch Pediatr Urug Volume: 80 Number: 3 Pages: 237-247

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

MATERIALS AND METHODS

1. Test material:

Test item:	Several pesticides were assessed but not specified.
Active substance(s):	Several active substances were assessed but not specified.
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: None

3. Test group:

Species:	Human
Age of test persons:	Newborn babies (The exposed mothers had an average age of 25 years (range: 12-45 years)) (Age of mothers from the control group not specified)
Sex:	Males and females

4. Test system:

Study type:	Epidemiological study for developmental toxicity
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Duration of study:	11 months (between March 28, 2006 and February 28, 2007)
Collection of data:	Questionnaire
Test group:	2414 newborn babies
Control group:	Controls were all healthy children of the same sex born immediately after the study period (February 28, 2007): up to 87 newborns
Application rate:	The concentration of the pesticides to which the mothers had been exposed was not specified.
Exposure frequency:	Not assessed.
Application technique:	Mainly fumigation

5. Observations/analyses:

Clinical history:	Not performed.
Clinical signs:	All persons during pregnancy.
Body weight:	Not performed.
Haematology:	Not performed.
Clinical chemistry:	Not performed.
Urine analysis:	Not performed.
Evaluation:	The test group consisted of all newborns recorded in the Regional Hospital of Encarnación during the observation period. A total of 2414 cases were recorded (mean value: 216/month). The mothers of the newborn were asked several questions such as where they live, if they store pesticides at home, if they work with pesticides, etc…. The region of Itapúa has mainly soya cultivation. Paraguay was declared by the FAO as a place of concern, since big amounts of pesticides are yearly used (approx. 24 million L of pesticides per year). Population living in the area are exposed to these agrochemicals via many pathways (mother's home proximity to treated fields, workplace, or private use of pesticides). According to the statistic conducted with the mothers 55% of them lived in urban areas, 82% worked as housewife.
Exposure situation:	19.9% of the mothers had had direct contact or accidentally with pesticides, 28.8% of the fathers had been exposed. 42.3% of the asked mothers lived near treated fields. 15.3% had pesticides at home for private use.
Record of malformations:	22 different types of congenital malformations were recorded and statistically assessed, such as ear, hand or arm malformation.
Clinical history:	Not performed.
Clinical signs:	All mothers during pregnancy.
Body weight:	Not performed.

Haematology:	Not performed.
Clinical chemistry:	Not performed.
Urine analysis:	Not performed.
Malformation assessment:	All newborns were examined for malformations 22 different types of congenital malformations were recorded and statistically assessed, such as ear, hand or arm malformation.
Statistics.	Yes, odds ratio statistic.

KLIMISCH EVALUATION

1. Reliability of study:	**Not reliable**
Comment:	Study design of epidemiological study for developmental toxicity insufficient for assessment, as well as methological 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.)
2. 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)
3. Klimisch code:	**3**

2. Literature Review of Neurotoxicity Publications

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; da Costa et al, 2003). 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). 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 (Kenborg et al., 2011) or general insecticide or herbicide exposure (Engel et al., 2001), 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). Human non-cancer epidemiologic outcomes related to glyphosate have recently been reviewed (Mink et al. 2012), 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), Negga et al. (2011) and Gui et al. (2012) all conducted glyphosate research in the above mentioned manner, all in very different test systems. Negga et al. (2011) 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. Atiz et al. (2009) and Gui et al. (2012) 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. (2003) evaluated 15 different pesticides for neurotoxic endpoints 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test person:

Sex:	Male
Age:	54 years
Body weight:	Not reported

4. Test system:

Study type:	Case report
Guideline:	Non-guideline study
GLP/GCP:	No
Guideline deviations:	Not applicable
Duration of study:	2 years
Exposure:	Acute accidental exposure of a 54-year-old male during spraying glyphosate in the garden. The man did not wear any protective gear (e.g. gloves, face mask). Exposure occurred as the breeze blew the spray back into his trunk, arms, legs, and face. The substance residues were washed off his body 30 minutes after exposure.
Application parameters:	Concentration – not reported; Total amount handled – not reported; Personal protection equipment – not worn
Additional factors:	Medical history – not reported; Lifestyle factors (smoking etc.) – not reported; Use of prescribed drugs – not reported

5. Observations/analyses:

Observations:	Six hours after exposure – severe conjunctival hyperemia and a generalised cutaneous rash One week after exposure – skin lesions became blistered; One month – rigidity and slowness in extremities; One year – slow tremor of hand, impaired short-term memory; Further on parkinsonian syndrome.
Statistics:	Not applicable

KLIMISCH EVALUATION

1. Reliability of study:	**Not assignable**
Comment:	Medical case report
2. 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.)
3. Klimisch code:	**4**

GTF Comment

- Single incident report of acute exposure to a glyphosate formulation while spraying a garden.
- See opening paragraph of this section.
- See Medical section 5.9.4 “Clinical signs and symptoms of poisoning and details of clinical tests”

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

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

MATERIALS AND METHODS

1. Test material:

Active substance(s):	**Zineb** (zinc ethylene-bis-dithiocarbamate) **Glyphosate** (N-phosphonomethyl-glycine) **Dimethoate** (O,O-dimethyl-S-methyl-carbamoyl-methyl phosphorodithioate)
Source:	Unknown local commercial sources & Instituto Nacional de Tecnologica Agropecuaria (INTA, Castelar, Argentina)
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle: Polyethylene-glycol (PEG-400)

3. Test animals:

Species:	Rat
Strain:	Wistar, SPF-free
Source:	Not reported
Age of test animals at study initiation:	Not reported
Sex:	Male
Body weight:	190 ± 20 g
Acclimation period:	1 week
Diet/Food:	Standard Purina chow from Ganave Co. (Santa Fe, Argentina)
Water:	Water, *ad libitum*
Housing::	Not reported
Environmental conditions:	Temperature: 25 ± 2°C

Humidity: Not reported
Air changes: Not reported
12-hour light/dark cycle

4. Test system:

Study type:	Sub-chronic study
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	5 weeks
Dose levels:	Untreated control; Vehicle control: polyethylene-glycol 400 (PEG-400); 15 mg zineb/kg body weight (b.w.) in PEG-400; 10 mg glyphosate/kg b.w. in PEG-400; 15 mg dimethoate/kg b.w. in PEG-400 15 mg zineb/kg b.w.+10 mg glyphosate/kg b.w. in PEG-400; 15 mg zineb/kg b.w.+15 mg dimethoate/kg b.w. in PEG-400; 10 mg glyphosate/kg b.w.+15 mg dimethoate/kg b.w. in PEG-400 15 mg zineb/kg b.w.+10 mg glyphosate/kg b.w.+15 mg dimethoate/kg b.w. in PEG-400
Animals per dose group	9 groups of 4 rats each
Exposure route:	i.p. injection
Dose volume:	Not reported
Frequency of administration:	3 times a week
Animal maintenance and handling:	According with the NIH guide for the care and use of laboratory animals

5. Observations/analyses:

Test substance preparations:	Not reported. There are also no information on achieved concentrations, homogeneity and stability of test substance preparations.
Mortality:	Not reported
Clinical signs:	Noted weekly
Body weight:	Measured weekly
Food- and water consumptions:	Assessed, but not reported
Haematology:	Not performed
Clinical chemistry:	Not performed
Urine analysis:	Not performed
Pathology:	No gross pathology performed
Organ weights:	Liver, cerebral cortes and substantia nigra of brains
Histopathology:	Not performed
Sample preparations:	Livers and brains (cerebral cortex (ventromedial areas directly connected with substantia nigra) and substantia nigra) each

	homogenized in HEPES 50 mM pH 7.4 with CHAPS 5 mM, dithiotreitol 5 mM, and aprotinin10 µg/ml, in a proportion of 300 µl buffer to 50 mg tissue. Cytosolic fractions for caspase and calpain measurements was prepared by homogenate centrifugation (20000 g, 15 min, 4°C). All samples were stored at -80°C until analyses.
	Mitochondrial fractions were prepared from all tissues by homogenisation with HEPES 10 mM, pH 7.5 containing ´mannitol 200 mM, sucrose 70 mM, and EGTA 1 mM followed by a combination of low- and high-speed centrifugation procedures. Samples of mitochondrial suspensions were treated with glutaraldehyde, impregnated and included in epoxyde-polymer for electron microscopy.
	DNA-samples were purified according to the Qiagen kit protocol after homogenisation of tissue.
Analytical methods:	To assess the integrity of the inner mitochondrial membrane (IMM), the electrochemical proton gradient ($\Delta\Psi$) was tested using a membrane potential-sensitive probe (JC-1) using MITO-ISO1 test kit, (Sigma Co.);
	The integrity of the outer mitochondrial membrane (OMM) was measured by determining the cytochromec (Cyt_c) oxidase activity, in the presence and absence of the detergent n-dodecyl β-D-maltoside using CYTOCOX1 kit (Sigma Co.);
	Milli- (m-) and micro-(µ-) calpain activities were measured in the cytoplasmic fractions. The assay involves the hydrolysis of whole ultra pure casein (Sigma, Chem. Co.) by calpain activity and the subsequent detection of trichloroacetic acid (TCA)-soluble peptidic fragments at 280 nm;
	Caspase-3 activieties were measured in tissue homogenates using a colorimetric assay kit (CASP-3-C).
	Total mitochondrial glutathione was measured by an adaptation of the Ellman's method using the purified mitochondrial suspension as sample;
	DNA fragmentation patterns were also analyzed using the DNA laddering technique;
	Protein content was determined according to the method of Lowry et al. (1951).
Lipid analysis:	Mitochondrial cardiolipin (CL) content was quantified by means of phosphorous measurement using the method of Chen et al. (1956). Colorimetric reactions were performed on lipid extracts previously obtained by the method of Folch et al. (1957). Samples were separated by high-performance thin layer chromatography (HPTLC).
Statistics:	The results were expressed as the mean ± standard error of four independent experiments. They were statistically analyzed by one-way analysis of variance (ANOVA) followed by a Tukey multiple comparison test, and were considered different with respect to control data at two levels of significance:*P<0.05 and **P<0.01.Linear and non-linear correlation coefficients were calculated.

KLIMISCH EVALUATION

1. 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

2. Relevance of study: **Not relevant** (intraperitoneal injection is a non-relevant route of exposure for humans)

3. Klimisch code: **3**

Response – GTF

- 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.
- Route of administration via intraperitoneal injection is not an appropriate route of exposure for human health or environmental risk assessment.
- 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 (*accepted manuscript*) Pages: not specified

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
Source of test items:	Sigma Aldrich (St. Louis, MO, USA)
Lot/Batch #:	Not specified
Purity:	Not specified

2. Vehicle and/or positive control: DMSO

3. Test system / cells:

Primary cell culture:	Differentiated PC12 cells
Source:	Not reported
Culture conditions:	Grown in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS, PAA), 100 U/ml penicillin, and 100 µg/ml Streptomycin (GIBCO). All cells were maintained in a humidified 5% CO_2 containing atmosphere at 37°C. For transfection experiments, PC12 cells were plated the day before transfection to achieve ~50% confluency.

4. Test methods:

Guideline:	Non-guideline assays (for all tests)
GLP:	No (for all tests)
Guideline deviations:	Not applicable (for all tests)

Preparation of test substance:	Glyphosate was dissolved in DMSO as a stock solution at −20◦C and diluted with culture medium to various working concentrations.

siRNA mediated silencing of Beclin-1

Conditions:	Cells were transfected with Beclin-1 siRNA or scrambled siRNA (Santa Cruz, CA, USA) using Lipofectamine 2000 reagent according to the manufacturer's instructions. After 48 h, cells were subjected to various treatments that were not further specified.

MTT assay — Assessment of cell viability

Conditions:	PC12 cells after various treatment were plated in 96-well culture plates (1.0×10^4 cells per well). 5 g/l MTT solution was added to the culture medium 4 h before the end of treatments.
Dose concentrations:	0, 5, 10, 20, 40 mM glyphosate
Exposure duration:	12, 24, 48, 72 h
Replicates:	Not reported

Apoptosis detection

Conditions:	Nuclei were stained with DAPI to detect chromatin condensation or nuclear fragmentation, which were characteristics of apoptosis. Following various treatments, cells were fixed with 4% paraformaldehyde for 20 min and then stained with DAPI for 10 min away from light at room temperature.
Dose concentrations:	Not reported
Exposure duration:	0, 24, 48, 72 h
Replicates:	3 per treatment

Autophagy detection

Conditions:	For LC3 punctate analyses experiments, cells were transiently transfected with EGFP empty vector or GFP-LC3 expression vector.
Dose concentrations:	Not reported
Exposure duration:	Not reported
Replicates:	Not reported

Western blot analysis

Conditions:	Cells after various treatment were lysed in ice-cold RIPA lysis buffer (1% Triton X100, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP40, 50 mM TrisHCl, pH 7.4) supplemented with protease inhibitor cocktail and phenylmethanesulfonyl fluoride for 30 min, followed by centrifugation at 12,000g for 30 min at 4 °C before collecting the supernatants.
Dose concentrations:	Not reported
Exposure duration:	Not reported

Replicates:	3 per treatment

5. Observations/analyses:

Measurements:	Cell viability, apoptosis and autophagy induction
siRNA mediated silencing of Beclin-1	Microscopy: In order to evaluate the effects of Beclin-1 siRNA or scrambled siRNA on GFP-LC3 puncta formation, cells were cotransfected with GFP-LC3 and either Beclin-1 siRNA or scrambled siRNA following various treatments and observed by confocal laser scanning microscopy. The Beclin-1 siRNA sequence (5'-CAGTTTGGACAATCAATA-3') efficiently targeted.
MTT assay	Detection: After DMSO was added to each well to dissolve the dark blue crystals the absorbance at 570 nm was interpreted on a microplate reader (Safire,TECAN).
Apoptosis detection	Microscopy: 24 h after transfection, the cells were subjected to various treatments before being observed under confocal laser scanning microscopy. In each experiment, no fewer than 200 transfected cells were counted. Analysis: The cell apoptosis was further determined using FITC Annexin V Apoptosis Detection Kit according to the manufacturer's recommendations. Cells were then analyzed by flow cytometry (FCM) on a linear scale, to detect apoptosis using a Becton Dickinson FACS Array.
Autophagy detection	Microscopy: 24 h after transfection, the cells were subjected to various treatments before being observed under confocal laser scanning microscopy. In each experiment, no fewer than 200 transfected cells were counted. Analysis: Only cells with at least five dots were scored as GFP-LC3-positive. The percentage of the positive cells was thus determined and expressed as the mean of four independent experiments. For western blot analyses, LC3-II/ LC3-I ratio was evaluated by band density analysis as the marker of cell autophagy.
Western blot analysis	Analysis: Protein extracts were quantified and equal amounts of lysates were resolved by SDS-PAGE, and then transferred into PVDF membrane (Millipore). After blocking with 3% BSA, appropriate primary antibodies and secondary antibodies were applied. The signals were developed with Immobilon Western Chemiluminescent HRP Substrate (Millipore). Band density values of interested bands were normalized to loading control and quantitative analyses were performed by imageJ software (Wayne Rasband, NIH).
Statistics:	Statistical analyses were done by SPSS software using a one-way ANOVA, followed by a two-tailed Student's *t*-test or multiple comparison test where appropriate. A $P < 0.05$ was considered significant for all analyses.

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: Not relevant (Due to reliability)

3. Klimisch code: **3**

Response - GTF

- 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 ¼ 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 anything will kill cells - but this does not mean that everything causes Parkinson's disease.
- There is no evidence that glyphosate causes Parkinson's disease. The authors cite two case reports of Parkinson's disease, discussed in the introduction of this neurotoxicity literature review.
    - The cited letter by Wang et al. (2011) reports a single patient, a 44 year old woman, who had worked in a glyphosate production unit for three years prior to developing Parkinson's. This provides no evidence for causation whatsoever.
    - The cited letter by Barbosa et al. (2001) is similarly a single case report, in this instance a 54 year old man who had dermal contact with a glyphosate formulation six months prior to developing 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 (Koller et al., 2012; Heu et al., 2012b) 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 LD-50 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) dosed rates 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) 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) achieved using doses of 400 mg/kg glyphosate. In short, the concentrations used in this work are massively higher than any concentration is blood (let alone brain tissue) that can be achieved following normal human exposures.

3. Literature Review of Carcinogenicity Publications

Over the 40 year product history of glyphosate based herbicides, regulatory expert and other authoritative review panels have evaluated multiple data sets to evaluated glyphosate safety, including potential for carcinogenicity. These multiple reviews over the decades have consistently drawn the same conclusion; glyphosate is not carcinogenic. These conclusions include those of the U.S. Environmental Protection Agency in 1993 and 1997 (Category E, evidence of non-carcinogenicity for humans -- based on the lack of convincing evidence of carcinogenicity in adequate studies); the European Commission's Health and Consumer Protection Directorate-General in 2002 (no evidence of carcinogenicity); the U.S. Forest Service (based on standard animal bioassays for carcinogenic activity *in vivo*, there is no basis for asserting that glyphosate is likely to pose a substantial risk); Canadian regulators (no evidence that glyphosate causes cancer); the World Health Organization and Food and Agriculture Organization of the United Nations in 2004 (long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study of carcinogenicity in mice, no toxic effects were observed at up to the highest dose tested (1000 mg/kg bw per day), and there was no evidence of carcinogenicity).

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.

One publication (George et al., 2009) utilized 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 for a positive control/comparator after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. This study is discussed in more detail 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; 2005), 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 current EU glyphosate acceptable operator exosure Level (AOEL). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95% of current EU glyphosate AOEL and 1.3% of the current EU glyphosate attapcable daily intake (ADI). Not surprisingly, even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Interestingly, the current European ADI is based on the NOAEL (highest dose tested) in an old 2-year rat carcinogenicity study; 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) 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) 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) reported an association between NHL and glyphosate use. McDuffie et al. (2001) reported a non-significant positive association between self-

reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009) 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), 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) specifically references De Roos (2005) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee et al. (2005) 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.

The follow epidemiology publications report a lack of association between glyphosate and specific cancer types.

- Alavanja et al. (2003) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigener et al, (2008) 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).
- 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).
- Lee et al. (2004) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
- Carreon et al. (2005) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel et al. (2005) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al (2004) reported AHS data on parental use of specific pesticides*a* and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti et al. (2009) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren et al. (2009) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake et al. (2011) reported a lack of association between glyphosate and Hodgkin's lymphoma.
- Pahwa et al. (2012) reported a lack of association between glyphosate and multiple myeloma.

In summarizing AHS publications, Weichenthal et al. (2010) 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.

Monge et al (2007) 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) and therefore should not be interpreted as an association with glyphosate.

Non-Hodgkin's Lymphoma (NHL)

Non-Hodgkin's lymphoma is not a specific disease, but rather a grouping of all lymphoma types, other than Hodgkin's lymphoma. This is a large group of different cancers of the immune system including Burkitt lymphoma, diffuse large B-cell lymphoma (NLPHL), follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, mycosis fungoides, anaplastic large cell lymphoma, and precursor T-lymphoblastic lymphoma (National Cancer Institute, http://cancer.gov/cancertopics/wyntk/non-hodgkin-lymphoma.pdf). Risk factors associated with NHL include weakened immune system (such as from an inherited condition or certain drugs used after an organ transplant), infections (Epstein-Barr virus, EBV; Human immunodeficiency virus, HIV; *Helicobacter pylori* bacteria; Human T-cell leukemia/lymphoma virus, HTLV-1; Hepatitis C virus; age). There are many different types of Non-Hodgkin's lymphomas, which are different lymphomas arising from different pathogeneses, and as such, should not be clustered together as a single disease with a common etiology for epidemiological investigation. When clustered together in epidemiological studies, further investigation to identify both the specific type of lymphoma and any underlying risk factors associated with individual reports of HNL is necessary.

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

Abstract*

BACKGROUND. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective 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. Uni-variate 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.
CONCLUSIONS. 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

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, impregnating agents, organic solvents
Active substance(s):	Glyphosate, phenoxyacetic acid, MCPA, 2,4-D, 2,4,5-T, DDT, Pyrethrins, mercurial seed dressing, chlorophenols, pentachlorophenol, arsenic, creosote
Description:	Not reported
Source of test medium:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human

Age of test persons:	≥25
Sex:	Males

4. Test system:

Study type:	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire
Total No. of cases analysed:	442
Total No. of controls:	741
No. of exposed cases to glyphosate:	4
No. of controls for glyphosate	3

5. Observations/analyses:

Working history:	All subjects
Additional information:	Smoking habits, previous diseases, and certain food habits were assessed.
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects.
Parameters determined:	Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis). NHLs with different pathogeneses were not distinguished.
Statistics:	Conditional logistic regression analysis for matched studies was performed with the SAS statistical program. Thereby, odds ratios (OR) and 95% confidence intervals (95% CI) were obtained. All 95% CIs were rounded outward, e.g., a 95% CI of 1.07– 4.52 is written 1.0 – 4.6. Both uni-variate and multi-variate analyses were performed. When exposure to different pesticides was analyzed, subjects with no pesticide exposure were taken as unexposed.

KLIMISCH EVALUATION

1. 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.

2. 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.

3. Klimisch code: **3**

Response 1 – Review by Mark R. Cullen, MD, Professor of Medicine and Epidemiology, Yale University School of Medicine, June 21 1999

This study is part of an ongoing effort of the investigators and their team to unravel the cause(s) of NHL, which has been increasing in incidence in Sweden and most developed countries for at least 2 decades. The premise, that the increase suggests an environmental cause or causes, is certainly correct.

The basic approach, the case control study using the superb existing tumor and population registries of Sweden, is appropriate to this challenge, and the investigators seem to have a clear grasp of the basic approach to such studies. Inclusion criteria for cases appear well considered, and the ability to recruit almost all is a strong plus for the study. The criteria for including controls, including the matching on vital status for comparability of information regarding past exposures is laudable, though, as discussed below, possibly unsuccessful despite careful consideration. The response of the subjects is encouragingly high.

Unfortunately the approach to exposure assessment for agricultural chemicals is very problematic. First, as I believe the data themselves ultimately demonstrate, it is not at all clear that even living subjects, let alone relatives of dead ones, can meaningfully assess or quantify exposure to herbicides and pesticides. It appears from the small number of phone interviews conducted (itself a problem, see below) that almost every subject provides different information or expanded information when directly contacted by phone. It is not at all obvious that the respondents can easily evaluate their exposures, which in many cases amount to an occasional use of a product many years before the survey, nor is it obvious that the surrogate measure of dose, i.e., days of use, is meaningful, especially given the remarkable difference which exists in actual biological exposure depending on how the products are used, information which was not even attempted here. In other words, the first problem is the degree to which this study classifies subjects in any biologically relevant way, or validly.

As if this were not problematic enough, there is evidence within the study results to suggest significant information or recall bias. When they were contacted because of ambiguous or missing information, a high proportion, possibly all subjects reported a positive history of exposure -- it is unclear from the report just how many such were contacted overall, but it appears that most were contacted to confirm positive histories, despite the evidence that the negative histories were more likely unreliable. I would worry greatly that cases, clearly aware of their disease status even if not the underlying hypothesis here, might be more thorough in their recollection of these distant events, whose recall is likely more subtle than recall of major industrial chemicals which likely would have involved (unforgettable) daily work exposures, unlike the chemical use with doses averaging about a month! The authors would have done well to interview everybody given this spareness, and the ubiquity of recall bias in such studies.

The third problem with the exposure assessment relates to co-linearity. For obvious reasons people exposed to one agricultural chemical have a non-independent (true) chance of exposure to another, and that recollection of one is likely to interact with recollection of others. The data presented are consistent with this, though the actual degree of overlapping exposures in the data are not fully disclosed. In any event, the effort to tease them apart using multi-variate regression unlikely gets at the fundamental issue, which is that information is hopelessly confounded. Even if one were not concerned about the other issues vitiating the exposure assessment, the attempt to distinguish one exposure from another within the herbicide category is, in my view, fatuous, though the investigators have drawn some rather sweeping inferences from it, and from the latency analysis which I believe suffers from the same recall issues.

One final comment, which I fear may betray a range of the authors preconceived ideas, is the inclusion of glyphosate in the uni-variate and multi-variate analyses, despite the fact that only 7 of 1145 subjects in the study gave exposure histories to this agent, and for a mean duration of what appears to be a few days! Since there is zero possibility that exposure to glyphosate could explain the Swedish excess of NHL which is the premise of the study, and since it is biologically absurd to imagine a few days exposure to virtually any short lived compound, let alone one with so little oncogenic potential based on its toxicologic profile, the inclusion of these data and the highlighting of them in the discussion - with a very biased review of the tox literature-- undermines even further the report.

In the end I think this study adds little to our overall knowledge of the cause(s) of NHL, though it continues to appear that farmers have increased risk, certainly an important clue for follow-up. However, it is unlikely that the roles of infection, other biological factors, UV light, diet and lifestyle issues or agricultural chemicals will be successfully unraveled by studies of this design. In particular, the evidence

regarding glyphosate in relation to NHL is meaningless, and it would be highly inappropriate to construe this as a positive study in that regard.

Response 2 – Review by Hans-Olav Adami, Professor of Epidemiology, Harvard School of Public Health and Dimitrios Trichopoulos, Vincent L. Gregory Professor of Cancer Prevention, Department of Epidemiology, Harvard School of Public Health.

We have classified our comments into those concerning study design and those concerning data analysis and interpretation, and we have concluded our evaluation with a short commentary and overall assessment.

Study design

The study base comprises men 25 years of age or older and living in any of seven Swedish counties from January 1, 1987 to December 31, 1990. The cases were divided according to their vital status at a time when the actual data collection took place. Of the 442 cases, 192 were deceased. The date of vital status ascertainment is not clearly indicated, as it should have been. Since, however, data were collected from 1993 to 1995, we assume that vital status was determined in 1993 or earlier.

The authors state that they have conducted a population-based study, but they have chosen their controls in a way that violates the defining characteristics of these studies. Sampling from the population register took place sometime after 1990, so that people who had migrated out of the area after the diagnosis of the corresponding case would have been incorrectly ineligible, whereas those who had migrated into the area after the diagnosis of the corresponding case would have been incorrectly eligible. Migration is generally related to socio-economic status, which is a plausible predictor of exposure to pesticides. Thus, important bias may have been introduced.

There are other issues that should have been addressed in the study design. Is it really possible to blind interviewers as to the case or control status of the interviewed person, so as to minimize interviewer-related information bias? And, what assurance is there that the substantial difference in response proportion between cases and controls did not introduce interviewee-related selection bias? It is certainly disturbing that all 17 reported odds ratios (Table 1 of the authors) were higher than the null value of 1, even though only marginally significant results were reported. It is also astonishing that there is no category of missing or unknown in any of the tables, even though about half of the exposure information was provided by proxy responders and this information was concerning compounds as complicated as 2,4-D/2,4,5-trichlorophenoxyacetic acid.

Analysis

The analysis is in many ways superficial and shows a surprising disregard to confounding. The authors appear so eager to report significant results, that when multi-variate analysis, *that is the proper analysis*, reduces all reported odds ratios to essentially non-significant values (table 7), they make the amazing statement that "regarding lymphomagenesis, the uni-variate analysis may be more informative than the multi-variate analysis". Moreover, they pay little attention to the multiplicity of comparisons and they attempt causal inferences with unacceptable disregard of the statistical limitations of their study. For example, for glyphosate, the p value is no less than 0.35 and for phenoxyacetic acids the multi-variate odds ratio has a p value of 0.25.

There are several other issues in the analysis. Although most of them are trivial, one deserves more attention. Non-Hodgkin lymphoma has been reported to be more common in some rural occupations. Exposure to pesticides is a possible explanation, but there are other plausible explanations, including exposure to infectious agents of animal origin and delayed establishment of herd immunity with concomitant increase in the average age at exposure to possible critical agents (the classical paradigm of paralytic polio has been invoked by several investigators in the study of the etiology of multiple sclerosis,

leukemias and lymphomas). In the latter two instances, occupation should be adjusted for in the analysis, in order to control for confounding.

Conclusion

This is a study that has limited power, was inadequately designed, poorly analysed and confusingly reported. Every epidemiological investigation should meet basic standards concerning selection bias, information bias, confounding and power. The investigation by Hardell and Eriksson does not provide reasonable confidence that it is free of information and selection bias, shows clear signs of uncontrolled confounding and lacks the power necessary to document agent-specific effects when several agents are inter-correlated, as they are in this situation. There is also evidence that the results were selectively interpreted by the investigators. For these reasons, the study cannot provide reliable information concerning possible associations between exposures to pesticides and risk for non-Hodgkin lymphoma

Response 3 – Monsanto Review by John Acquavella, PhD and Donna Farmer, PhD

Executive Summary

Hardell and Erikkson 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 finding needs to be evaluated in light of the limitations of the study, mentioned above, and the wealth of toxicologic information that has resulted in glyphosate being judged to be non-mutagenic and noncarcinogenic by the U.S. Environmental Protection Agency and the World Health Organization. Systematic error or chance seem the most likely explanations for the findings reported for glyphosate in this study.

Hardell and Eriksson[1] conducted an epidemiologic study to look for associations between self-reported pesticide use and non-Hodgkin's lymphoma (hereafter NHL). The rationale for conducting this research was previous studies by the first author[2,3] and by investigators at the U.S. National Cancer Institute[4,5], which found associations between reported use of phenoxyacetic acids (primarily 2,4-D) and NHL. The results of these studies were determined to be inconclusive by a special Science Advisory Panel convened in the early 1990s by the U.S. Environmental Protection Agency (EPA).[6]

The present study presents new data about phenoxyacetic acids and other commonly used pesticides. Herein, we review the methods and results of this recent study.

Study design

Hardell and Eriksson employed a case control design for their research. In case control studies, subjects are selected on the basis of their disease status. Those with the disease of interest (in this case those with NHL) are the cases; disease free study participants are the controls. Information about presumptive etiologic factors are collected from cases and controls using similar methodology.

The controls in a case control study provide an estimate of the exposure prevalence (in this case the prevalence of self-reported pesticide use) in the base population that gave rise to the cases and controls[7]. The exposure odds for the cases is then compared to the exposure odds for the controls. The resulting ratio of exposure odds - called the odds ratio (OR) - estimates the ratio of disease rates for exposed versus unexposed subjects[8]. The ratio of disease rates is the fundamental measure of association in epidemiologic studies.

The interpretation of the OR is straightforward. An OR of 1.0 implies that the disease rate (in this case the rate of NHL) is the same for exposed members of the base population and for unexposed members and indicates no association between exposure and disease. An OR greater than 1.0 or less than 1.0 implies that the disease rate is different for the exposed population than for the unexposed population and, if valid, may indicate an exposure disease relationship. Exposure disease relationships can be "positive" (viz. the OR is greater than 1.0) - where exposure is associated with increased rates of disease - or inverse (viz. the OR is less than 1.0) - where exposure is associated with decreased rates of disease (viz. exposure prevents disease). For example, an OR of 2.0 is consistent with a disease rate among exposed persons that is twice the disease rate for unexposed persons; likewise, an OR of 0.5 is consistent with a disease rate for exposed persons that is half the disease rate for unexposed persons.

Interpreting ORs at face value requires the assumption that there is no confounding or other bias in a study. Much of the evaluation of epidemiologic studies hinges on whether there are discernible sources of bias or potential for bias, which, if present, compromise the validity of findings. Often it is not possible to pinpoint specific sources of bias, but methodologic limitations can usually be identified and the results interpreted accordingly.

A major validity concern in case control studies is recall bias: that is when cases or their next-of-kin are more likely to recall (real or imagined) specific exposures than are controls. This can result in differential exposure misclassification whereby cases are more likely to be classified as exposed than are controls, despite no real difference in exposure prevalence. Recall bias is particularly an issue in cancer studies; cancer being a disease that stimulates introspection about presumptive causes. Other important validity concerns are selection bias (cases or controls as selected are unrepresentative) or uncontrolled confounding factors. Proper reporting of an epidemiologic study requires consideration of potential biases and their likely impact on study results.

Finally, findings are also evaluated according to how likely they are to have occurred by chance alone if there is not, in fact, a true relationship between exposure and disease. This is evaluated by calculating a probability (called a p-value) for seeing results at least as extreme as those observed if the null hypothesis of no true effect is true. By convention, only findings where the p value is less than 0.05 are considered "statistically significant." Hardell and Erikkson did not actually calculate p values in their study. Instead, they calculated 95% confidence intervals for the OR. The 95% CI is defined as the range of values that are consistent with the data observed in a study with 95% confidence. For example, a CI of 0.4 to 13.0 means the data are consistent with an OR as low as 0.4 (implying a 60% reduced rate with exposure) or as high as 13.0 (implying a 13-fold elevated rate with exposure). A finding is statistically significant when the OR of 1.0 is not included in the 95% CI.

Study subjects

The study included 404 NHL cases, diagnosed during the period 1987-1990, from the four most northern counties of Sweden. These cases (or their next-of-kin when cases were deceased) and 741 controls (or their next-of-kin when controls were deceased) were sent a mailed 18 page questionnaire that addressed a variety of (self-reported, viz. undocumented) factors including pesticide use, work history and chemical exposures, smoking habits, previous diseases, and certain dietary habits.

Controls were selected to be similar to cases in terms of age and vital status (i.e. living cases were matched to living controls and deceased cases were matched to deceased controls). Matching subjects on vital status was intended to minimize recall bias to the extent that the fact of death, but not death from a

specific cause, might affect recollections of pesticide use. Approximately 43% of cases were deceased, hence next-of-kin information a significant component of this study.

Exposure Assessment

There was no exposure assessment, per se, in this study. Exposure was presumed based on reported use of specific pesticides. This can be an inaccurate indicator of exposure for two reasons: 1) inaccurate recall or 2) negligible exposure from use. An example of the latter would be glyphosate which has very low skin penetrability[9], so reported use is not equivalent to (meaningful) exposure. A recent study of forestry sprayers by Lavy et al. found indications of significant dermal exposure, but no indication, based on biomonitoring, of an absorbed dose of glyphosate.[10]

Statistical analysis

The data analysis involved standard techniques to estimate the OR and control, in a very limited sense, for coincident pesticide exposures as potential confounding factors. These statistical techniques included uni-variate and multi-variate logistic regression analysis. The analysis was primarily restricted to a crude dichotomous classification of reported pesticide use (ever use versus never use). There were too few "exposed" subjects to conduct dose response analyses for most specific chemicals. The authors also estimated 95% CIs as a measure of the statistical variability of the ORs.

Results

The authors found modest, though statistically significant, associations between NHL and reported use of any herbicide (OR = 1.6, 95% CI 1.0-2.5) reported use of any fungicide (OR = 3.7, 95%
CI 1.1-13.0) and reported use of 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR = 2.7, 95% CI 1.07.0).
Through various analyses, the authors concluded that only exposure in the two decades preceding diagnosis was associated with increased risk.

The authors also reported findings for glyphosate, none of which were statistically significant. The overall OR for glyphosate was 2.3 (95% CI 0.4-13.0) based on 4 cases (1% of cases) and 3 controls (0.4% of controls) reporting glyphosate use. The authors also mentioned an additional analysis where glyphosate and phenoxyacetic acids were considered jointly in attempt to control for confounding from phenoxyacetic acids on the glyphosate/NHL association. In this instance, the OR for glyphosate was 5.8 (95% CI 0.6-54.0) and the OR for phenoxyacetic acids was 1.4 (95% CI 0.8-2.2). The description of this analysis was insufficient to know what the authors actually did or even to know the number of cases who reported using glyphosate. But it was clear that there was no systematic attempt to assess the association between glyphosate and NHL while controlling for exposures other than phenoxyacetic acids.

Authors' conclusions

The authors interpreted their results as supportive of a role for chemical pesticides in the etiology of NHL. They speculated, since NHL is known to be related to immunosuppression from studies of transplant patients[11], that phenoxyacetic acids might produce NHL by an immunosuppressive mechanism. In fact, they interpreted selected papers from the literature as supportive of an immunotoxic effect for phenoxyacetic acids and chlorophenols.[12,13,14]

The authors reached less definite conclusions about other pesticides and specifically about glyphosate. They noted the elevated OR for glyphosate, an elevated OR for glyphosate from another study of theirs[15] concerning hairy cell leukemia (OR = 3.1, 95% CI 0.8-12.0, based on 4 cases who reported use of glyphosate), and selected toxicologic data[16-21] as indicative that glyphosate is, at least, deserving of further epidemiologic study.

The authors considered several potential biases in interpreting their results. They ruled out selection bias by arguing that they had good response rates from cases and controls and included most cases who were diagnosed during the study period. They felt they minimized recall bias by matching cases and controls on vital status and collecting information from all study subjects using similar (blinded) methodology.

Critique

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.

In epidemiologic studies results can be:

- real (viz. disease is due to exposure)
- biased (viz. the results are invalid)
- due to chance (viz. the association is unbiased, but non causal).

It is by exclusion of the latter two possibilities and application of generally accepted criteria for causality[22] that scientists come to believe that an exposure disease association is causal. The most important causal criteria are strength of association (judged by the size of the OR), dose response (judged by whether the OR increases or decreases with increasing exposure), temporality (exposure should precede the onset of disease by an appropriate induction/latent period), consistency of findings across studies, and biological plausibility. I'll return to each of these criteria subsequently.

The major potential sources of bias in this study are recall bias, confounding bias, and selection bias. Recall bias is a major concern in cancer case control studies because cancer cases, and especially their next-of-kin, tend to scrutinize their lives hoping to understand the cause(s) of their disease. Hardell and Eriksson's matching of study subjects on vital status does not address the specific recall bias issue for cancers. Other investigators have found elevated ORs for the popular herbicide 2,4-D based on next-of-kin responses, but not based on responses of direct informants.23 Results based on a substantial number of next-of-kin respondents are usually considered less persuasive than data from actual study subjects. It would have been informative had Hardell and Erikkson analyzed their data separately for next-of-kin respondents to see whether the elevated ORs were determined primarily by next-of-kin responses. That would be difficult in the present study due to the limited number of cases who reported using most specific pesticides.

A second important limitation of the study was the inability to control for potential confounding factors. Confounding refers to finding spurious exposure-disease associations resulting from other correlated factors. The confounding factor must also be a risk factor for the disease in question. Relatively little is known about the etiology of NHL, other than there seems to be a relationship with immunosuppression.[24] It is difficult to control for confounding factors when little is known about etiologic factors. In addition, in light of the high correlation between reported use of various pesticides, it is difficult in such a study, given the small number of exposed subjects, to separate the putative effects of one pesticide from another. Therefore, associations reported for any specific pesticide might be due to effects from other pesticides.

The final source of bias to be considered is selection bias. There is no way to know whether the cases or controls who participated in the study were a biased sample, but the relatively high participation rates for cases and controls would make selection bias a less likely explanation for the findings in this study.

Specific results in an epidemiology study can be due to chance, especially when many statistical associations have been evaluated. The convention is that a p value of 0.05 or less is considered unlikely to have occurred by chance and is therefore "statistically significant." The p values for the glyphosate findings are well in excess of 0.05, approximately 0.30 or greater by my estimation, so neither of the

elevated ORs for glyphosate are close to the conventional criterion for statistical significance. They could easily be chance findings. It is noteworthy that if even one exposed case was misclassified, the OR would be approximately 1.8 (95% CI 0.6-9.9, p value 0.43); two misclassified exposed cases would give an OR of 1.2 (95% CI 0-6.2, p value 0.99). Hence, the elevated OR for glyphosate hinges on the classification of a single case or two and an exposure assessment methodology of questionable accuracy.

It is helpful at this point to assess how the findings in the present study for glyphosate (and for most of the other herbicides) match up with the causal criteria generally accepted by epidemiologists.
Specifically:

- strength of association - the findings of the present study show a weak to moderate non significant association between glyphosate use and NHL. The association is statistically imprecise and, even assuming an absence of bias, is not convincing.
- temporality - in this study, the presumed exposures would precede disease onset satisfying, in general, the temporality criterion. However, the authors did not have enough exposed subjects to consider issues of disease induction/latency as they tried to do for the phenoxyacetic acids.
- dose response - there was insufficient data in this study to consider dose response. Also, in light of glyphosate's very low skin penetrability9, one can question whether any meaningful range of
- exposure occurred among study subjects.
- consistency - there are no other studies that have reported an association between glyphosate and NHL. Hence the consistency criterion cannot be met.
- biological plausibility - Hardell and Erikkson characterized the available glyphosate toxicologic data as showing: excess mutations and chromosome aberrations in studies with mouse lymphoma cells[16-19], excess sister chromatid exchanges (SCEs) in cultures of human lymphocytes[20], and a somewhat increased incidence of various cancers in one carcinogenicity study of mice.21 However, five of the six references cited did not use glyphosate as the test material.[16-19,21] In these studies the test material was sulfosate - the trimesium salt of glyphosate. Sulfosate has a somewhat different toxicology profile than glyphosate. Nonetheless, it is worth pointing out that Hardell and Erikkson's assessment of these studies is not shared by regulatory agencies. For example, the U.S. Environmental Protection Agency (EPA) considered the mouse lymphoma findings[16-19] to be false positives due to sulfosate's acidity; sulfosate was not mutagenic in this assay when the pH was adjusted to a physiological level.[25] Also, EPA characterized the sulfosate mouse carcinogenicity study[21] as showing "… no evidence of carcinogenicity … at the doses tested" and classified sulfosate as category E - no evidence for carcinogenicity in humans.[25]

The one glyphosate toxicology study cited[20] showed weak positive findings for sister chromatid exchange in human lymphocytes in vitro. This study had many limitations and numerous, more specific, mutagenicity assays have not shown positive results for glyphosate.[26] Extensive reviews of the available toxicologic data have been completed recently by the U.S. Environmental Protection Agency[27,28] (EPA) and the World Health Organization.[29] These agencies concluded that glyphosate is not mutagenic or carcinogenic. EPA classified glyphosate as category E.[27,28] This would argue against the biological plausibility of the findings reported by Hardell and Erikkson.

In conclusion, the study by Hardell and Eriksson found a modest association between NHL and several chemical pesticides - most notably for MCPA and the collective group of fungicides. The reported weak to moderate associations for glyphosate are not statistically significant and could be due to chance or to recall or confounding bias. It is clear, however, that the widespread use of glyphosate and concerns about pesticide related health effects for farmers and their families will raise the "index of concern" for glyphosate in future agricultural epidemiologic studies.

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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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, impregnating agents, organic solvents
Active substance(s):	Glyphosate, phenoxyacetic acid, MCPA, 2,4-D, 2,4,5-T, DDT, Pyrethrins, mercurial seed dressing, chlorophenols, pentachlorophenol, arsenic, creosote
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

(in the following data only presented for exposures to glyphosate and total number of subjects)

Species:	Human
Age of test persons:	≥25
Sex:	Males

4. Test system:

Study type:	Epidemilogical study for Non-Hodgkin´s Lymphoma (NHL) and Hairy cell Leukemia (HCL)
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire & telephone interviews
No. of exposed persons with NHL or HCL:	NHL study: 404 HCL study:111 Total: 515
No. of control persons:	NHL study: 404 HCL study:111 Total: 515
No. of persons with NHL or HCL exposed to glyphosate:	8
No. of persons in control group:	8

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects. For analysis only subjects with a minimum exposure of 1 working day (8h) and a

tumour induction period of at least one year were included.

Parameters determined: Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis). NHLs with different pathogeneses were not distinguished.

Statistics. Conditional logistic regression analysis for matched studies was performed with SAS statistical program. Odds ratios and 95% confidence intervals were obtained. Both uni-variate and multi-variate analyses were done. In the pooled analysis an adjustment was made for study, study area and vital status. When risk estimates for different pesticide exposures were analysed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999) and HCL (Nordstrom,et al., 1998). 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.

2. Relevance of study: **Not relevant** (Due to reliability of data set drawn from Hardell and Eriksson, 1999)

3. Klimisch code: **3**

Response – GTF

- This study pools NHL data from the previously reviewed publication by Hardell and Eriksson (1999) with HCL data from Nordstrom et al. (1998). Therefore the responses to Hardell and Eriksson (1999), the methodology and data issues, also apply to the NHL data set used in Hardell et al. (2002). It is of interest to note that Hardell was also a coauthor of Nordstrom et al. (1998).
- Each individual study reported non-statistically significant associations between glyphosate and NHL or HCL.
- Each study was based on few exposed cases, 4 each. The pooled analysis combined these cases.
- The uni-variate odds ratio was similar to those in the two individual studies (OR = 3.04; 95% CI: 1.08–8.52), the multi-variate adjusted odds ratio was attenuated (OR = 1.85; 95% CI: 0.55–6.20)
- These data fail to demonstrate convincing evidence for an association between glyphosate and NHL or HCL.

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Organophosphates, organochlorines, phenoxy herbicides, other herbicides, and other pesticides
Active substance(s):	Glyphosate and others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	20-74
Sex:	Males and females

4. Test system:

Study type:	Occupational exposure study to assess exposure to pesticides and risk of non-Hodgkin’s lymphoma

Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire Histopathological confirmation of NHL was done by an experienced pathologist.
No. of exposed persons with NHL:	694
No. of control persons:	694
Pesticide use frequency:	Not reported

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	The questionnaire included a diary with a detailed lifetime history of each job the subject had held for 1 year or more. Information obtained on each job included job title, employer, industry, start and finish years, number of hours worked per day, and number of days worked per week.
Parameters determined:	A pesticide-crop matrix was developed for assistance with exposure assessment. Levels of exposure were considered according to time-weighted average threshold limit values. Frequency of exposure was allocated as number of 8-hour days per year and was calculated using responses to the task questions. If no data on frequency of exposure were available (n=4), subjects were assumed to have been exposed for 2 days per year.
Statistics:	Logistic regression was used to calculate odds ratios (as estimates of relative risk) for non-Hodgkin's lymphoma associated with exposure to any pesticide and exposure to each pesticide subtype in each amount category (substantial or nonsubstantial), with adjustment for age, sex, ethnic origin, and state of residence. In addition, logistic regression analyses were carried out for exposure to any pesticide after restricting the sample to males only and after excluding cases that were not on the electoral roll. We also examined the odds of non-Hodgkin's lymphoma using the following metrics of exposure to any pesticide: maximum exposure level (low, medium, high); ever being exposed before 1985 (yes, no); maximum frequency of exposure (0, ≤4, or >4 days/year); and total number of years exposed (0, ≤5, or >5 years). For the latter two metrics, 4 days per year and 5 years were the median frequency and duration, respectively, in control subjects. All *p* values were two-sided.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: No information about exposure duration, used glyphosate products, exposure duration and application rates. Documentation is insufficient for assessment.

2. Relevance of study: **Not relevant** (Multiple pesticide exposures. No definitive association between NHL and glyphosate can be made.)

3. Klimisch code: **3**

Author(s)	Year	Study title
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 Volume: 60 Number: 9 Pages: -E11

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides (in total 47)
Active substance(s):	Glyphosate and 46 others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	≥ 21
Sex:	Males

4. Test system:

Study type:	Epidemiological studies for Non-Hodgkin´s Lymphoma (NHL) in male farm workers exposed to pesticides

	Pooled data from three population based cased control studies conducted in Nebraska, Iowa and Minesota and Kansas.
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Selection of test persons:	Nebraska: Persons identified by Nebraska Lymphoma Study Group and area hospitals (Time of diagnosis: July 1983 – June 1986). Iowa and Minesota: Ascertained from records of the Iowa State Health Registry; Surveillance system of Minnesota hospitals and pathology laboratories (Time of diagnosis: 1980 - 1983) Kansas: A random sample of cases from the statewide cancer registry run by the University of Kansas Cancer Data Service (Time of diagnosis: 1979 – 1981)
Selection of control persons:	Randomly; Same geographical areas as the cases; Frequency matched to cases by race, sex, age, and vital status at the time of interview
Collection of data:	Questionnaire / Interview

(in the following data only presented for exposures to glyphosate and total number of subjects)

No. of exposed persons with NHL:	870
No. of control persons:	2569
No. of persons with NHL or HCL exposed to glyphosate:	36
No. of persons in control group:	61
Pesticide use frequency:	≥ 20 per person

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects. For analysis only subjects with a minimum exposure of 1 working day (8h) and a tumour induction period of at least one year were included. No analysis of actual exposure duration or frequency was included.
Parameters determined:	Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis)
Analyses and statistics:	Standard logistic regression (maximum likelihood estimation); Hierarchical regression, calculating odds ratios to estimate the relative risk associated with each pesticide Models included variables for age (coded as a quadratic spline variable with one knot at 50 years) and indicator variables for study site Other factors known or suspected to be associated with NHL, including first degree relative with haematopoietic cancer, education, and smoking, were evaluated and found not to be important confounders of the associations between NHL and pesticides

Conditional logistic regression analysis for matched studies was performed with SAS statistical program. Odds ratios and 95% confidence intervals were obtained. Both uni-variate and multi-variate analyses were done. In the pooled analysis an adjustment was made for study, study area and vital status. When risk estimates for different pesticide exposures were analysed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

The standard logistic regression models did not assume any prior distribution of pesticide effects, in contrast to the hierarchical regression modelling

KLIMISCH EVALUATION

1. 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.

2. 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)

3. Klimisch code: **3**

Response – GTF

- The authors pooled data from three case-control studies conducted in Iowa and Minnesota, Nebraska, and Kansas
- The data available in this study did not permit analyses of duration or frequency of use.
- No consideration of types of NHL of varying pathogeneses was presented.
- The reported logistic regression analysis noted, a statistically significant odds ratio for ever use of glyphosate and NHL (OR = 2.1; 95% CI: 1.1–4.0).
- The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL (OR = 2.1; 95% CI: 1.1–4.0) (OR = 1.6; 95% CI: 0.9–2.8).
- Authors introduce the phraseology "a possible increase" in NHL incidence establishing their criteria for this category as OR >1.3 and lower confidence limit >0.8.

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various pesticides
Active substance(s):	Glyphosate and 50 others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	Up to 70 years
Sex:	Males and females

4. Test system:

Study type:	Prospective cohort study
Data collection:	Self-administered enrolment questionnaire
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
No. of persons analyzed:	54315

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Collected comprehensive-use data on 22 pesticides, ever/never use information for 28 additional pesticides, and general information on pesticide application methods, personal protective equipment, pesticide mixing, and equipment repair. Data were also collected on basic demographic and lifestyle factors. Glyphosate exposure metrics for this analysis: *a*) ever personally mixed or applied products containing glyphosate (ever/never); *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days per year, categorized in tertiles among users: 1–20, 21–56, 57–2,678); and *c*) intensity-weighted cumulative exposure days (years of use × days per year × intensity level, categorized in tertiles: 0.1–79.5, 79.6–337.1, 337.2–18,241).
Parameters determined:	The median time of follow-up for occurring cancers was 6.7 years.
Statistics:	Differences between the exposure groups were tested using the chi-square statistics and associated *p*-values. Poisson regression analyses were carried out for all cancers combined and specific cancer sites to estimate rate ratios (RRs) and 95% confidence intervals (CIs) associated with glyphosate exposure metrics; the effect of each metric was evaluated in a separate model for each cancer. Tertile exposure variables were analyzed in separate models using either the lowest tertile–exposed or never-exposed subjects as the reference category. For each exposure metric, RRs were adjusted for emographic and lifestyle factors, including age at enrolment (continuous), education (dichotomous: ≤ high school graduate or GED/education beyond high school), pack-years of cigarette smoking [indicator variables: never, pack-years at or below the median (12 pack years), pack-years above the median], alcohol consumption in the past year [indicator variables: none, frequency at or below the median (72 drinks), frequency above

the median], family history of cancer in first-degree relatives (dichotomous: yes/no), and state of residence (dichotomous: Iowa/North Carolina).

Potential confounding from exposure to other pesticides was explored by adjusting for the five pesticides for which cumulative exposure-day variables were most highly associated with glyphosate cumulative exposure days [(2,4-dichlorophenoxy)acetic acid (2,4-D), alachlor, atrazine, metolachlor, trifluralin].

Tests for trend across tertiles were conducted by creating a continuous variable with assigned values equal to the median value of cumulative exposure days (or intensity weighted exposure days) within each tertile; the *p*-value for the trend test was that from the Poisson model coefficient for this continuous variable. **P**-values < 0.10 were considered as indicative of a trend.

Additional analyses were conducted for cancers for which we observed elevated RRs, and for NHL (non-Hodgkin lymphoma) because of its association with glyphosate in previous studies. These included analyses stratified by state and analyses across quartiles and quintiles (where numbers allowed) of exposure day's metrics.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: **Relevant** (Evaluation focussed on glyphosate, although other pesticides were also considered in the data evaluation)

3. Klimisch code: **2**

Response 1 – summary from Letter to the Editor by Donna Farmer, PhD (Monsanto), Timothy Lash, PhD (Boston University) and John Acquavella PhD (Monsanto)

- 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)
- Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004) 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.

  Note: Farmer et al. (2005) is referenced in Doc L Table 3 and included in Doc K.

Response 2 – summary from Lash (2007)

- Table 2 of De Roos et al. (2005) 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), 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.

  Note: Lash (2007) was captured in the literature search, is referenced in Doc L Table 2 and included in Doc K.

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, rodenticides, and impregnating agents
Active substance(s):	Glyphosate and others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

(in the following data only presented for exposures to glyphosate and total number of subjects)

Species:	Human
Age of test persons:	18-74
Sex:	Males and females

4. Test system:

Study type:	Epidemological study for pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire

No. of exposed persons with NHL:	910
No. of control persons:	1016
No. of persons with Non-Hodgkin lymphoma (NHL) exposed to glyphosate:	29
No. of persons in control group:	18
Pesticide use frequency:	Glyphosate exposed / control group ≤ 10 days: 1/9 persons ≥ 10 days: 17/9 persons
Application rates:	Not reported

5. Observations/analyses:

Working history:	All subjects
Other:	Smoking habits, medications, leisure time activities, proximity from home to certain industrial installations (these factors were not reported)
Detailed assessment of exposure:	Questionnaire included a total work history with in depth questions regarding exposure to pesticides, organic solvents and several other chemicals. For all pesticides not only numbers of years and numbers of days per year, but also approximate length of exposure per day were questioned. Since most work with pesticides was performed in an individualized manner, no job-exposure matrix was judged to be applicable.
Parameters determined:	Regarding phenoxy herbicides and glyphosate an analysis was made taken the latency period for exposure into account
Statistics:	Unconditional logistic regression analysis (Stata/SE 8.2 for Windows) was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis (cases) or enrolment (controls). In the uni-variate analysis, different pesticides were analyzed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides. When analyzing subgroups of NHL all controls were used in the separate analyses. In the dose-response calculations made for agents with at least 20 exposed subjects, median number of days of exposure among controls was used as cut-off. Latency period calculations and multi-variate analyses included agents with statistically significant increased OR, or with an OR > 1.50 and at least 10 exposed subjects

KLIMISCH EVALUATION

1. 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.
2. Relevance of study:	**Relevant** with reservation

3. Klimisch code: 3

Response –Review by Professor Pamela Mink, PhD, Rollins School of Public Health, Emory University, Atlanta Georgia, USA

Study Overview and Main Findings

The authors (Eriksson et al. 2008) 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) and pooled analysis (Hardell et al. 2002) 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), 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; McDuffie et al. 2001; De Roos et al. 2005) 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) 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), 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), 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) 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; Cantor et al. 1992; De Roos et al. 2003; Hardell and Eriksson 1999; Hardell et al. 2002; Lee et al. 2004; McDuffie et al. 2001; Nordstrom et al. 1998) (Tables A and B). It is notable that Hardell et al. (2002) 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). 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 A. 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	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 Also adjusted for other pesticides
	337.2-18,241 vs. 0.1-79.5 Intensity-Weighted Exposure Days[b]	22	RR	0.8	0.5-1.4	

[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. 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
Cantor et al. 1992	Agricultural exposure based on ever living or working on a farm	Nonfarmer	266	547	1.0	Referent	Vital status, state, age, smoking, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures
		Farmer	356	698	1.2	1.0-1.5	
	Farmers with specific pesticide exposures (ever mixing, handling, or applying) compared to nonfarmers	Glyphosate	26	49	1.1	0.7-1.9	
De Roos et al. 2003	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	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	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. 2004a	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	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
Nordstrom et al. 1998	Exposure to specific herbicides, insecticides, and fungicides	Glyphosate	4	5	3.1	0.8-12	Age and country (matching factors)
Eriksson et al. 2008	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-III	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

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, calcyclin, 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 calcyclin, 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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Original ®
Active substance(s):	Glyphosate
Source:	Monsanto Company St. Louis, USA (obtained from a local market)
Lot/Batch #:	Not reported
Purity:	360 g/L glyphosate salt equivalent as the isopropylamine salt
Co-formulants:	The formulation contained 15% POEA (polyethoxylated tallow amine)

2. Vehicle and positive controls:

50% ethanol
12-o-tetradecanoylphorbol-13-acetate (TPA);
7, 12-dimethylbenz[a]anthracene (DMBA).

3. Test animals:

Species:	Mice
Strain:	Swiss albino
Source:	Indian Institute of Toxicology Research (IITR)
Age of test animals at study initiation:	Not reported
Sex:	Male
Body weight:	12-15 g
Acclimation period:	1 week

Diet/Food:	Synthetic pellet basal diet (Ashirwad, Chandigarh, India), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 23 ± 2°C
	Humidity: 55 ± 5%
	Air changes: Not reported
	Light/dark cycle Not reported

4. Test system:

Study type:	**Proteomic study in mouse skin**
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	32 weeks
Dose groups:	**Group I** – Untreated control (No treatment). **Group II** – Glyphosate alone (25 mg/kg bw, topically 3 times per week). **Group III** – DMBA+TPA (Single topical application of DMBA, 52 µg/mouse followed 1 week later by thrice a week application of TPA, 5 µg/mouse). **Group IV** – Glyphosate (s)+TPA (Single topical application of glyphosate, 25 mg/kg bw followed 1 week later by TPA application as in group III). **Group V** – Glyphosate (m)+TPA (Thrice a week topical application of glyphosate, 25 mg/kg bw for 3 weeks [total of 9 applications], followed1 weeklater byTPA application as in group III). **Group VI** – DMBA (Single topical application of DMBA, 52 µg/ mouse). **Group VII** – TPA (Thrice a week topical application of TPA, 5 µg/mouse). **Group VIII** – DMBA+glyphosate (Single topical application of DMBA [as in group III], followed 1 week later by topical treatment of glyphosate, 25mg/kg bw thrice per week).
Animals per dose group:	8 groups of 20 animals each

Study type:	**Proteomic study**
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Dose groups:	**Group I** – Untreated controls (No treatment). **Group II** – Glyphosate (Single topical application, 50 mg/kg bw/mouse). **Group III** – DMBA (Single topical application of DMBA, 104 µg/mouse). **Group IV** – TPA (Single topical application of TPA, 10 µg/mouse).
Animals per dose group:	4 groups of 4 animals each

Sampling and sample preparation:	24 h after application animals were sacrificed and skin tissues from the treatment site were excised. Hair and subcutaneous fat was removed, and small pieces of cleaned skin tissues of each mouse from all the groups were then homogenised (10 % w/v) individually, in 2-DE lysis buffer. The lysed samples were soniocated, centrifuged and pooled for the respective group. After quantification of proteins by Lowry´s method, the supernants were stored at -80°C until use fro electrophoresis.

5. Observations/analyses:

Carcinogenicity study in mouse skin

Body weight:	Measured weekly
Development:	Examined weekly
Gross morphological changes:	Volume of squamous cell papillomas (tumors) locally on the skin was examined during the entire study period. Tumors larger than 1 mm diameter were included in the total number of tumors.
Mortality:	Not reported
Clinical signs:	Not reported
Food- and water consumptions:	Not reported
Test substance intake:	Not reported
Haematology:	Not reported
Clinical chemistry:	Not reported
Urine analysis:	Not reported
Sacrifice/pathology:	Not reported
Organ weights:	Not reported
Histopathology:	Not reported

Proteomic study

Protein quantification:	Quantification of proteins in the supernants prepared for 2-DE by Lowry's method.
Protein expression profile:	2-D electrophoresis (2-DE) IEF was carried out using commercially dedicated equipment, Protean IEF. IEF was performed for each individual sample to a total of 45.5 kVh. All IEF steps were carried out at 20 °C. After the first-dimensional IEF, focused IPG strips were placed in an equilibration solution. Separation in the second dimension was carried out using Protean II xi electrophoresis equipment. Each experiment was performed in triplicate to obtain the reproducible results. After completion of the second-dimension electrophoresis, the gels were fixed and stained by using a fast silver staining protocol with neutral silver nitrate. Analysis of the 2D-gels including background subtraction, spot detection, volume normalization and differences in protein

	expression levels among samples were analyzed by using PDQuest software Ver. 7.4.0. To determine the variation, 3 gels were prepared for each sample. The protein spots that varied >2 fold change and were specific for the test groups and the control group were manually labeled and considered for MS analysis.
Protein identification	Matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF/TOF) and liquid chromatography mass spectrometry (LC-MS) Differential protein spots of interest were excised manually and washed with deionised water. After in-gel digestion, trypsinised samples were dissolved and mixed with matrix (α-cyano-4-hydroxy cinnamic acid). Following drying the peptides were spotted on ground steel plate and subjected to Bruker Ultraflex MALDI-TOF/TOF and 2D Nano LC-ESI-Trap (Agilent) for mass spectrometric identification. Data acquisition and analysis was performed using flex control and flex analysis/biotools version 2.2 software, respectively. Data was acquired in reflectron positive mode using 15–18% laser power. Mass tolerance and monoisotopic values (50 ppm/100 ppm for peptide mass fingerprint and peptide mass tolerance of 2 Da for MS/MS spectra) were used for searching. The datasets of the MS spectra, including peptide sequence information, were searched against the SWISS-PROT and NCBInr database using Mascot Daemon as a client attached to the Mascot search protocol.
Verification of calcyclin, calgranulin-B and SOD 1:	The differential proteins screened with 2-DE were confirmed by Western blot analysis. Skin tissue samples were lysed in lysis buffer, resolved on a 12-15% polyacrylamid gel, and electro-transferred onto polyvinylidene fluoride membranes. After blocking, the membranes were immunoblotted with antibodies of calcyclin, calgranulin-B and superoxide dismutase (SOD 1) and beta-actin at dilutions recommended by the suppliers. Horse radish conjugated secondary antibodies and chemiluminescence kit, were used for detection. Protein expression was visualized by Versa Doc Imaging System. The intensity was given in terms of relative pixel density for each band normalized to band of beta-actin. The intensity of the bands was measured using software UNSCAN-IT automated digital system version.
Statistics:	The skin tumour incidence was analyzed by one-way analysis of variance (ANOVA) test in untreated control and treated groups, $p<0.05$ value was considered as significant. Protein expression data for untreated control and treated groups are expressed as the mean±SD of 3 replicate gels for fold changes of normalized spot volumes. For the statistical analysis of data, Student-t-test was used and $p<0.05$ was considered as significant. Hierarchical clustering analysis using Ward's minimum variance was performed by NCSS software.

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Relevant with restrictions** (Glyphosate formulation not glyphosate alone was tested.)

3. Klimisch code: **2**

Response – GTF

It is important to note that 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 in Section IIA 5.9.9 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).

Proteomics is an emerging science, not yet yielding validated test methods for establishing human health endpoints. 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. Further research and validation in this field will be necessary before such studies may prove useful in human health risk assessment.

4. Literature Review of Genotoxicity Publications

The following genotoxicity literature review was conducted by an expert in the field of genotoxicology. Relevant OECD Tier II-like summaries and Klimisch ratings (Klimisch, 1997), as described in introduction of the overall literature review, follow this genotoxicity literature review.

Review of Genotoxicity of Glyphosate and Glyphosate Based Formulations,
Larry D. Kier, PhD, Genotoxicology Consultant, Buena Vista, CO

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, tallowamine ethoxylate; SCE, sister chromatid exchange; SCGE, single cell gel electrophoresis (comet).

Abstract
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). 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 endpoint 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.

1. Introduction
Glyphosate is the active ingredient in very widely used herbicide formulations in crop production, industrial turf, ornamental plants, forestry, roadsides, home lawns and gardens. Accordingly, the toxicity of glyphosate and its formulated products has been extensively studied. An earlier thorough review of glyphosate and glyphosate formulation safety and risk assessment included descriptions and analyses of genetic toxicology studies of glyphosate, the original Roundup™ formulation and other glyphosate based formulations (GBFs) (Williams et al., 2000). Subsequently, a fairly large number of genetic toxicology studies of glyphosate and GBFs have been published. These studies include a wide variety of test systems and endpoints. The number and diversity of the studies warrant careful examination and integration of their findings with the previous results to produce an updated assessment of the overall genotoxicity profile of glyphosate and GBFs.

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 GBFs that also contained searchable terms which indicated that genotoxicity studies were performed. Literature search utilized 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 Science[SM], 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 endpoint. 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 Organization 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 1 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 summarized 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 ([redacted] containing 41% isopropyl amine glyphosate salt and 15.4% MON 0818 (a polyethoxylated tallowamine 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 (Dearfield et al., 2011; Muller and Kasper, 2000; Scott et al., 1991; Thybaud et al., 2007b; Thybaud et al., 2007a). 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 1. 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)
In vitro 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	
In vivo 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	
In vitro 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	
In vitro 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)	
In vitro 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	
In vivo 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	
In vivo 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 2 presents a summary of genotoxicity test results for glyphosate and GBFs published subsequent to Williams et al. (2000). Test results are organized by the major genotoxicity assay categories of gene mutation, chromosome effects and DNA damage and other endpoints. 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 (Williams et al., 2000) rather than the original references reviewed in (Williams et al., 2000).

Table 2. Genetic Toxicology Studies of Glyphosate and Glyphosate Formulations Published On or After 2000

Endpoint	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vitro Gene Mutation						
Point mutation	Ames strains	Perzocyd 10 SL formulation	2 µg/plate (toxic)	Negative	TA1535 not used	Chruscielska et al., 2000
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
In Vitro Chromosome Effects—Mammalian Systems						
Cytokinesis block micronucleus	Bovine lymphocytes	Glyphosate formulation (62% glyphosate Monsanto source)	560 µM 48 h –S9	Positive?	PH, MA, SC, TO	Piesova, 2004
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
Chromosome aberration	Mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear. PH, MA, SC, TO, RE	Am er et al., 2006
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
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
Chromosome aberration	Human lymphocytes	Glyphosate (96%)	6 mM (not toxic)	Negative	MA, IC, RE	Manas et al., 2009b
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., 2009a
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., 2009b

Endpoint	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vitro Chromosome Effects— Non Mammalian Systems						
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
Micronucleus	Onion root tip meristem	Roundup formulation (Bulgaria)	1% active ingredient (estimated 4.4-5.9 mM)	Negative	TO, RE	Dimitrov et al., 2006
In Vivo Chromosome Effects—Mammalian Systems						
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	Chruscielska et al., 2000
Bone marrow erythrocyte micronucleus	Mouse	Roundup 69 formulation	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	Coutinho do Nascimento and Grisolia, 2000
Bone marrow erythrocyte micronucleus	Mouse	Roundup™ formulation (Monsanto)	2 x 200 mg/kg i.p.	Negative	TO, SC, IE, RE	Grisolia, 2002
Bone marrow Chromosome aberration	Rabbit	Roundup™ formulation	750 ppm in drinking water	Positive?	DL, PC, TO, SC, IC	Helal and Moussa, 2005
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	Amer et al., 2006
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	Amer et al., 2006
Bone marrow Chromosome aberration	Mouse	Roundup formulation (Bulgaria)	1080 mg/kg p.o. (1/2 LD_{50})	Negative	DL, TO, IC, RE	Dimitrov et al., 2006
Bone marrow erythrocyte micronucleus	Mouse	Analytical glyphosate (96%)	2 x 200 mg/kg i.p.	Positive	Erythrocytes scored? TO, SC, IC, RE	Manas et al., 2009b
Bone marrow Chromosome aberration	Mouse	Roundup™ formulation (Monsanto)	50 mg/kg i.p.	Positive	DL, SC, IC, RE	Prasad et al., 2009

Endpoint	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vivo Chromosome Effects—Non-Mammalian Systems						
Erythrocyte micronucleus	Oreochromis niloticus (Tilapia)	Roundup 69	170 mg/kg i.p. (maximum tolerated)	Negative?[c]	TO, RE	Coutinho do Nascimento and Grisolia, 2000
Wing spot test	Drosophila	Glyphosate (96%)	10 mM in larval stage	Positive/inconclusive[b]		Kaya et al., 2000
Erythrocyte micronucleus	Tilapia	Roundup™ formulation (Monsanto)	170 mg/kg (abdominal injection)	Positive	TO, RE	Grisolia, 2002
Erythrocyte micronucleus	*Crasseus auratus* (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, IE, RE	Cavas and Konen, 2007
	Prochilodus lineatus (tropical fish)	Roundup™ formulation (75% of 96 h LC50)	10 mg/l (6, 12 and 24 h) in water	Negative	DL, TO, SC, RE	Cavalcante et al., 2008
Erythrocyte micronucleus	Caiman eggs	Roundup® Full II formulation	1750 ug/egg	Positive	RE	Poetta et al., 2009
Erythrocyte micronucleus	Caiman eggs	Roundup® Full II formulation	Sprayed 2x with 100 litres of 3%/ha 30 days apart	Positive	DL, TO, RE	Poetta et al., 2010
In Vitro DNA Damage Mammalian Systems						
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
Sister chromatid exchange	mouse spleen cells	herbazed formulation	50 µM?	Positive	Concentrations used not clear MA, PH, TO, SC, RE	Amer et al., 2006
Sister chromatid exchange	bovine lymphocytes	Glyphosate formulation (62% glyphosate, Monsanto)	1.12 mM (toxic)	Positive	PH, SC, RE	Sivikova and Dianovsky, 2006
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., 2009b
Alkaline SCGE	Human lymphocytes	Glyphosate (technical, 96%)	580 µg/ml (toxic) (est. 3.43 mM)	Positive (-S9) Positive (+S9)		Mladinic et al., 2009a

Endpoint	Test System	Test Material	Maximum Dose	Result	Comment[a]	Reference
In Vitro DNA Damage Non-Mammalian Systems						
SOS	*E. coli*	Roundup BIO formulation	2.5 ug/sample	Positive		Raipulis J, 2009
Alkaline SCGE	Tradescantia flowers and nuclei	Glyphosate(technical, 96%)	700 µM	Positive	PH, SC	Alvarez-Moya et al., 2011
Bone marrow SCE	Mouse	herbazed formulation (84% glyphosate)	200 mg/kg p.o.	Positive	TO, SC, RE	Amer et al., 2006
Sperm abnormality	Mouse	herbazed formulation (84% glyphosate)	200 mg/kg p.o. (5 days)	Positive	TO, SC, RE	Amer et al., 2006
In Vivo DNA Damage Non-Mammalian Systems						
Erythrocyte Alkaline SCGE	Freshwater mussel larvae	Roundup formulation	5 mg/liter	Negative	TO, SC	Conners and Black, 2004
Erythrocyte alkaline SCGE	*Crasseus auratus* (goldfish)	Roundup formulation	15 ppm glyphosate in water (2, 4 and 6 days)	Positive	TO, RE	Cavas and Konen, 2007
Erythrocyte and gill cell alkaline SCGE	*Prochilodus lineatus* (tropical fish)	Roundup™ formulation (75% of 96 h LC50)	10 mg/l (6, 12 and 24 h) in water	Positive	DL, TO, RE	Cavalcante et al., 2008
Erythrocyte alkaline SCGE	Caiman eggs/hatchlings	Roundup® Full II formulation	1750 µg/egg	Positive	RE	Poletta et al., 2009
Erythrocyte alkaline SCGE	European eel	Roundup formulation	166 µg/liter	Positive	DL, SC, RE	Guilherme et al., 2010
Erythrocyte alkaline SCGE	Caiman eggs/hatchlings	Roundup® Full II formulation	Sprayed 2x with 100 l of 3%/ha 30 days apart	Positive	DL, RE	Poletta et al., 2010

[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.

3. Structure Activity Analysis

Glyphosate was evaluated using Derek for Windows (Llhasa 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.

4. Gene Mutation

As reviewed by Williams et al., (2000), 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) review only two gene mutation studies have been reported (Table 2). One negative Ames/*Salmonella* assay result was reported for a GBF of undefined composition, Percozyd 10 SL (Chruscielska et al., 2000). 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., 2004). 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.

5. Chromosome Effects

Assays to detect chromosome effects such as structural chromosome aberrations and micronucleus incidence constitute a second major genotoxicity endpoint category. A large number of publications with chromosome effects endpoints have been reported since the Williams et al. (2000) review. These are described in Table 2 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.

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). 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 2 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) 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 1). 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.

5.1.2 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., 2009b; Mladinic et al., 2009a; Mladinic et al., 2009b). Negative results for the micronucleus or chromosome aberration endpoints 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; Piesova, 2005). 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). For 48 hours of treatment a statistically significant increase in micronucleus frequency was observed in one donor at 280 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. The second publication reported negative results for the cytokinesis block micronucleus assay in bovine lymphocytes incubated with glyphosate formulation up to 560 µM for two hours in the absence and presence of a mammalian metabolic activation system (Piesova, 2005). 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 µM but not at 560 µM and in a second donor at 560 µM but not 280 µM. 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; Sivikova and Dianovsky, 2006). 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) only examined effects detectable by staining of chromosome 1 and did not report positive control results (Holeckova, 2006). 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 µg/ml, ≈ 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., 2009a). Also, the negative control level of such endpoints as necrosis and alkaline SCGE tail moment was significantly increased in the presence of S9 (Mladinic et al., 2009a). It should be noted that glyphosate is mostly excreted unmetabolized *in vivo* in mammals with only very small levels of aminomethylphosphonic acid (AMPA) or an AMPA-related structure observed (Anadon et al., 2009; Brewster et al., 1991). 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. It is possible that such effects might be generated by *in vitro* S9-mediated processes that are not relevant to *in vivo* processes such as genotoxic effects of low pH observed in the presence of S9 in *in vitro* assays (Cifone et al., 1987). 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) and a bovine lymphocyte cytokinesis block micronucleus study (Piesova, 2005). 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 endpoints 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) 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., 2009a; Mladinic et al., 2009b) 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 2) 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) and a negative result in the presence of S9 for the micronucleus endpoint in bovine lymphocytes (Piesova, 2005). 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.

5.1.3 Results for GBFs

Amer et al. (2006) 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 characterized, 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 µM) 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). 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).

5.2 In vivo Chromosome Effects—Mammalian Systems

The Williams et al. (2000) 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 2, 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.

5.2.1 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 (Chruscielska et al., 2000). 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 (Manas et al., 2009b). 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 (Williams et al., 2000) confirms that positive results are not simply due to repeat dosing. The reported negative result (Chruscielska et al., 2000) 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 (Williams et al., 2000). Also, the apparent scoring of micronuclei in erythrocytes rather than just polychromatic erythrocytes raises a significant methodological question for the reported positive study.

5.2.2 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 (Coutinho do Nascimento and Grisolia, 2000; Grisolia, 2002). 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 (Dimitrov et al., 2006) 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. (Prasad et al., 2009). 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 2 and Williams et al., 2000). 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 (Heydens et al., 2008). 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 (Helal and Moussa, 2005). 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 (Saunders, 2005). 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; Savage, 1976). 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 (Chruscielska et al., 2000). 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 (Amer et al., 2006). 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

micronucleus test of mice treated with glyphosate up to 50,000 ppm in feed for 13 weeks (Williams et al., 2000) 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 Amer et al. (2006) studies. Thus, it appears likely that these effects were due to some component(s) of the specific herbazed GBF tested rather than glyphosate.
In vivo mammalian assays for chromosome effects are an important category for characterizing 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.

5.3 In vivo Chromosome Effects—Non-Mammalian Systems
The Williams et al. (2000) 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 endpoint 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. (Coutinho do Nascimento and Grisolia, 2000). 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 (Cavalcante et al., 2008). 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 (Grisolia, 2002). 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 (Cavas and Konen, 2007). 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/liter in water).
Results for an unusual test system of exposed caiman eggs are reported in two publications. In one study eggs were topically exposed in a laboratory setting to Roundup Full II GBF, and erythrocyte micronucleus formation was measured in hatchlings (Poletta et al., 2009). The GBF tested was reported to contain the potassium salt of glyphosate and alkoxylated 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 endpoint (Wolf et al., 2008). 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.
A second publication by (Poletta et al., 2011) described two field experiments evaluating caiman hatched from eggs in artificial nests that were sprayed on incubation days 5 and 35. Experiment 1 dosed with two applications of Roundup Full II GBF and experiment 2, twelve months later, with the same dosing regimen except the second application at incubation day 35 included cotreatment with cypermethrin and endosulfan formulations. Increases in micronucleated erythrocyte frequency in hatchlings were reported

for both experiments. Additional measurements of growth showed small but statistically significant differences in total length and snout-vent length in 3 month old, but not 12 month old animals in both experiments. Alanine aminotransferase enzyme levels in serum of 3 month old animals in both experiments were significantly elevated (>2-fold control values). Alterations in these parameters suggest that the treated groups had some persistent biological differences from control group animals either as a result of treatment or some other factor. It is certainly possible that the micronucleus effects in both publications are associated with these persistent biological differences rather than from genotoxic effects induced in the embryos.

One published study reported a weak positive result in a *Drosophila* wing spot assay (Kaya et al., 2004). 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.

6. DNA Damage and Other Endpoints

A number of studies of glyphosate and GBFs have been published since 2000 which used various DNA damage endpoints in a variety of *in vitro* and *in vivo* systems. The DNA damage category includes endpoints such as sister chromatid exchange and DNA repair response in bacteria, but the most common DNA damage endpoint encountered was the alkaline single cell gel electrophoresis endpoint (alkaline SCGE) also commonly referred to as the "comet" assay. The alkaline SCGE endpoint 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 endpoints are not specific indicators of genotoxicity per se. These include sperm morphology and carcinogenicity studies.

6.1 In vitro DNA Damage Studies

Some positive results for glyphosate or GBFs in the SCE endpoint were reported in cultured human and bovine lymphocytes in the earlier review (Williams et al., 2000). 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 endpoint 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 uncharacterized herbazed GBF in mouse spleen cells (Amer et al., 2006). 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 endpoint 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). 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). 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 endpoint 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).

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. Characterization 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., 2009b). 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., 2009a). 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 endpoints (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) and in the *E. coli* SOS chromotest for DNA damage conducted on a Roundup BIO GBF (Raipulis J, 2009). 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 (ICHS2(R1), 2008; Parry et al., 2010). 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 (Henderson et al. 1998; Storer et al. 1996; Tice et al. 2000). 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 (Storer et al. 1996). 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., 2009a) 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.

6.2 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 (Williams et al., 2000).

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 uncharacterized herbazed GBF (Amer et al., 2006). 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/liter of a Roundup GBF in water (Cavalcante et al., 2008). 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, *Carasseus auratus*, exposed to 5, 10 and 15 ppm of a Roundup GBF for 2, 4 or 6 days (Cavas and Konen, 2007). Similar effects were observed for other endpoints (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 (Guilherme et al., 2010). 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/liter 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. Negative alkaline SCGE results were reported in cells of freshwater mussel larvae exposed to 5 mg/liter of a Roundup GBF in water for 24 hours (Conners and Black, 2004). This concentration was reported to be one-half of a no observable effect concentration and the 24-hour LC50 for this GBF was reported to be 18.3 mg/liter in parallel experiments.

6.3 Significance of DNA Damage Endpoint Results

DNA damage endpoints such as SCE or alkaline SCGE are generally regarded as supplementary to the gene mutation and chromosome effects endpoint categories. DNA damage endpoints do not directly measure effects on heritable mutations or events closely associated with chromosome mutations. It is widely recognized 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.

There are numerous examples of SCE positive responses which are unique compared to other genotoxic endpoints, are not concordant with carcinogenicity, or which are induced by oxidant stress (Benigni, 1989; Bradley et al., 1979; Decuyper-Debergh et al., 1989; Djelic et al., 2006; Eckl et al., 1993; Speit, 1986; Tayama and Nakagawa, 1994; Zeiger et al., 1990). These examples indicate that the SCE endpoint,

particularly in *in vitro* assays, should not be assumed to indicate DNA reactive genotoxicity or to have the same weight as genotoxicity assays using other endpoints such as gene mutation or chromosome effects.
Similarly, there are abundant data supporting the concept that induction of DNA strand breakage or alkaline SCGE effects can be secondary to necrotic or apoptotic processes (Amin et al., 2000; Henderson et al., 1998; Kiffe et al., 2003; Storer et al., 1996; Tice et al., 2000). Several clear specific examples exist of *in vitro* induction of alkaline SCGE effects in mammalian cells by conditions which do not appear to be relevant to genotoxic potential at lower doses or which occur by mechanisms that do not involve direct interaction with DNA. These include induction of alkaline SCGE effects by apoptosis inducers which inhibit topoisomerases (Boos and Stopper, 2000; Gieseler et al., 1999); cytokine treatment of cultured cells (Delaney et al., 1997); sodium dodecyl sulfate and potassium cyanide (Henderson et al., 1998); colchicine, dl-menthol and sodium acetate (Kiffe et al., 2003); luteolin (Michels et al., 2005); gossypol (Quintana et al., 2000), carbon tetrachloride (Sasaki et al., 1998) and vitamin C (Anderson et al., 1994). The reported positive results for vitamin C by Anderson et al. (1994) are interesting because comet effects were observed in the same 1-10 mM concentration range as reported for glyphosate or GBFs in *in vitro* alkaline SCGE assays. Further examples of alkaline SCGE effects of questionable genotoxic biological significance include dietary flavonoids quercitin, myricetin and silymarin (Duthie *et al.* 1997); hemoglobin (Glei *et al.* 2005); olive oil extracts (Nousis *et al.* 2005) and capsaicin (Richeux *et al.* 1999).
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, polyoxyethylenealkalylmine (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 (Levine et al., 2007). 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.
Some data suggest better concordance of the alkaline SCGE assay with other genotoxic endpoints or carcinogenicity in *in vivo* mammalian studies (Brendler-Schwaab et al., 2005; Hartmann et al., 2004; Kirkland and Speit, 2008). However there are examples of *in vivo* studies of alkaline SCGE effects with questionable genotoxicity significance because of negative results for other *in vivo* genotoxic endpoints or carcinogenicity assays or which appear to be due to toxicity. Some examples of positive results for non-carcinogens include thiabendazole, saccharine, tartrazine and ortho-phenylphenol (Brendler-Schwaab et al., 2005). Discordance between carcinogenicity species specificity and *in vitro* alkaline SCGE has also been observed (Sekihashi et al., 2002) as well as other positive results for non-carcinogens (Kirkland and Speit, 2008). Another example of questionable *in vivo* genotoxic significance is positive alkaline SCGE effects produced in lymphocytes of exercising humans that were not accompanied by micronucleus induction (Hartmann et al., 1998)
It is has long been recognized that alkaline SCGE effects, even in *in vivo* studies, can arise from processes that do not involve direct DNA-reactivity such as cytotoxicity and induction of apoptosis (Tice et al., 2000; Hartmann et al., 2003; Burlinson et al., 2007). Concurrent assessment of cytotoxicity is recommended in *in vivo* studies. The reported "gold standard" for cytotoxicity is histopathological evaluation of the tissues or cells being evaluated (Burlinson et al., 2007). Other measures for evaluating cytotoxicity include neutral pH SCGE to detect double strand breaks associated with apoptosis or necrosis and measurement of "hedgehogs" which are nuclei in which almost all of the DNA is in the tail (Tice et al., 2000). The latter are thought to represent dead or dying cells severely damaged by cytotoxicity. While "hedgehogs" are usually not included in tabulation of alkaline SCGE effects, they may be used as an additional measure of toxic effects (Smith et al., 2008). 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.

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 summarized in Table 3.

Table 3. Studies of Human and Environmental Populations with Reported or Assumed Glyphosate Exposure

Exposed Population	Endpoint	Exposures	Result	Reference
Human Studies				
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	Andre V, 2007
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	Bolognesi et al., 2009
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	Bolognesi et al., 2004
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	Bolognesi et al., 2002
Agricultural workers	Buccal cell micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increase in MN	Bolognesi et al., 2009
Workers exposed to pesticides	Lymphocyte SCE, micronucleus, chromosome aberration (CA)	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increases in MN and SCE but not chromosome aberrations	Costa et al., 2006
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	Lebailly et al., 2003
Agricultural workers	Lymphocyte SCE; buccal cell micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increase in SCE in lymphocytes and micronucleus frequency in buccal cells	Martinez-Valenzuela et al., 2009
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	Pastor et al., 2003
Individuals on or near glyphosate spraying	Lymphocyte alkaline SCGE	Glyphosate formulation aerially sprayed within 3 km	Statistically significant increases in damaged cells	Paz-y-Mino C, 2007

Exposed Population	Endpoint	Exposures	Result	Reference
Greenhouse Farmers	Lymphocyte SCE	Glyphosate formulation use reported in 99/102 workers; numerous other pesticides used	Statistically significant increases in SCE	Shaham et al., 2001
Workers exposed to pesticides	Lymphocyte alkaline SCGE	Glyphosate formulation use reported along with numerous other pesticides	Statistically significant increases in damaged cells	Simoniello et al., 2008
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	Vlastos D, 2006
Environmental Studies				
Meadow voles living on golf cousres	Blood cell alkaline SCGE; erythrocyte micronucleus	Glyphosate formulation use reported along with numerous other pesticides	Some effects judged possibly related to Daconil® fungicide	Knopper et al., 2005
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	Salvagni J, 2011

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 (Andre V, 2007; Bolognesi et al., 2004; Lebailly et al., 2003; Pastor et al., 2003; Vlastos D, 2006).

Several other studies did not indicate the extent of usage of specific pesticides but simply listed a large number of pesticides used by the populations (Bortoli et al., 2009; Costa et al., 2006; Martinez-Valenzuela et al., 2009; Simoniello et al., 2008). In other studies, incidence of GBF use by the population studied was significant but high incidence of use of other pesticides was also reported (Bolognesi et al., 2002; Shaham et al., 2001). 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 (Paz-y-Mino C, 2007). 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 tallowamine 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 liters/ha which was stated to be 20 times the maximum recommended application rate. Some of the reported human health effects described by Paz-y-Mino (2007) 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 (Menkes et al., 1991). Given the considerably favorable general toxicology profile of glyphosate as reported by the WHO/FAO Joint Meeting on Pesticide Residues (WHO/FAO, 2004) and in Williams et al. (2000), 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 (Bolognesi et al., 2009). 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 Bolognesi et al. (2009) 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 binuclated 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 Bolognesi et al. (2009) 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 uncharacterized 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 attritubable to glyphosate formulation exposure. This conclusion is reinforced by Acquavella et al. (2004), 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 (Knopper et al., 2005). 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 (Salvagni J, 2011). 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 interpretted 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.

8. DNA-Reactivity and Carcinogenesis

As noted in the earlier review, ^{32}P-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 (Peluso et al., 1998; Williams et al., 2000). 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 (Bolognesi et al., 1997; Williams et al., 2000). 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 ^{32}P-postlabelling and DNA strand breakage and 8-OHdG studies (Heydens et al., 2008). 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 ^{32}P-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 organizations (Williams et al., 2000).

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, tallowamine ethoxylate, POEA). Today a wide variety of surfactant systems are employed by different companies for different regions and end uses.

In the earlier review, summarized 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 (Williams et al., 2000). 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 (Manas et al., 2009a). 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 endpoints (Fellows and O'Donovan, 2007).

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) The maximum dose level was much higher than those used by Manas et al. (2009a) Although Manas 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 (OECD474, 1997). PCE/NCE ratio data from the Manas et al. (2009a) 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 Manas et al. (2009a) 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). 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. These POEA effects have been noted in aquatic species of several taxa (Folmar et al., 1979; Moore et al., 2011; Perkins et al., 2000; Tsui and Chu, 2003; Wan et al., 1989). 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). 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). A transcription profiling study of a Roundup GBF in yeast produced responses similar to those produced by detergent and oil treatments, and glyphosate alone did not produce effects at equivalent concentrations (Sirisattha et al., 2004). Effects on mammalian cells consistent with membrane disruption and consequent cytotoxicity were observed for POEA (Benachour and Seralini, 2009).

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 (Williams et al., 2000). 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., 2009a; Mladinic et al., 2009b). An enrichment for centomeric-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 characterized.

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 endpoint 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 endpoints for initial core testing, particularly for *in vitro* testing (Cimino, 2006; Eastmond et al., 2009; ICHS2(R1), 2008). 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 endpoints.

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 uncharacterized 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 (Cavalcante et al., 2008; Manas et al., 2009b; Mladinic et al., 2009a; Sivikova and Dianovsky, 2006). 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 endpoint. 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 endpoint 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 (Hartmann et al., 1998). 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). 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 Aquavella et al. (2004) farmer biomonitoring study would be on the order of 24nM 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 endpoint 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 endpoint 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; EPA, 1993; WHO/FAO, 2004) (AVPMA, 2010). 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.

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 *Tradescantia* bioassays Genetics and Molecular Biology Volume: 34 Number: 1 Pages: 127-130

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 utilized 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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate (N-(phosphonomethyl)-glycine)
Active substance(s):	Glyphosate
Source of test items:	Aldrich
Lot/Batch #:	09816PE
Purity:	Glyphosate: 96%

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells / organism:

Species:	*Tradescentia*
Strain:	Clone (4430) (hybrid *T. subacaulis X T. hirsutiflora*)
Source:	Not reported
Growth conditions:	Daytime temperature: 22°C Night-time temperature: 16-18°C

4. Test methods:

Pink mutation assay:	Assessment of DNA-damage in nuclei from treated plants
Comet assay:	Assessment of DNA-damage in nuclei from treated plants (in vivo assay), or in nuclei from untreated plants exposed in vitro

(in vitro assay)

Guideline: Non-guideline assays

GLP: No

Guideline deviations: Not applicable

Treatment: Plants:
30 inflorescences, corresponding to about 15 flowers (1500 to 3000 stamen hairs), were immersed for 3 h in 250 mL of test substance solution, or negative or positive controls. The test was carried out in duplicate. After exposure inflorescences were washed with distilled water and placed in Hoagland´s solution until further processing.

A part of the inflorescences were used for the pink mutation assay, the other part were used for the comet assay.

Nuclei from untreated plants:
After slide preparation (as described below) slides with nuclei extracted from untreated plants were exposed for 3 h at 25°C to the test substance preparations or controls, washed and stored at 4°C.

Dose levels: 0.7, 0.07, 0.007, 0.0007 mM; vehicle used for preparation not reported

Negative control: Hoaglands solution

Positive control: 1 mM nitrosodiethylamine (NDEA) or 1 mM ethylmethane sulfonate. (Reporting deficiency: in the method section of the report NDA is positive control, in the figure in the results section EMS is positive control!)

Test conditions: Pink mutation assay:

Based on the results of the assay, the authors qualified the assay as unsuitable for Genotoxicity assessment of glyphosate. Therefore this assay in not further described.

Comet assay:

1) Extraction of staminal hair cell nuclei from treated or untreated plants

The stamens of ten treated flowers for each experimental point, obtained on the 6th day after treatment, were homogenised for 2 min using a mortar and Honda buffer (0.44 M sucrose, 2.5% Ficoll (type 400), 5% Dextran-T-40, 25 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol and 2.5 % Triton-X-100). The homogenate was filtered and the nuclei separated by centrifugation. Nuclei were washed 3 x in washing solution (sucrose 0.4 M, Tris-Base 50 mM, $MgCl_2$, pH 8.5) and resuspended in 200 µL of the same solution. Slides for electrophoresis were prepared according to Singh *et al*., 1988 [Exp. Cell Res., 175, 184-191].

Nuclei from untreated plants were prepared accordingly, using stamens from 10 untreated flowers.

2) Electrophoresis

Slides were immersed in lysis buffer (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris-HCl, 1 % sodium lauryl sarcosine, 1 % Triton X-100, and 10 % DMSO, pH 10) for 1 h at 4°C, then placed in a horizontal electrophoresis system with a high pH buffer (30 mM NaOH, 1 mM Na_2EDTA, pH 13) for 45 min prior to electrophoresis. Electrophoresis were carried out for 15 min at 1.0 V/cm and approx. 200 mA.

3) Microscopy

Slides were washed, neutralised and stained with ethidiumbromide. After further washing coverslips were added and the slides were evaluated using a fluorescence microscope. Nuclei were observed at 40 x magnification, and migration was determined by visual scoring of tail length, according to published protocols.

Replicates per dose level: 2

5. Observations/analyses:

Measurements: Comet assay: tail length, DNA migration

Statistics: The data obtained were submitted to one-way analysis of variance testing (ANOVA). Dunnett´s test was used for comparing the negative control with data from the nuclei of exposed plants and the exposed healthy ones.

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Not relevant** (Due to reliability, and exposure conditions of plants and inappropriate test model.).

3. 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
CAS-No.:	1071-83-6
Source:	Societa Italiana Chimici, Rome, Italy
Purity:	99.9 %
Test item:	Roundup ®
Active substance(s):	Glyphosate
Concentration:	30.4% glyphosate
Source:	Monsanto Italiana, Milan, Italy

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells / animals:

Primary cell culture:	Human lymphocytes
Source:	Heparinised veneous blood samples from two healthy female donors.
Culture conditions:	Whole blood (0.5 mL) was added to 4.5 mL of RPMI 1640 medium supplemented with 17% foetal bovine serum. After addition of 50 µL phytohemoaggluthin (PHA) and 50 µL of 1 mM bromodeoxyuridine (BUdR), cultures were incubated in

	complete darkness at 37 °C.
Animals:	
Species:	Mice
Strain:	Swiss CD1
Source:	Charles River, Como, Italy
Age at dosing:	8-10 weeks
Sex:	Male
Number of animals/group:	3
Weight at dosing:	30 - 40 g
Acclimation period:	Not reported
Diet/Food:	Not reported
Water:	Not reported
Housing:	Not reported
Environmental conditions:	Not reported

4. Test methods:

GLP:	No (for all tests)
***In vitro* sister chromatide exchange (SCE) test:**	Assessment of cytogenicity
Guideline:	None
Guideline deviations:	Not applicable
Dose levels:	Glyphosate: 0, 0,33, 1, 3, 6 mg/mL Roundup: 0, 0.1, 0.33 mg/mL Solvent used for preparation not reported.
Positive control:	None
Negative control:	Culture medium
Test conduct:	24 h after PHA stimulation of the cultured lymphocytes the test substances were added and cultured were further incubated for 48 h. Two hours before determination, 75 µL of Colcemid was added. At termination, 72 h from onset of culture, slides were prepared according to standard methods and stained.
Exposure duration:	Last 48 h of the culture duration of 72 h
Replicates per dose level:	2
Number of cells analysed	At least 50 metaphases were scored for each experimental point by two observers.
***In vivo* alkaline elution assay:**	Assessment of DNA damage
Guideline:	None
Guideline deviations:	Not applicable
Dose levels:	Glyphosate: 300 mg/kg bw Roundup: 900 mg/kg bw (≅ 270 mg/kg bw glyphosate)
Test substance preparations:	Test substances were prepared in physiological saline. The pH of each solution was checked and adjusted to pH 7.0 before treatment.
Positive control:	None

Negative control:	Yes, but details not provided
Conduct of test:	Groups of 3 male mice were treated by i.p. injection with a single dose of the test substance preparations or control. Animals were sacrificed 4 and 24 hours after the injection. Liver and kidney were removed and processed to obtain crude nuclei free from adhering tissues. These nuclei further processed and subjected to alkaline elution assay.
Exposure duration	4 h and 24 h
Replicates per dose level:	3
8-OHdG-levels:	Assessment of oxidative DNA damage
Guideline:	None
Guideline deviations:	Not applicable
Dose levels:	Glyphosate: 300 mg/kg bw
	Roundup: 900 mg/kg bw (≅ 270 mg/kg bw glyphosate)
Test substance preparations:	Test substances were prepared in physiological saline. The pH of each solution was checked and adjusted to pH 7.0 before treatment.
Positive control:	None
Negative control:	Yes, but details not provided
Conduct of test:	Same as described for the alkaline elution assay (see above).
Exposure duration	4 h and 24 h
Replicates per dose level:	3
Tissue sampling and processing:	Livers and kidneys were removed and homogenised in 5 mL PBS. Nuclei were obtained by centrifugation and further processed for DNA extraction. Aliquots of DNA are hydrolised with Nuclease P and alkaline phosphatise, and filtered through cellulose acetate filter units (0.22 mm).
***In vivo* Micronucleus test (MNT):**	Assessment of cytogenicity
Guideline:	None
Guideline deviations:	Not applicable
Dose levels:	Glyphosate: 300 mg/kg bw
	Roundup: 450 mg/kg bw (≅ 135 mg/kg bw glyphosate)
Positive control:	Methyl methanesulfonate (MMS)
Negative control:	Not reported
Animals per dose group:	3
Application:	i.p. injections at 24 h interval
Number of treatments:	2 (test substance groups); 1 (control groups)
Sacrifice:	6, 24 h after the second injection
Sampling and sample processing:	Bone marrow smears were prepared from both femoral bones following the method described by Schmid (1975) with minor modifications.

5. Observations/analyses:

***In vitro* SCE**	
Measurements:	SCEs were determined in at least 50 metaphase cells per culture

***In vivo* alkaline elution assay**

Measurements: DNA elution rate. Fluorometric determination of DNA was performed with Hoechst 33258 reagent.

Results were expressed as elution rate constant K
$K(mL^{-1})$ = -ln fraction of DNA retained on filter / eluted volume

8-OHdG-levels

Measurements: Approximately 80 µg of DNA per sample is injected in HPLC for 8-OHdG determination.

The separation of 8-OHdG and normal deoxynucleosides is performed in a LC-18-DB column (Supelco, 75 x 4.6 mm) equipped with an LC-18-DB guard column cartridge. UV-detection was accomplished at 254 nm, and electrochemical analysis was carried out by a pulsed electrochemical detector.

The 8-OHdG levels are referred to the amount of deoxyguanidin (dG) detected by UV-absorbance at 254 nm. The amount of DNA is determined by a calibration curve vs known amounts of calf thymus DNA.

8-OHdG-levels are expressed as the number of 8-OHdG-adducts per 10^5 dG bases.

***In vivo* MNT**

Mortality/clinical signs: Not reported

Measurements: In order to score micronuclei about 1000 erythrocytes per animal were analysed.
To evaluate bone marrow toxicity, 1000 erythrocytes were counted and the ratio polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) was calculated

Statistics for all tests: The standard deviation and the nonparametric test of Mann-Whitney were used for the statistical analysis.

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Not relevant** (Due methodological and reporting deficiencies data considered to be supplemental information. i.p. exposure route is not relevant for human exposure)

3. 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 Mutagnesis Volume: 17 Number: 5 Pages: 391-397

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

MATERIALS AND METHODS

1. Test material:

Test item:	≥ 50 pesticides
Active substance(s):	≥ 50, including glyphosate
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	Exposed group: 17-59 y; non-exposed: 16-53 y
Sex:	Exposed group: 92 male, 15 females non-exposed group: 41 males, 19 females
Smoking habits:	Exposed group: 23 smokers, 36 former smokers, 48 non-smokers Non-exposed group: 20 smokers, 19 former smokers, 22 non-smokers

Persons per group:	107 exposed; 61 non-exposed (control)
Exposure duration:	2-70 years (mean 27.8 ± 15.5)

4. Test system:

Study type:	Epidemilogical study for cytogenicity – Micronucleus assay
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Duration of study:	1 year
Application rate:	Not specified
Persons per group:	107 exposed; 61 non-exposed (control)
Application technique:	Not specified
Mixing/loading performed:	Yes: 88 No: 19
Use of personal protective equipmet (PPE):	Yes: 90 No: 17
Cultivation conditions:	Greenhouses: 19 Open field: 49 Both: 39
Crops:	Ornamentals: 81 Vegetables & ornamentals: 26
Exposure conditions:	75.7 % harvesting ornamentals 24.3% harvesting of ornamentals & vegetables 82.2% preparing pesticides
Blood sampling:	Blood samples were obtained from each subject by venipuncture. All blood samples were collected in sterile sodium heparin tubes. The specimen were received in the laboratory within a few hours of collection and were processed immediately.
Cell cultures:	Whole blood was added to 4.5 mL of RPMI 1640 complete medium with 10 % FCS, and 1% phytohaemoagglutinin. Cells were cultured for 72 hours at 37°C, with cytochalasin B being added after 44 h (concentration: 6 µg/mL). At the end of the incubation period, whole blood cultures were centrifuged, washed, and cells were fixed twice in cold fixative (methanol : acetic acid 3:1) for 20 min at room temperature. Samples for microscopic evaluation were loaded onto wet slides, air dried and stained with 3% Giemsa.

5. Observations/analyses:

Questionnaire:	All subjects. The following information was provided: Demographic information, personal data, smoking habits, history of recent illness and medical treatment. Exposed group: in addition, kind of crops handled, pesticide use, exposure duration, work activity, protective measures
Microscopic micronuclei determination:	2000 binucleated lymphocytes with preserved cytoplasm were scored for each subject on coded slides. The number of binucleated cells with micronuclei (BNMN)

were determined.

Statistics. Parametric and non-parametric statistical test were used. Student´s t-test for independent samples was applied to detect differences in the mean of BNMN in the exposed and non-exposed subjects. Differences among the group means were evaluated by non-parametric Mann-Whitney U-test. The relationship between BNMN and use of protective measures was evaluated using regression analysis.

KLIMISCH EVALUATION

1. 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 resulte unreliable for glyphosate.

2. Relevance of study: **Not relevant** (Due to the exposure of multiple pesticides, only general conclusions about pesticide exposure and cytogenicity possible. Not relevant to glyphosate).

3. 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 centrometric probe Mutation Research Volume: 557 Number: 2 Pages: 109-117

Abstract*

Flower production in greenhouses associated with a heavy use of pesticides is very wide-spread 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

MATERIALS AND METHODS

1. Test material:

Test item:	≥ 50 pesticides
Active substance(s):	≥ 50, including glyphosate
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	Exposed group: 50.16 ± 13.67 y; Non-exposed: 46.83±10.59 y
Sex:	Exposed group: 44 male, 7 females non-exposed group: 15 males, 9 females
Smoking habits:	Exposed group: 11 smokers, 11 former smokers, 29 non-

	smokers Non-exposed group: 11 smokers, 5 former smokers, 8 non-smokers
Persons per group:	51 exposed; 24 non-exposed (control)
Exposure duration:	2-10 years (mean 26.34 ± 14.46)

4. Test system:

Study type:	Epidemilogical study for cytogenicity – Micronucleus assay + FISH
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Application rate:	Not specified.
Persons per group:	51 exposed; 24 non-exposed (control)
Application technique:	Not specified
Mixing/loading performed:	Yes: 36 No: 15
Use of personal protective equipmet (PPE):	Yes: 44 No: 7
Cultivation conditions:	Greenhouses
Crops:	Ornamentals
Exposure conditions:	Not reported
Blood sampling:	Blood samples were obtained from each subject by venipuncture. All blood samples were collected in sterile sodium heparin tubes. The specimen were received in the laboratory within a few hours of collection and were processed immediately.
Cell cultures:	Whole blood was added to 4.5 mL of RPMI 1640 complete medium with 10 % FCS, and 1% phytohaemoagglutinin. Cells were cultured for 72 hours at 37°C, with cytochalasin B being added after 44 h (concentration: 6 µg/mL). At the end of the incubation period, whole blood cultures were centrifuged, washed, and cells were fixed twice in cold fixative (methanol : acetic acid 3:1) for 20 min at room temperature. Samples for microscopic evaluation were loaded onto wet slides, air dried and stained with 3% Giemsa or hybridized within 1 week of preparation
Fluorescence in situ hybridisation (FISH):	Centrometric FISH was performed using an alphoid centromer-specific biotinylated probe for all centromers, which was previously tested onmetaphase chromosome for centromer-specific labelling. Prepared slides were processed for hybridisation. The hybridisation mixture containing the probe (2.5 µg/mL) and 500 µg/mL salmon sperm DNA in 2 x SCC was denatured at 70°C for 5 min, followed by chilling on ice for 4 min. An aliquot of 50 µg per slide was applied. The slides were covered with coverslips and sealed with rubber cement. Hybridisation was performed for 16 h at 37°Y in a moist chamber. Afterwards, the slides were washed, and incubated with

blocking reagent (5% skimmed milk in 4 x SCC) at 37°C for 10 min. The slides were washed with 4 x SCC, covered with a 1.250 dilution of anti-biotin-antibody in IB (immunological buffer: 0.5% skimmed milk in 4 x SCC) and incubated at 37°C for 30 min. Afterward, slides were washed in and incubated in a 1:20-dilution of FITC-conjugated anti-mouse antibody, followed by incubation with a 1:20-dilution of FITC-conjugated anti-sheep antibody for 30 min at 37°C. All incubations were performed in a moist chamber, and were followed by washes in Tween-20 buffer. After the last wash, slides were dehydrated with ethanol and stained with propidium iodide in anti-dde solution.

5. Observations/analyses:

Questionnaire: All subjects. The following information was provided:

Demographic information, personal data, smoking habits, history of recent illness and medical treatment.

Exposed group: in addition, kind of crops handled, pesticide use, exposure duration, work activity, protective measures

Microscopic analyses: Giemsa stained slides:

2000 binucleated lymphocytes with preserved cytoplasm were scored for each subject. The MN frequency was calculated as the number of binucleated cells with micronuclei (BNMN).

FISH:

Slides were scored with a microscope with fluorescence equipment. The micronuclei present in the bi-nucleated lymphocytes with intact cytoplasm were examined for the presence of one or more centrometric spots and were classified as centromer-positive (C+MN) or centromer-negative (C – MN). 2000 binucleated lymphocytes with preserved cytoplasm were scored for each subject

Statistics. Parametric and non-parametric statistical test were used. Student´s t-test for independent samples was applied to detect differences in the mean of BNMN in the exposed and non-exposed subjects. Differences among the group means and between the percentages of C + MN and C – MN analysed by FIH technique were evaluated by non-parametric Mann-Whitney U-test. The relationship between C + MN and age was evaluated using regression analysis. The level of significance was taken as $p \leq 0.05$.

KLIMISCH EVALUATION

1. 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

2. 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).

3. Klimisch code: **2**

Author(s)	Year	Study title
Cavas, T., Könen S.	2007	Detection of cytogenetic and DNA damage in peripheral erythrocytes of goldfish (Carassius auratus) exposed to a glyphosate formulation using the micronucleus test and the comet assay Mutagenesis 22 263-268

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

MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Test item:	Roundup®
Active substance:	Glyphosate
Source:	Not reported
Lot/Batch #:	Not reported
Purity:	480 g/L isopropylammonium salt (equivalent to 360 g/L glyphosate)
Stability of test compound:	Not reported

2. Vehicle and/or positive control: Specified under the respective tests

3. Test animals:

Species:	Goldfish, *C. auratus*
Strain:	Linneaus, 1758
Family:	*Cyprinidae*
Source:	Local market
Age:	Not specified
Length:	6 ± 1 cm
Weight:	5 ± 1 g
Acclimation period:	3 weeks
Conditions:	At a population density of 15 per 20 L aquaria
Diet/Food:	Once per day with commercial fish pellets. Amount not specified.

Environmental conditions:	Temperature: 25°C 12 hours light/dark cycle

4. Test methods:

Micronucleus test (MNT):	Assessment of cytogenicity
Comet assay:	Assessment of cytogenicity, DNA damage
Guideline:	Not stated
GLP:	No
Guideline deviations:	Not applicable
Exposure conditions:	Goldfish were placed in four different aquaria containing declorinated tap water and three different concentrations of Roundup®, corresponding to 5, 10 and 15 ppm glyphosate obtained by serial dilutions of Roundup®. The test water was renewed every 2 days.
Exposure duration:	2, 4, or 6 days
Dose levels:	0, 5, 10, 15 ppm glyphosate
Negative control:	Tap water
Positive control:	Cyclophosphamid (5 mg/L)
Group size:	5 fish per dose per duration
Blood sampling:	At the end of each exposure period fish were killed by cervical dislocation. Blood samples were obtained from the caudal vein of the fish.
Sample processing and slide preparation:	For the MNT blood smears were prepared immediately after sampling onto pre-cleaned slides. After fixation in pure ethanol for 20 min, slides were allowed to dry and stained with 10 % Giemsa for 25 min. All slides were coded and scored blind. Five slides were prepared for each fish, and 1500 cells were scored from each slide. For the Comet assay, about 0.5 mL of blood was diluted with 1 mL of phosphate-buffered saline. The Comet assay was performed according to *Tice et al, 2000* [*Env. Mol. Mutagen.*, 35, 206-222] with some modifications. Electrophoresis conditions were: 0.66V/cm, 300 mM, for 25 min. Slides were neutralised and stained with ethidium-bromide and evaluated using a fluorescence microscope. From each fish five slides were prepared and from each slide 200 cells were scored.

5. Observations/analyses:

Measurements:	MNT: Non-refractive, circular or ovoid chromatin bodies, smaller than the one-third of the main nucleus, were scored as micronuclei. Nuclear abnormalities (NA) other than micronuclei in erythrocytes were classified into 5 groups: binucleated cells, blebbed nuclei, lobed nuclei, notched nuclei

Comet assay: DNA-damage was quantified by visual classification of cells into five categories (comets) corresponding to the tail length:

Type 0: undamaged
Type 1: low-level damage
Type 2: medium-level damage
Type 3: high-level damage
Type 4: complete damage
The extent of DNA damage was expressed as the mean % of cells with medium, high ad complete damageg DNA, which was calculated as the sum of cells with damage types 2, 3, 4. From the arbitrary values assigned to the different categories, a genetic damage index (GDI) was calculated for each fish.

Statistics: After assessing the normality of distribution of the data, both parametric and non-parametric tests were used to detect the level of significance at the 0.05 level. Differences between mean values were compared using the Student´s t-test and least significant difference test for the micronuclei data and the Mann-Whitney U-test for the Comet assay data.

KLIMISCH EVALUATION

1. 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).

2. Relevance of study: **Relevant with restrictions** (Due to reliability. Discussion confuses glyphosate with glyphosate formulated products)

3. Klimisch code: **3**

Author(s)	Year	Study title
Guilherme, S. Gaivao, I. Santos, M.A. Pacheco, M.	2010	European eel (*Anguilla Anguilla*) genotoxic and pro-oxidant responses following short-term exposure to Roundup® - a glyphosate-based herbicide. Mutagenesis Volume: 25 Number: 5 Pages: 523-530

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 *m*g/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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup®
Active substance(s):	Glyphosate
Source of test items:	Bayer CropScience Portugal
Lot/Batch #:	Not reported
Purity:	485 g/L isopropylammonium salt of glyphosate (equivalent to 360 g/L or 30.8% of glyphosate)

2. Vehicle and/or positive control: No positive control

3. Test organism:

Species:	European eel (A. anguilla L.)
Source:	Captured from an unpolluted area of Aveiro Lagoon-Murtosa, Portugal
Length:	Average 25 ± 3 cm

Body weight:	32 ± 5 g (yellow eel stage)
Acclimation period:	12 days
Diet/Food:	Not fed during experimental period
Maintenance conditions:	During acclimatisation eels were kept in 80 L aquaria under a natural photoperiod, in aerated, filtered, dechlorinated and recirculated tap water
Physicochemical conditions of water:	Salinity: 0; Temperature: 20 ± 1 C; pH: 7.3 ± 0.2; Ammonia: <0.1 mg/L; Dissolved oxygen: 8.1 ± 0.5 mg/L

4. Test methods:

Comet assay:	Assessment of DNA strand breaks and alkali labelled sites
ENA assay:	Detection of micronuclei and other nuclear anomalies, clastogenicity, and aneugenicity
Catalase (CAT) activity:	Indicator of pro-oxidant state
Glutathion-S-transferase (GST) activity:	Indicator of pro-oxidant state
Indicator of pro-oxidant state Glutathion- peroxidise (GPx) activity:	Indicator of pro-oxidant state
Glutathion-reductase (GR) activity:	Indicator of pro-oxidant state
Total glutathion content (GSHt)-quantification:	Indicator of pro-oxidant state
Thiobarbituric acid reactive substances (TBARS) quantification:	Estimation for lipid peroxidation
Guideline:	None. Comet assay was done according to Collins (2004), Mol. Biotechnol. 26, 249-261M; with slight modifications
GLP:	No
Guideline deviations:	Not applicable
Dose levels:	58 µg/L (2 aquaria) and 116 µg/L (2 aquaria) of Roundup® (equivalent to 18 and 36 µg/L of glyphosate, respectively); 2 aquaria as controls with clean water.
Exposure duration:	For each pesticide concentration, 1- and 3-day exposures were tested, corresponding to the two different aquaria
Animals per dose group	36 eels divided in 6 aquaria
Exposure conditions:	The test was carried out in 20 L aquaria, in a static mode, under the same conditions as described for the acclimation period.
Blood sampling:	At the end of the exposure period blood was sampled from the posterior cardinal vein. Blood smears were immediately prepared for ENA assay. 2 µL of blood were diluted in 1 mL of phosphate-buffered saline for comet assay.. The remainder volume was stored at -80°C until further analyses for oxidative stress.
Tissue preparation and fractioning:	Whole-blood samples were lysed through homogenisation in a 1:15 ratio (blood : buffer, v/v), using a homogeniser in chilled phosphate buffer (0.2 M, pH 7.4). The lysate was divided into 3

aliquots for TBARS, GSHt quantification, as well as for post-mitochoindrial supernant (PMS) preparation. The PMS fraction was obtained by centrifugation (13400 g, 20 min, 4°C).

Test conditions: Comet assay: Two gel replicates each containing ca. 2 x 10^4 cells (using the blood samples) in 70 µL of 1% low melting point agarose in phosphate-buffered saline, were placed on glass mmicroscope slide, precoated with 1 % normal melting point agarose. Gels were covered with glass coverslips, left for ± 5 min at 4°C to sodify agarose and then immersed in lysis solution (2.5 M NaCl, 0.1 M ethylenediaminetetraacetic acid, 10 mM tris and 1 % Triton X-100, pH 10) at 4°C, for 1 h. Slides were immediately processed according to the conventional comet assay.

ENA assay:
This assay was carried out in mature peripheral erythrocytes according to the procedure of Pacheco and Santos.

Blood smear per animal was fixed with methanol for 10 min and stained with Giemsa (5%) for 30 min.

Replicates per dose level: Comet assay: 2

5. Observations/analyses:

Measurements: Comet assay: One slide with 2 gels (100 nucleotids/gel) was observed for each fish.
The DNA damage was quantified by visual classification of nucleoids into five comet classes, according to the tail intensity and length, from 0 (no tail) to 4 (almost all DNA in tail). The total score expressed as a genetic damage index (GDI) was calculated multiplying the mean percentage of nucleoids in each class by the corresponding factor.

Results were expressed as 'arbitrary units' in a scale of 0–400 per 100 scored nucleoids (as average value for the two gels observed per fish). Besides the GDI, the frequency of nucleoids observed in each comet class was also expressed as recommended by Azqueta et al.

ENA assay: From each smear 1000 erythrocytes were scored to determine the frequency of the following nuclear lesion categories: kidney shaped nuclei, lobed nuclei, binucleate or segmented nuclei, miclonuclei, and notched nuclei.

CAT activity: CAT activity was assayed (at 25 C) by the method of Claiborne as described by Giri et al. Change in absorbance was recorded spectrophotometrically at 240 nm and CAT activity was calculated in terms of micromoles H_2O_2 consumed per minute per milligram of protein (ε = 43.5/M cm).

GST activity: GST activity was determined (at 25 C) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, according to the method of Habig et al. Absorbance was recorded spectrophotometrically at 340 nm for 3 min. The enzyme activity was calculated as nanomoles CDNB conjugate formed per minute per milligram of protein (ε = 9.6/mM cm).

GPx activity: GPx activity was determined (at 25 C) according

to the method of Mohandas et al. NADPH oxidation was recorded spectrophotometrically at 340 nm and GPx activity was calculated in terms of nanomoles NADPH oxidised per minute per milligram of protein ($\varepsilon = 6.22 \times 10^3/M\ cm$).

GR activity: GR activity was assayed (at 25 C) by the method of Cribb et al. The assay determines indirectly the GR activity by measuring the NADPH disappearance associated to the reduction of oxidised glutathione catalysed by GR. Change in absorbance was registered spectrophotometrically at 340 nm during 3 min and GR activity calculated as nanomoles of NADPH oxidised per minute per milligram of protein ($\varepsilon = 6.22 \times 10^3/M\ cm$).

GSHt quantification: For GSHt quantification – the rate of TNB production is proportional to the concentration of glutathione in the sample. Formation of TNB was measured by spectrophotometry at 412 nm and the results expressed as nanomoles TNB formed per minute per milligram of protein ($\varepsilon = 14.1/mM\ cm$).

TBARS quantification: As estimation of lipid peroxidation (LPO), TBARS quantification was carried out in the previously prepared lysate as adapted by Filho et al. The absorbance was measured at 535 nm and the rate of LPO was expressed in nanomoles of TBARS formed per milligram of fresh tissue ($\varepsilon = 1.56 \times 10^5/M\ cm$).

Total protein: Total protein contents were determined according to the Biuret method, using bovine serum albumin as a standard

Statistics: SigmaStat software (SPSS Inc.) was used for statistical analyses. All data were first tested for normality and homogeneity of variance to meet statistical demands. One-way analysis of variance was used to compare the different treatments within the same exposure duration as well as to compare the same treatment in different exposure durations. The Tukey's test was applied for post-hoc comparison. Whenever the assumptions for parametric statistics failed, the non-parametric correspondent test (Kruskall–Wallis) was performed, followed by the non-parametric all pairwise multiple comparison procedure (Dunn's test). Differences between means were considered significant when $P<0.05$. The relationship between the assessed parameters was explored using linear regression analyses. The correlation coefficient (r) was calculated and its statistical significance (P) was determined from the table of critical values for the correlation coefficient.

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: **Not Relevant** (Non-standard test system, no positive controls to verify test method/study validity.)

3. Klimisch code: **3**

Annex point	Author(s)	Year	Study title
IIA 5.10	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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Pesticides: Ambush and Galecron; Herbicides: Treflan, Blazer, Roundup, 2,4-D Amine, Crossbow, Pramitol, Pondmaster
Active substance(s):	Ambush – permethrin Galecron – chlordimeform Treflan – trifluralin Blazer – scifluorfen Roundup – glyphosate 2,4-D Amine – 2,4-(Dichlorophenoxy) acetic acid Crossbow – 2,4,5-(Trichlorophenoxy) acetic acid Pramitol – prometon Pondmaster – glyphosate
Source:	Madison County Co-Op, Huntsville, Alabama
Lot/Batch #:	Not reported
Purity:	Not reported

Positive Contol	No positive control
2. Vehicle:	Distilled water
3. Test animals:	
Species:	Drosophila
Strains:	*Basc* genotype (females) and Canton-S (males, as a wild type)
Source:	Department of Biology, Alabama A. & M. University, Normal
Diet/Food:	Food pan was kept in the cage for 6 to 12 h
Collection of eggs:	The pan with eggs was kept at 25 °C for 24 h; Larvae were collected by adding 15% NaCl to the pan and then decanting the solution into a separatory funnel. The larvae were washed down with distilled water and collected on a piece of nylon gauze.
4. Test system:	
Study type:	Drosophila sex-linked recessive lethal (SLRL) test
Guideline:	Similar to, but not adhering to OECD 477
Guideline deviations:	No reference substances used; wild type male treatment age (treatment of larvae).
GLP:	No
Duration of study:	Not reported
Dose levels:	Control – only reported for Ambush – 0.1 ppm; Treflan – 1000 ppm; Blazer – 10000 ppm; Roundup – 1 ppm; 2,4-D – 10000 ppm; Crossbow – 10000 ppm; Galecron – 10000 ppm; Pramitol – 1000 ppm; Pondmaster – 0.1 ppm.
Treatment period:	Larval stage until the pupation
Treatment:	5 mL of each dilution was added to 3-4 mL of instant medium in vials. Each dilution was in sets with at least three vials. Larvae were added to the vials which were maintained in the hood at 25 C. In the control set of vials, 5 mL distilled water was added instead of the test chemical. Experiments were performed at LC_{50} except for very toxic compounds, where even lower survival points were used.
Reproduction procedure:	1 day old adult males treated as larvae were individually mated to six *Basc* virgins for 2 days to obtain the first brood. The males were then separated and each male was provided with a second set of virgins for the second brood. This procedure was repeated to obtain 6 consecutive broods

from individual males.

Inseminated females were allowed to lay eggs for 6 days and then discarded.

F1 progeny from each male were pair-mated separately in all broods.

Pair-matings were scored from 15 to 18 days after culturing.

The absence of wild-type males was used as the criterion for determining the lethal mutation.

Control experiments were performed similarly with untreated Canton-S males and virgin *Basc* females in order to determine the spontaneous mutation frequency.

5. Observations/analyses:

Measurements: Interbrood variations of sex-linked recessive lethal mutations;
Induction of sex-linked recessive lethals

Statistics: Simple χ^2 statistic was used

KLIMISCH EVALUATION

1. 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.)

2. Relevance of study: **Not Relevant to glyposate** (Glyphosate not tested; formulation tested)

3. Klimisch code: **3**

Annex point	Author(s)	Year	Study title
IIA 5.10	Manas, F. Peralta, L. Raviolo, J. 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	AMPA (aminomethylphosphonic acid)
Active substance(s):	AMPA (aminomethylphosphonic acid)
CAS-No.:	1066-51-9
Source:	Sigma-Aldrich, Argentina
Lot / Batch #:	Not reported
Purity:	Analytical grade. Not further specified.

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells / animals:

Cell lines:	Hep-2
Source:	Asociación Banco Argentino de Células (ABAC, Pergamino, Argentina)
Maintenance medium:	EMEM, supplemented with 200 mM L-glutamine, 2% (v/v) fetal calf serum, and antibiotics (penicillin, 100 U/ml, streptomycin 100 and 0.25 mg/ml anfotericine B)
Plate cultures:	96-well tissue culture plate (TPP® Zellkultur testplate 96F Switzerland) (8.25 x 10^5 cells/ml; 200 ml/well)
Culture conditions:	Cells were grown during 24 h at 37°C in an atmosphere of 5% CO_2 in air with 100% humidity to obtain confluent monolayers. Afterwards medium was removed and wells were replenished with MEM.

Primary cell culture:	Human lymphocytes
Source:	Six healthy donors, three females and three males, from 18 to 33 years old. The donors had no history of pesticide exposure and were non-smokers.
Culture conditions:	Lymphocytes were cultured for 72 h at 37 °C according to conventional methods.
Animals:	
Species:	Mice
Strain:	Balb-c
Source:	Not reported
Age at dosing:	8-12 weeks
Sex:	Males and females
Number of animals/group:	5
Weight at dosing:	Not reported
Acclimation period:	Not reported
Diet/Food:	Rodent diet, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Not reported

4. Test methods:

GLP:	No (for all tests)
Single-cell gel electrophoresis assay (Comet assay):	Assessment of DNA damage
Guideline:	Non-guideline study. Study carried out according to Singh et al. (1988). (Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 84–191.)
Guideline deviations:	Not applicable, but slight modifications from Singh et al., 1988 regarding sample preparations.
Dose levels:	2.5, 4.5, 5.5, 6.5, 7.5, 9 and 10.0 mM AMPA
Positive control:	Mitomycin C, 0.01 mM
Negative control:	MEM medium
Conduct of test:	The test was conducted in 96-well tissue culture plates. In all cases the cultures were diluted to 200 µL/well final volume.
Exposure duration	4 h
Replicates per dose level:	2
Chromosome aberration (CA) test:	Assessment of cytogenicity
Guideline:	OECD 473 (1997) cited
Guideline deviations:	Lower number of used analysable concentrations, lower number of metaphases analysed.
Dose levels:	0.9 and 1.8 mM AMPA. The test substance was previously solved in 1 mL of culture medium and adjusted to pH 7.2-7.4.

Positive control:	Mitomycin C, 0.9 µM
Negative control:	Culture medium
Exposure duration:	Last 48 h of the culture duration of 72 h
Replicates per dose level:	2
Number of cells analysed	2000/replicate
***In vivo* Micronucleus test (MNT):**	Assessment of chromosome damage
Guideline:	OECD 474 (1997)
Guideline deviations:	Higher number of immature erythrocytes per animal scored for the incidence of micronucleated immature erythrocytes
Dose levels:	100, 200 mg/kg bw
Positive control:	Cyclophosphamid 20 mg/kg bw
Negative control:	Saline solution
Animals per dose group:	5
Exposure route:	i.p. injections at 24 h intervals
Number of treatments:	2 (test substance groups); 1 (control groups)
Sacrifice:	24 h after the second injection
Sampling and sample processing:	Bone marrow smears were prepared from the femoral bones. Smears were stained with May Grunwald Giemsa as described by Schmid (1975)

5. Observations/analyses:

Comet assay	
Measurements:	Cell viability (by tryptan blue exclusion technique), tail moment (TM), DNA percentage in tail (% of DNA) and tail length (TL)
Chromosome aberration-test	
Measurements:	Mitotic index determined for 2000 cells/replicate Chromosome aberrations: 100 metaphase cells were analysed for chromosome aberrations and classified into the following categories: Chromatid breaks (ctb) and gaps (ctg), chromosome breaks (csb) and gaps (csg), dicentric chromosomes (dic), acentric fragments (ace) and endoreduplicated (end) cells
***In vivo* MNT**	
Mortality/clinical signs:	Not reported
Measurements:	In order to score micronuclei about 1000 erythrocytes per animal were analysed. To evaluate bone marrow toxicity, 500 erythrocytes were counted and the ratio polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) was calculated
Statistics for all tests:	The mean scores were calculated from the experiments of each duplicate treatment. The Kolmogorov–Smirnov test was performed to verify whether the results follow a normal distribution. The non-parametric Kruskal–Wallis Analysis of Variance on Ranks ($p<0.05$) test followed by the Dunn's Multiple Comparisons Test were used for comparing the means of each treatment with their negative and positive control in the

Comet assessment. The Pearson statistical test was used to examine possible dose–response effects. In all cases, the level of significance was set at α=0.05.

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Not relevant** (Due to reliability)

3. 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.	2009b	Genotoxicity of glyphosate assessed by the comet assay and cytogenic tests Environmental Toxicology and Pharmacology Volume: 28 Pages: 37-41

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate [*N*-(phosphonomethyl) glycine]
Active substance(s):	Glyphosate [*N*-(phosphonomethyl) glycine]
CAS-No.:	1071-83-6
Source:	Sigma-Aldrich, Argentina
Purity:	96%

2. Vehicle and/or positive control: Specified under the respective assays (see below)

3. Test system / cells / animals:

Cell lines:	Hep-2
Source:	Not reported
Maintenance medium:	EMEM, supplemented with 200 mM L-glutamine, 2% (v/v) fetal calf serum, and antibiotics (penicillin, 100 U/ml, streptomycin 100 and 0.25 mg/ml anfotericine B)
Plate cultures:	96-well tissue culture plate (8.25 x 10^5 cells/ml; 200 ml/well)
Culture conditions:	Not reported
Primary cell culture:	Human lymphocytes
Source:	Six healthy donors, three females and three males, from 18 to 33 years old. The donors had no history of pesticide exposure and were non-smokers.
Culture conditions:	Lymphocytes were cultured for 72 h at 37 °C according to conventional methods.

Animals:	Detailed parameters for mice are given only for the *in vivo* MNT, not for the TBARs, SOD and CAT determinations
Species:	Mice
Strain:	Balb-c
Source:	Not reported
Age at dosing:	8-12 weeks
Sex:	Males and females
Number of animals/group:	5
Weight at dosing:	Not reported
Acclimation period:	Not reported
Diet/Food:	Rodent diet, *ad libitum*
Water:	Water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Not reported

4. Test methods:

GLP:	No (for all tests)
Single-cell gel electrophoresis assay (Comet assay):	Assessment of DNA damage
Guideline:	Non-guideline study. Study carried out according to Singh et al. (1988). (Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 84–191.)
Guideline deviations:	Not applicable, but slight modifications from Singh et al., 1988 regarding sample preparations.
Dose levels:	3.00, 4.50, 6.00, 7.50, 9.00, 12.0 and 15.0 mM glyphosate
Positive control:	Mitomycin C, 0.01 mM
Negative control:	MEM medium
Conduct of test:	The test was conducted in 96-well tissue culture plates. In all cases the cultures were diluted to 200 µL/well final volume.
Exposure duration	4 h
Replicates per dose level:	2
Chromosome aberration (CA) test:	Assessment of cytogenicity
Guideline:	OECD 473 (1997)
Guideline deviations:	Lower number of metaphases analysed than required
Dose levels:	0.20, 1.20, 6.00 mM glyphosate. The test substance was previously solved in 1 mL of culture medium and adjusted to pH 7.2-7.4.
Positive control:	Mitomycin C, 0.89 µM
Negative control:	Culture medium
Exposure duration:	Last 48 h of the culture duration of 72 h
Replicates per dose level:	2
Number of cells analysed	2000/replicate

***In vivo* Micronucleus test (MNT):**	Assessment of chromosome damage
Guideline:	OECD 474 (1997)
Guideline deviations:	Higher number of immature erythrocytes per animal scored for the incidence of micronucleated immature erythrocytes
Dose levels:	50, 100, 200 mg/kg bw
Positive control:	Cyclophosphamid 20 mg/kg bw
Negative control:	Saline solution
Animals per dose group:	5
Exposure route:	i.p. injections at 24 h intervals
Number of treatments:	2 (test substance groups); 1 (control groups)
Sacrifice:	24 h after the second injection
Sampling and sample processing:	Bone marrow smears were prepared from the femoral bones. Smears were stained with May Grunwald Giemsa as described by Schmid (1975)
***In vivo* TBARs, SOD, and CAT assay:**	*In vivo* determination of oxidant markers TBARs: thiobarbituric acid reaction products, SOD: superoxide dismutase, CAT: catalase
Guideline:	No
Guideline deviations:	Not applicable
Dose levels:	400 mg/kg bw
Positive control:	none
Negative control:	Saline solution
Animals per dose group:	5
Exposure route:	i.p. injection
Number of treatments:	1
Sacrifice:	1 and 2 h after the injection by cervical dislocation
Sampling and sample processing:	Livers, kidneys, hearts and lungs were removed. Tissue homogenates (10%) were prepared in chilled 0.05 M potassium phosphate buffer, pH 7.4

5. Observations/analyses:

Comet assay	
Measurements:	Cell viability (by tryptan blue exclusion technique), tail moment (TM), DNA percentage in tail (% of DNA) and tail length (TL)
Chromosome aberration-test	
Measurements:	The slides were scored blind by two observers. Mitotic index determined for 2000 cells/replicate Chromosome aberrations: 100 metaphase cells were analysed for chromosome aberrations and classified into the following categories: Chromatid breaks (ctb) and gaps (ctg), chromosome breaks (csb) and gaps (csg), dicentric chromosomes (dic), acentric fragments (ace) and endoreduplicated (end) cells

***In vivo* MNT**	
Mortality/clinical signs:	assessed
Measurements:	In order to score micronuclei about 1000 erythrocytes per animal were analysed. To evaluate bone marrow toxicity, 500 erythrocytes were counted and the ratio polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) was calculated
***In vivo* TBARs, SOD, and CAT assay**	TBARs concentrations, expressed as nmol of malondialdehyde (MDA)/g of tissue were measured spectrophotometrically at 532nm in liver and kidney homogenates. The concentrations were determined using standard curves of MDA. Superoxide dismutase activity was assayed spectrophotometrically in the supernatants of liver homogenates. One unit of enzymatic activity has been defined as the amount of enzyme which causes 50% inhibition of auto oxidation of epinephrine. Catalase activity was measured at 240 nm by the decomposition of the H_2O_2.
Statistics for all tests:	Oneway ANOVA and Dunnett as “a posteriori” test were used in all the experiments. The Pearson statistical test was used to examine possible dose-response effects. In all cases, the level of significance was set at α=0.05.

KLIMISCH EVALUATION

1. 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.
2. Relevance of study:	**Not relevant** (Due to guideline deviations and reporting deficiencies)
3. Klimisch code:	**3**

Annex point	Author(s)	Year	Study title
IIA 5.10	Mladinic, M. Berend, S. Vrdoljak, A.L. Kopjar, N. Radic, B. Zeljezic, D.	2009	Evaluation of Genome Damage and Its Relation to Oxidative Stress Induced by Glyphosate in Human Lymphocytes in Vitro Environmental and Molecular Mutagenesis Volume: 50 Number: 9 Pages: 800-807

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 μg/mL, and increased tail intensity (1.88%) at 92.8 μg/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 μg/mL. Using the hOGG1 comet assay, a significant increase in tail intensity was observed at 2.91 μg/mL with S9 and 580 μg/mL without S9. Without S9, the frequency of micronuclei, nuclear buds and nucleoplasmic bridges slightly increased at concentrations 3.5 μg/mL and higher. The presence of S9 significantly elevated the frequency of nuclear instabilities only for 580 μg/mL. FRAP values slightly increased only at 580 μg/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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Description:	Not reported
Source:	Supelco, Sigma, St. Louis, MO, US
Lot/Batch #:	Not reported
Purity:	98%

2. Vehicle and/or positive control: Yes

Vehicle control:	Standard physiological solution

3. Test group:

Species:	Human
Tissue:	Blood
Age of test animals at study initiation:	Not reported
Sex:	Male

Dose levels: 0.5, 2.91, 3.5, 92.8, and 580 µg/mL

Metabolic activation: With and without (human liver mix)

Positive controls: Without metabolic activation: ethyl methanesulfonate, 200 µg/mL

With metabolic activation: cyclophosphamide, 30 µg/mL

4. Observations/analyses:

Antioxidant capacity

Test system: Ferric-reducing ability of plasma (FRAP)

To assess the antioxidant capacity of plasma its ability to reduce Fe^{3+} to Fe^{2+} the FRAP assay was measured by the FRAP assay. Fe^{3+} to Fe^{2+} reduction results in the formation of a coloured Fe^{2+}-TPTZ-complex with absorbance at 593 nm.

Working FRAP reagent was prepared as required by mixing 20 mL acetate buffer, 2.0 mL 2,4,6-tri[2-pyridyl]-s-triatine (TPTZ) solution, 2.0 mL $FeCl_3$ solution and 2.4 m distilled water. 30 µL of centrifuged plasma sample diluted in saline (1:1) was then added to 1 mL of freshly prepared reagent warmed at 37 °C.

Water solutions of known $FeSO_4$ concentration, in the range of 0.1–1.0 mM, were used for obtaining the calibration curve. As a positive control, 0.5 mL whole blood was treated with vitamin C at a final concentration of 100 µg/mL.

Lipid peroxidation

Test system: Thiobarbituric Acid Reactive Substances (TBARS)

Thiobarbituric acid (TBA) reacts calorimetrically with malondialdehyde (MDA), a secondary product of lipid peroxidation and gives an index of the extent of lipid peroxidation.

5 µL 0.2% (w/v) butylated hydroxytoluene (BHT) and 750 µL 1% (v/v) phosphoric acid was added to 50 µL plasma sample. After mixing, 250 µL 0.6% (w/w) TBA and 445 µL H_2O were added and the reaction mixture was incubated in a water bath at 90 °C for 30 min. The mixture was cooled and absorbance was measured at 532 nm.

The concentration of TBARS was calculated using standard curves of increasing 1,1,3,3-tetramethoxypropane concentrations, and expressed as µmol/L.

Cell viability and necrosis

Test system: Vital Staining

The indices of cell viability and necrosis were obtained from differential staining with acridine orange and ethidium bromide, using fluorescence microscopy. Both dyes intercalate with the DNA, but acridine orange is cell-permeable in contrast to ethidium bromide.

50 µL of treated blood was mixed with the same amount of acridine orange (100 µg/mL) and ethidium bromide (100 µg/mL, 1:1; v/v). The suspension mixed with dye was covered with a cover slip and analyzed under the epifluorescence microscope.

400 lymphocytes were analyzed (200 per duplicate culture) for each lymphocyte culture (concentration), counting the unstained (viable) cells. The nuclei of vital cells emitted a green fluorescence; apoptotic lymphocytes emitted a green fluorescence surrounded by a red echo and necrotic cells red fluorescence.

DNA damage

Test system: Alkaline and hOGG1Modified Comet Assay

The comet assay measures DNA strand breaks by embedding cells in agarose and lysing the cells with detergent and high salt. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy. The intensity of the comet tail relative to the head reflects the number of DNA breaks.

Blood samples (8 µL) were mixed with 0.5% low melting point agarose, were immersed in freshly prepared ice-cold lysis solution (pH 10) and stored at 4 °C overnight. For the alkaline comet assay the standard procedure was followed.

In addition, the hOGG1Modified Comet Assay is able to infer the type of DNA damage from the substrate specificity of human 8-oxoguanine DNA glycosylase 1 (hOGG1). For this assay, the hOGG1 FLARE™ Assay Kit (Trevigen) was used.

Comet assay analysis was done in duplicates.

Chromosomal and Nuclear Instability

Test system: Fluorescence in situ hybridization (FISH)

Cultivation of lymphocytes gained from the blood samples and slide preparation was done according to standard protocol (Fenech, 2006).

Cytokinesis was arrested using cytochalasin B at a final concentration of 6 µg/mL and added to the culture after 44 hr of incubation. Cells were centrifuged, washed in 0.9% NaCl solution and fixed with 3:1 (v/v) methanol/acetic acid solution. Slides were stained with 5% Giemsa.

One thousand binucleated cells with well-preserved cytoplasm were scored per subject, to determine the total number of micronuclei in binucleated lymphocytes (MN), nuclear buds (NBs), and nucleoplasmic bridges (NPBs). The cytokinesis-block proliferation index (CBPI) was evaluated by classifying 1000 cells per number of nuclei, according to the formula: CBPI = [M1+2M2+3(M3+M4)]/N, where M1-M4 indicate the number of cells with 1–4 nuclei respectively, and N the total number of cells scored. To detect the ratio of micronuclei (C+MN), nuclear buds (C+NB), and nucleoplasmic bridges (C+NPB) originating from whole chromosomes that contain centromeres, and the number of DAPI signal positive micronuclei (+MN), slides were kept in dark for a month.

Slides were hybridized with All Human Centromere Satellite Probes directly labelled with a red fluorophore (Texas Red spectrum) following the supplier's instructions. Slides were counterstained with DAPI prepared in an antifade solution. 1000 binucleated lymphocytes were analyzed for each

concentration.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable with restrictions**

Comment: Non-GLP, non-guideline *in vitro* study, meeting scientific principles

2. Relevance of study: Relevant with restrictions (Assessment of Genotoxicity *in vitro* 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.

3. Klimisch code: **2**

Annex point	Author(s)	Year	Study title
IIA 5.10	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

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

MATERIALS AND METHODS

1. Test material:

Test items:	Glyphosate, terbuthylazine, and carbofuran
Active substance(s):	Glyphosate, terbuthylazine, and carbofuran
Description:	Not reported
Source of test medium:	Supelco, Sigma, St. Louis, MO, USA
Lot/Batch #:	Not reported
Purity:	98%

2. Vehicle, negative and/or positive control:

Vehicle: PBS, pH 7.2

Positive control: ethyl methanesulfonate (-S9, 200 µg/mL) and cyclophosphamide (+S9, 30 µg/mL)

Negative control: Standard physiological solution

3. Test system / cells:

Cell cultures:	Lymphocytes
Species:	Human
Source:	Three young, healthy, non-smoking voluntary donors that were not exposed to any physical or chemical agent that could interfere the results 12 month prior to blood sampling.

Culture medium	RPMI 1640 medium without mitogen and newborn calf serum
Culture conditions:	Temperature: 37°C

4. Test method:

Study type:	FISH cytome analysis
Guideline:	Non-guideline study, but similar to OECD 487 with additional analysis
GLP:	No
Guideline deviations:	Not applicable
Test conditions:	Each culture was treated with glyphosate diluted in PBS. The test substance was tested with and without metabolic activation. After the treatment period samples were washed two times in 0.5 mL of culture medium and centrifuged. The supernatant was removed and pellet was used to set up cultures by adding it to 6 mL of culture medium supplemented with 15% foetal calf serum, and 1% antibiotics (penicillin and streptomycin). Then lymphocytes were stimulated by 1% phytohaemagglutinin and incubated for 72 h at 37°C. Cultivation of lymphocytes gained from the blood samples and slide preparation was done according to standard protocol (Fenech, 2006). Cytokinesis was arrested by means of cytochalasin B (6 µg/mL) added to the culture after 44 h incubation. Cells were centrifuged, washed in 0.9% NaCl solution and fixed with 3:1 (v/v) methanol/acetic acid solution. Slides were stained with 5% Giemsa.
Metabolic activation:	10% w/w of human liver S9 mix
Dose levels:	0.50, 2.91, 3.50, 92.8, and 580 µg/mL glyphosate
Exposure duration:	4 h (test samples) and 72 h (positive control cultures)
Replicates:	2 per human donor

5. Observations/analyses:

Measurements:	1000 binucleated cells with well-preserved cytoplasm were scored per subject for determination of the total number of micronuclei (MN), nuclear buds (NBs), and nucleoplasmic bridges (NPBs). The cytokinesis-block proliferation index (CBPI) was assessed by classifying 1000 cells per number of nuclei, according to the formula: CBPI = [M1+2M2+3(M3+M4)]/N, where M1-M4 designate the number of cells with 1–4 nuclei respectively, and N the total number of cells scored. Slides were kept in dark for a month for detection of the ratio of micronuclei (C+MN), nuclear buds (C+NB), and nucleoplasmic bridges (C+NPB) originating from whole chromosomes containing centromeres, and the number of DAPI signal positive MN (DAPI+MN) and DAPI signal positive NB (DAPI+NB). Slides were hybridized with All Human Centromere Satellite Probes directly labelled with a red fluorophore (Texas Red

spectrum) following the supplier's instructions. Slides were counterstained with DAPI prepared in an antifade solution. 1000 binucleated lymphocytes were analyzed for each concentration.

Ratio of centromere-positive micronuclei (C+MN) was calculated by dividing the number of MN containing the centromere signal with the total number of MN counted for the specific treatment. The same approach was used for calculating ratio of C+NB, and C+NPB. Ratio of DAPI signal positive micronuclei (DAPI+MN) was obtained by dividing the number of MN displaying intensive DAPI signal with total number of MN counted for specific treatment.

Statistics: Evaluation was done in triplicate. Results are presented as mean ± SD. Differences in the number of different parameters between treated and control cultures and with and without S9 were evaluated using the Fisher LSD test. The correlations between different measured parameters were analysed by means of Spearman correlation test.

KLIMISCH EVALUATION

1. 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.

2. 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 verified *in vivo*.)

3. 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

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 mu m) compared to the control group (comet length = 25.94 mu m). These results suggest that in the formulation used during aerial spraying glyphosate had a genotoxic effect on the exposed individuals.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Ultra®
Active substance(s):	Glyphosate
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	43.9 % glyphosate

2. Vehicle and/or positive control: None

3. Test group:

Species:	Human
Age of test persons:	Exposed group: 17-59 y; non-exposed: 16-53 y
Sex:	Exposed group: 1 male, 23 females non-exposed group: 4 males, 17 females

4. Test system:

Study type:	Epidemilogical study for cytogenicity – Comet assay
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	About 3 month
Application rate:	23.4 L product/ha (= 10.3 L glyphosate/ha); The application of the glyphosatew product was combined with the adjuvant “Cosmoflux 411F”, that increases the adherence or absorption of the herbicide. The concentration of the adjuvant in the spray solution is not specified.

Persons per group:	24 exposed; 21 non-exposed (control)
Application technique:	Aerial spraying
Test conditions:	The exposed group consisted of 24 randomly selected individuals who lived ≤ 3 km from an area where a glyphosate-based herbicide was applied. Exposure occurred on three consecutive days followed by sporadic aerial spraying over a three-week period. One-half of this group were exposed due to direct spray application over their houses; the other half lived within 200 m to 3 km from spray areas. The non-exposed group consisted of 21 healthy individuals living 80 km away from the spraying area. None of the persons (exposed, non-exposed) were involved in application of pesticides. Activities performed were mainly in the house and sometimes cultivation and harvesting.
Blood sampling:	Exposed group: venous blood (5 mL) was taken from the exposed individuals between 2 weeks and 2 month after their exposure and processed immediately after collection. Non-exposed group: Blood samples were collected and processed as for the exposed group, but not concomitantly.

5. Observations/analyses:

Clinical history:	Exposed persons only
Clinical signs:	Exposed persons only
Body weight:	All persons
Cytogenicity:	Comet assay with venous blood
Haematology:	Not performed.
Clinical chemistry	Not performed.
Urine analysis	Not performed.
Statistics.	Mann-Whitney U test was applied to determine the differences between exposed and non-exposed group in the comet assay.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Documentation of Comet assay insufficient for assessment.

2. 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.)

3. 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup ®
Active substance(s):	Glyphosate (as isopropylammonium salt)
Source:	Monsanto, Milan, Italy
Purity:	30.4% isopropylammonium salt
Test item:	Formulated isopropylammonium salt of glyphosate with surfactant
Source:	Societa Italiana Chimici, Rome, Italy

2. Vehicle: Dimethylsulfoxide (DMSO)/ olive oil

3. Test animals:

Species:	Mice
Strain:	Swiss CD1
Source:	Charles River, Como, Italy
Age of test animals at study initiation:	8-10 weeks
Sex:	Males and females
Body weight:	Not reported
Acclimation period:	Not reported
Diet/Food:	Not reported
Water:	Not reported

Housing: Not reported

Environmental conditions: Not reported

4. Test system:

Study type: ^{32}P-postlabeling detection of DNA adducts in mice

Guideline: Non-guideline study

GLP: No

Guideline deviations: Not applicable

Duration of study: 24 h

Dose levels: Controls; and

Roundup: 400, 500, and 600 mg/kg bw, corresponding to 122, 152, and 182 mg/kg bw of glyphosate salt.

Isopropylammonium salt of glyphosate: 130 and 270 mg/kg bw.

Animals per dose group: DMSO/olive oil – 6 animals;

Roundup:
400 mg/kg (122 mg/kg of glyphosate salt) – 6 animals;
500 mg/kg (152 mg/kg of glyphosate salt) – 3 animals;
600 mg/kg (182 mg/kg of glyphosate salt) – 3 animals.

Isopropylammonium salt of glyphosate:
130 mg/kg – 6 animals;
270 mg/kg – 3 animals;

Route of exposure: Intra-peritoneal (i.p.)

5. Observations/analyses:

Test substance preparations: Stability, achieved concentrations, and homogeneity not reported

Sample preparation: Kidneys and liver were separately pooled and DNA was isolated by a procedure involving enzymatic digestion of protein and RNA and solvent extraction.

Measurements: ^{32}P-postlabeling. The level of DNA adducts was determined by excising areas of chromatograms and measuring the levels of radioactivity present by Cerenkov counting. Quantitation of normal nucleotides was carried out as previously described [Taningher et al., 1995].

Statistics: Not reported

KLIMISCH EVALUATION

1. 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) reporting the same study type with a glyphosate formulated product and an appropriate control; formulation blank without glyphosate.
2. Relevance of study:	**Not relevant** (i.p. administration of high doses of a surfactant containing formulation a relevant exposure scanario 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.)
3. 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 (*Caiman latirostris*) evidenced by the Comet assay and Micronucleus test Mutation Research Volume: 672 Number: 2 Pages: 95-102

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup® Full
Active substance(s):	Glyphosate (as potassium salt)
Source:	Agroservicios Humboldt, Santa Fe, Argentina
Purity:	66.2% glyphosate
Lot/Batch #:	Not reported

2. Vehicle and/or positive control: Vehicle – distilled water;
Positive control – cyclophosphamide (CP)

3. Test system:

Species:	*Caiman latirostris*
Test system:	Eggs
Source:	Fisco field ($30^0$11'26''S; $61^0$0'27''W), Santa Fe Province, Argentina
Sex:	Not reported
No. of eggs:	Experiment #1 – 100 Experiment #2 - 84
Egg weight:	Experiment #1 – 67.5 ± 4.89 g;

Experiment #2 – 72.7 ± 7.21 g

Acclimation period: Not reported

Environmental conditions: All nests used in each experiment were collected within 5 days after oviposition, on the same day and maintained under the same conditions from harvest to treatment assignment.

4. Test methods:

Study type: Genotoxicity study: Comet assay, micronucleus assay

Duration of study: Not specified

Dose levels: <u>Experiment #1</u>
Control group – distilled water, 50 µL;
Positive control groups – CP, 700 or 1400 µg/egg;
Treatment groups – 50, 100, 200, 300, 400, 500 and 1000 µg/egg

<u>Experiment #2</u>
Control group – distilled water, 50 µL;
Positive control group – CP, 700 µg/egg;
Treatment groups – 500, 750, 1000, 1250 and 1750 µg/egg

Eggs per dose group: <u>Experiment #1</u>
10 groups of 10 eggs each (5 eggs per each, two replicas)
<u>Experiment #2</u>
7 groups of 12 eggs each (6 eggs per each, two replicas)

Administration: All treatments were presumably a single dose, applied topically to the eggshell dissolved in 50 µL of distilled water (CP and Roundup treatments). Applications were done at early embryonic stage, within the first 5 days after oviposition, based on the opaque eggshell banding development

Experimental conditions: Artificial incubator;
Temperature: 31 ± 1°C
Humidity: 95%
Peripheral blood samples (0.5 ml) were obtained from each hatchling from the spinal vein, with heparinized disposable syringes.

Test methods:

<u>Micronucleus test</u>

Guideline: OECD 474

GLP: No

Guideline deviations: Modified to be applied in *C. latirostris* erythrocytes; housing and feeding conditions of parents not specified; sex not distinguished.

1) Modification
The MN assay originally performed in peripheral blood lymphocytes was modified to be applied in *C. latirostris*: application on eggs (within 5 days after oviposition), blood sampling after hatching.

2) Preparation
Two smears were prepared from each animal, coded for 'blind' analysis and stained with Acridine Orange supravital stain at

the moment of analysis.

Comet assay

Guideline: Non- guideline

GLP: No

Guideline deviations: Modified to be applied in *C. latirostris*

1) Cell preparation
Cell viability was determined before the application of the SCGE by fluorescent DNA-binding dyes. The cell suspension was mixed with a dye-mix working solution of 100 µg/ml Acridine Orange and 100 µg/ml ethidium bromide, prepared in Ca^{2+} - and Mg^{2+} -free PBS and then examined under a fluorescent microscope (40×). A total of 100 cells were counted per sample and the percentage of viable cells was determined.

2) Electrophoresis
The alkaline Comet assay was performed as described by Singh et al. with modifications required by *C. latirostris* erythrocytes, determined in previous studies: blood samples were diluted 1:19 (v/v) with RPMI-1640 medium and 1.5µL of the dilution (4.0×10^3 erythrocytes, approximately) were used to prepare each of two slides per blood sample, following standard protocol. Slides were immersed in lysis buffer for 24 h, incubated in alkaline buffer for 10 min and electrophoresed at 300 mA and 25V (0.90 V/cm) during 10 min also.

To demonstrate the electrophoresis conditions, positive controls were included in each electrophoresis carried out. The result of each electrophoresis was considered only if the positive controls showed positive results.

5. Observations/analyses:

Test substance preparations: Stability, achieved concentrations, homogeneity not reported

Mortality: Not reported

Clinical signs: Not reported

Body weight: Measured (OHAUS® Compact scale CS200, precision 0.1)

Body length: Measured (tape measure, precision 0.5 cm)

Snout-vent length: Measured (tape measure, precision 0.5 cm)

Identification: Individually identified by two numbered webbing tags in the hindlegs (National Band and Tag Co., Newport, KY)

Food- and water consumptions: Not relevant

Haematology: Not reported

Clinical chemistry: Not reported

Urine analysis: Not reported

Sacrifice/pathology: Not reported

Organ weights: Not reported

Histology: Not reported

Measurements: Micronucleus test
Microscopy: The frequency of MN was manually scored using

a fluorescent microscope (Olympus CX 40) equipped with a U-RFLT 50 excitation filter.

For each individual, 1000 erythrocytes were analysed in two replicated slides and the frequencies of micronucleated cells among them were recorded.

Comet assay

Microscopy: All samples were coded and evaluated blindly. At the moment of analysis, the slides were stained with ethidium bromide (2µg/mL). Comet images were analyzed using the fluorescent microscope.

Images of 100 randomly selected cells (50 cells from each of two replicated slides) were scored from each animal. Cells were visually classified into five classes according to tail size and intensity (from undamaged, class 0, to maximally damaged, class 4), resulting in a single DNA damage score (damage index, DI = $n1$+2 $n2$+3 $n3$+4 $n4$) for each animal.

Statistics: Mean values ± standard error of MN and DI were calculated from data of animals of each experimental group. Statistical analysis was performed using the software SPSS 14.0 for Windows. Variables were tested for normality with Kolmogorov–Smirnov test and homogeneity of variances between groups was verified by Levene test. One-way ANOVA followed by Dunnett's test was used for the comparison of MN frequencies and DI between each group exposed to Roundup or CP and the negative control. A difference of $p < 0.05$ was considered statistically significant. Linear regressions were carried out to determine the existence of a concentration-dependent effect of Roundup on DI and MN frequencies. Data from Experiment 1 and Experiment 2 groups (from Roundup 50 to Roundup 1750) were considered together, taking into account that experiment conditions were exactly the same for both experiments except for Roundup concentrations applied. Concentration-dependent analysis was performed on MN and DI total data as well as on MN and DI mean values of each Roundup experimental group.

KLIMISCH EVALUATION

1. 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.

2. 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.

3. 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

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 spectrophometric and morphologic analyses. At the concentration limit recommended for agricultural purposes, Roundup® promoted 100% of hemolysis. The D_{50}Roundup® 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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup®
Active substance(s):	Glyphosate
Source:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Tissue:	Blood
Number of test persons:	8
Age:	24 ± 3 years
Sex:	Not reported
Inclusion criteria:	o healthy o non-smokers o non-users of drugs or medications o non-consumers of alcoholic beverages

Species:	Mice
Strain:	Swiss
Source	Iquego, Goiânia, Goiás, Brazil
Number of animals:	Not reported
Age:	7-12 weeks
Sex:	Not reported
Environmental conditions:	o 26 ± 2 °C o 12 h light/dark cycle o *ad libitum* access to food and water

4. Test system:

Type:	Determination of the stability of human erythrocytes
Guideline:	Non-guideline study
GLP / GCP:	No
Dose levels:	*In physiologic saline solution:* 0 to 40 µL/100 mL Roundup® (40 µL/dL) in 0.9%NaCl, (concentration range within the acceptable limit for use of the herbicide in agriculture according to the manufacturer) *In aqueous solution:* 0 to 40 µL/100 mL Roundup® (40 µL/dL) as aqueous solution
Sample collection:	Blood samples (3 mL) were collected from volunteers through intravenous puncture after an 8-12 h fasting period. The blood collections were performed in evacuated tubes containing 50 µL of 25 mmol/L K_2EDTA or 50 µL of heparin as anticoagulant.

Type:	Micronuclei frequency in Mice
Guideline:	Non-guideline study
GLP / GCP:	No
Dose levels:	0, 0.148, 0.754 and 1.28 mg/kg bw
Negative control:	Saline solution
Positive control:	Cyclophosphamide; 250 mg/kg bw
Sample collection:	Drugs were prepared in 0.2 mL of sterile saline solution and intraperitoneally administrated. 24 h after treatment, the animals were sacrificed by cervical dislocation, and then the bone marrow of both femurs was collected for the preparation of slides. The bone marrow was collected with the aid of a 1 mL syringe filled with saline. The femur was washed with saline and cell suspension was collected in a test tube containing saline. The cell suspension was centrifuged for 5 minutes at 1300 g, discarding the supernatant and retaining a volume of 0.5 mL in the tube for later re-suspension and homogenization of the cell precipitate. From the resulting suspension, a small drop was removed and placed in one extremity of the blade for the performance of smears in duplicate.

5. Observations / analyses:

Type: Determination of the stability of human erythrocytes

Measurements: Duplicate sets of test tubes with physiologic saline solutions (NaCl 0.9%) or aqueous solutions with Roundup® were pre-incubated at 37 °C for 5 min. After the addition of 10 µL of blood samples, homogenization and incubation at 37 °C for 30 min, the flasks were centrifuged for 10 min at 1300 g and the supernatant was analyzed by spectrophotometry at 540 nm. The supernatant and the precipitate were stained with Leishman's stain and analyzed by light microscopy.

Calculations: The dependence of the A_{540} values on the Roundup® concentrations were adjusted by sigmoidal regression lines, given by the Boltzmann equation:

$$A540 = \frac{A1 - A2}{1 + e^{(D - D50)/dD}} + A2$$

where A_1 and A_2 are the A_{540} values that represent the minimum and maximum hemolysis plateaus, D is the Roundup® concentration, D_{50} represents the Roundup® concentration that causes 50% of hemolysis, and dD is the amplitude of the sigmoidal transition between A_1 and A_2.

Type: Micronuclei Frequency in Mice

Observations: The smears were stained with Leishman's stain after drying and the slides were dried at room temperature.

No information on micronucleus evaluation (e.g. number and type of cells evaluated, in- and exclusion criteria, etc.) given.

Statistics: The regression lines were only considered significant when p was lower than 0.05. The comparisons of means between groups were performed by analysis of variance (ANOVA), with $p < 0.05$ indicating statistically significant differences.

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 (2008)

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).

2. Relevance of study: **Not Relevant** (Test material containing surfactant is not appropriately evaluated in either model).

3. 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

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-((trichloromethyl)thio)-4-cyclo-hexene-1, 2-dicarboximide, because of toxic levels of the fungicide or solvent used.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Captan; Dexon; Roundup
Active substance(s):	**Captan** - cis-N-((trichloromethyl)thio)-4-cyclo-hexene-1, 2-dicarboximide **Dexon** - sodium (4-(dimethylamino)phenyl)diazene sulfonate; **Roundup** - isopropylamide salt of N-(phosphonomethyl)glycine
Source:	Not reported
Purity:	Not reported

2. Positive control: Ethylmethane sulfonate (EMS)

3. Test material:

Material:	Human lymphocytes
Species:	Human
Sex:	No data
Source:	Two subjects were chosen to act as regular donors of blood cells (not further specified)
Culture medium:	McCoys 5A medium (Gibco) with the addition of 10% fetal calf serum, 1% PHA (Gibco), 1% pen-strep solution (10,000 units penicillin and 10,000 µg streptomycin/mL), and 30.7 µg/mL 5-bromodeoxyuridine (10^{-4} M)

4. Test method:

Study type:	*In vitro* sister chromatid exchange test in human lymphocytes
Guideline:	No

GLP:	No
Guideline deviations:	Not applicable
Duration of treatment:	72h
Dose levels:	Except controls, each 5-mL culture contained: EMS: 1.24, 12.4, 124 mg/mL (10^{-5} , 10^{-4}, 10^{-3} M); Dexon: 2.5, 25.0, 250 mg/mL (10^{-5}, 10^{-4}, 10^{-3} M); Captan: 3.0, 30.0, 300.0 mg/mL (10^{-5}, 10^{-4}, 10^{-3} M); Roundup: 0.25, 2.5, 25.0 mg/mL ($65x10^{-5}$, $65x10^{-4}$, $65x10^{-3}$ M).
Solvents:	Captan dissolved in 7% ethanol followed by a 1:4 dilution in water; All other chemicals were dissolved in distilled water.
No. of replicates:	none

5. Observations/analyses:

Measurements:	For each sample and concentration, 50 well spread and completely differentially stained metaphases were analyzed for SCE frequency from each subject.
Preparation of material:	Stained by the FPG technique
Statistics:	Paired Student's *t*-test were determined for all pairs of data in both subjects

KLIMISCH EVALUATION

1. 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.

2. Relevance of study: **Not Relevant** (Limited data set, internally consistent findings, no statistics conducted and no dose-response)

3. Klimisch code: **3**

5. Literature Review of Category 'E' Publications and Other Publications

This section reviews other peer reviewed publications not captured in the previous four subject area reviews, as well as Category 'E' peer reviewed citations, as described in the introduction to this literature review. Publications are presented in OECD Tier II style summaries followed by Klimisch ratings (Klimisch, 1997) then responses/comments on the paper. The first four *in vitro* publications from the same research group are commented on collectively after the fourth summary/Klimisch rating, below.

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

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

MATERIALS AND METHODS

1. Test material:

Test item: Roundup
Active substance(s): Glyphosate

Source of test items:	Not specified
Lot/Batch #:	Not specified
Purity:	Not specified (Roundup is presented as a commonly used pesticide/herbicide containing glyphosate (is formulated with surfactants and permeabilisants) as active ingredient.

2. Vehicle and/or positive control: Not specified

3. Test system / cells / organism:

Species:	*Not specified (sea urchin embryo used)*
Strain:	Not specified
Source:	Not specified
Growth conditions:	Not specified

4. Test methods:

Assessment of DNA-damage/cell division in sea urchin embryo

Guideline:	Non-guideline assays
GLP:	Not specified
Guideline deviations:	Not applicable
Treatment:	Sea urchin embryos were exposed to Roundup at different concentration levels
Dose levels:	The evaluated concentration levels were 500 – 2500 fold lower than commonly recommended by the producers were used. Not further specified
Negative control:	Not specified
Positive control:	Not specified; Roundup was evaluated together with different pesticide cocktails.
Test conditions:	Not further specified
Replicates per dose level:	No specified

5. Observations/analyses:

Measurements:	Not specified
Statistics:	Not specified

KLIMISCH EVALUATION

1. 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.

2. 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.)

3. Klimisch code: **4**

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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup
Active substance(s):	Glyphosate isopropylamine salt
Source of test items:	Amersham (France)
Lot/Batch #:	Not specified
Purity:	170 g/L isopropylamine glyphosate salt

2. Vehicle and/or positive control: Vehicle: Millipore-filtered seawater
Positive control: Not reported

3. Test animals:

Species:	Sea urchin (*Sphaerechinus granularis*)
Source:	Brest area (France), not further specified
Acclimation period:	5 days
Acclimation environment:	Seawater
Environmental conditions:	Not reported

4. Test system:

Study type:	Embryonic cell cycle for risk assessment of pesticides at the molecular level
Guideline:	Non-guideline study
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Treatment for up to 200 min
Dose levels:	0.8 % Roundup (corresponding to 8 mM isopropylamine glyphosate)
Replicates per dose group:	Not reported
Treatment:	Pesticide solutions were adjusted to pH 7.5 and added to the embryo suspension in Millipore filtered seawater. Thousands of embryos were incubated for each experimental determination.
Culturing conditions:	Cultures were performed at 16 °C with constant stirring.

Test methods:

Handling of eggs and embryos

Experiments were performed only on batches exhibiting greater than 90% fertilization and for each experiment, using gametes isolated from a single female. Cultures were observed at short time intervals by Nomarski differential interference contrast (DIC) microscopy for developmental progression.

Preparation of whole embryo extracts and affinity purification of CDK1

Whole extracts from the embryos were prepared by pelleting 200 µL of a 5% egg suspension. The pellets were suspended in electrophoresis sample buffer, vortexed for 15 s and boiled for 3 min. The extracts were clarified by centrifugation.

The CDK1 protein was affinity-purified at different times after fertilization using $p13^{suc1}$-Sepharose beads. Extracts for the measurement of the activation state of CDK1 protein were prepared. For extract preparation for cyclin B protein immunodetection the embryo suspension was rapidly packed by centrifugation. Egg pellets were vortexed in 1 mL ice-cold buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.3, 50 mM sodium fluoride, 10 mM pyrophosphate, 10 mM phenyl-phosphate, 1 mM sodium vanadate, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 0.1 mg/ml soybean trypsin inhibitor, and 0.1% Triton), then immediately frozen in liquid nitrogen and kept at –80 °C. Thawed extracts were homogenized through a 25-gauge needle and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was incubated at 4 °C for 45 min under constant rotation with 25 µL packed $p13^{suc1}$-Sepharose beads. The beads were washed twice in 1 mL ice-cold bead buffer (50 mM Tris-HCl pH 7.4, 5 mM sodium fluoride, 250 mM NaCl, 5 mM ethylene diamine tetraacetic acid (EDTA), 0.1% nonidet-P40 (NP-40), 5 mM ethylene glycol tetraacetic acid (EGTA), 10 µg/mL 4-(2-aminoethyl)-benzenesulfonyl fluoride, 10 µg/mL soybean trypsin inhibitor, 100 µM benzamidine) and once in 1 mL ice-cold 50 mM Tris-HCl pH 7.5. Beads were suspended in 35 µL electrophoresis sample buffer and boiled for 3 min.

Analysis of in-vivo CDK1 activation
The assays were performed by incubation of 10 µL beads affinity purified CDK1 protein with 1 µCi [γ-^{32}P]-ATP, 10 µM unlabeled ATP, 2.5 µg histone III-SS in 50 µl buffer containing 60 mM β-glycerophosphate, 30 mM p-nitrophenylphosphate, 25 mM 3-N-morpholino propane sulfonic acid, pH 7.2, 5 mM EGTA, 15 mM MgCl2, 1 mM dithiotreitol, 0.1 mM sodium orthovanadate. After 10 min at 30 °C, the reaction was stopped by chilling the tubes in ice.
Cyclin B Immunodetection
15 µL of the embryo whole extracts or of the affinity-purified CDK1 extracts were resolved by one-dimensional electrophoresis (SDS-polyacrylamide gel). Proteins were transferred to nitrocellulose, stained by Ponceau Red and processed for Western blotting.

5. Observations/analyses:

Cytological observations	Approximately 100 embryos were scored for the developmental stage.
CDK1/Cyclin B activation *in vivo*	The kinase activity of affinity-purified CDK1/cyclin B complex was assayed in standard conditions using histone H1 as a substrate. Duplicate aliquots of 10 µL were spotted on Whatman P81 phosphocellulose papers, which were washed five times in 1% phosphoric acid and counted in water in a 1450 MicroBetacounter (Wallac, EG & G Instruments).
Analysis of Cyclin B by Immunoblot:	Western blots were performed using the anti-cyclin B antibody detected with goat anti-rabbit IgG-AP conjugate (Bio-Rad) using the BCIP-NBT reagent (Fluka). Densitometric analyses of the immunoblots were performed using the domain public NIH image program.
Statistics:	Not reported

KLIMISCH EVALUATION

1. 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.

2. 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.)

3. 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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Amega, Cargly, Cosmic, Roundup Biovert, Roundup 3plus
Active substance(s):	Glyphosate isopropylamine salt
Source:	Amega – CFPI Nufarm; Cargly – Cardel; Cosmic – Calliope; Roundup Biovert and Roundup 3plus – Monsanto.
Lot/Batch #:	Not reported
Purity:	Amega – 360 g/L glyphosate; Cargly – 360 g/L glyphosate; Cosmic – 360 g/L glyphosate; Roundup Biovert – 360 g/L glyphosate; Roundup 3plus – 170 g/L glyphosate.

2. Vehicle and/or positive control: Vehicle: Millipore-filtered seawater
Positive control: Not reported

3. Test animals:

Species:	Sea urchin (*Sphaerechinus granularis*)
Source:	Brest area (France), not further specified
Acclimation period:	5 days
Acclimation environment:	Seawater
Environmental conditions:	Not reported

4. Test system:

Study type:	Cell cycle analysis by means of microscopy

Guideline:	Non-guideline study
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Treatment for up to 240 min
Dose levels:	Amega: Control, 0.1 mM, 1 mM; Cargly: Control, 0.1 mM, 1 mM; Cosmic: Control, 0.1 mM, 1 mM; Roundup 3plus: Control, 2 mM, 8 mM; Roundup Biovert: Control, 1 mM, 10 mM;
Replicates per dose group:	5 - 10 independent experiments.
Treatment:	Pesticide solutions were adjusted to pH 7.5 before addition to the embryos suspended in Millipore-filtered seawater. Thousands of embryos were incubated for each experimental determination from which around one hundred were scored for the developmental stage.
Culturing conditions:	Embryos were cultured at 16 °C with constant stirring.
Test methods:	Handling of eggs and embryos Spawning of gametes was induced by intracoelomic injection of 0.1 M acetylcholine. Eggs were collected in 0.22 µm-Millipore-filtered seawater, rinsed and collected by centrifugation. For fertilization, eggs were suspended in Millipore-filtered sea-water (5 % suspension) containing 0.1 % glycine. Dilute sperm was added to the eggs and withdrawn after fertilization membrane elevation. Experiments were only performed on batches exhibiting greater than 90 % fertilization and each experiment used gametes from a single female. Sea urchin development and cytological observations Embryos were observed at short time intervals by phase contrast microscopy for developmental progression. At various times after fertilization, 0.2 mL aliquots of the egg suspension were fixed for at least 2 hours in 0.5 mL methanol/glycerol (3:1) in the presence of the DNA dye bisbenzimide (0.1 µg/mL), mounted in 50 % glycerol and observed under fluorescence microscopy.

5. Observations/analyses:

Measurements:	At different times following fertilization, developmental stage and chromatin state were observed by phase contrast microscopy and fluorescence microscopy after DNA staining, respectively.
Statistics:	Not reported

KLIMISCH EVALUATION

1. Reliability of study:	**Not Reliable**
Comment:	Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.
2. 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.

3. Klimisch code: **3**

Author(s)	Year	Study title
Marc, J. Belle, R. Morales, J. Cormier, P. Mulner-Lorillon, O.	2004b	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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup 3plus
Active substance(s):	Glyphosate isopropylamine salt
Source:	Cluzeau Info Labo (France)
Lot/Batch #:	Not reported
Purity:	170 g/L glyphosate isopropylamine salt

2. Vehicle and/or positive control: Vehicle: Millipore-filtered seawater
Positive control: aphidicolin (DNA polymerase I inhibitor)

3. Test animals:

Species:	Sea urchin (*Sphaerechinus granularis*)
Source:	Brest area (France), not further specified
Acclimation period:	5 days
Acclimation environment:	Seawater
Environmental conditions:	Not reported

4. Test system:

Study type:	Cell cycle analysis: DNA-response checkpoint, cell cycle transition (G2/M)
Guideline:	Non-guideline study
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	Treatment for up to 175 min
Dose levels:	Control; and 1 % formulated glyphosate i.e. 10 mM equivalent glyphosate (Formulated glyphosate).
Replicates per dose group:	3 - 4 independent experiments.
Treatment:	Pesticide solutions were adjusted to pH 7.5 before addition to the embryos suspended in Millipore-filtered seawater. Thousands of embryos were incubated for each experimental determination from which around one hundred were scored for the developmental stage.
Culturing conditions:	Embryos were cultured at 16 °C with constant stirring.
Test conditions:	<u>Handling of eggs and embryos and cytological observations</u> Spawning of gametes was induced by intracoelomic injection of 0.1 M acetylcholine. Eggs were collected in 0.22 µm Millipore-filtered seawater and rinsed twice. For DNA synthesis determination experiments, eggs were dejellied by swirling in 3.5 mM citric acid (pH 5) and rinsed in filtered seawater prior to fertilization. For fertilization, eggs were suspended in Millipore-filtered seawater (5% suspension) containing 0.1% glycine. Diluted sperm was added to the eggs and withdrawn after fertilization envelope elevation. Experiments were only performed on batches exhibiting greater than 90% fertilization, and each experiment used gametes from a single female. For some experiments, 0.2 mL aliquots of the embryo suspension at various times after fertilization were fixed in methanol/glycerol (3:1) in the presence of the DNA dye bisbenzimide (0.1 µg/mL) and were mounted in 50% glycerol. Chromatin state observation under fluorescence microscopy was used to determine metaphase stage timing. <u>Affinity purification of CDK1/cyclin B from embryos</u> The CDK1 protein was affinity purified at different times after fertilization using $p13^{suc1}$-Sepharose beads. Every 10 min after fertilization, embryos were rapidly packed by centrifugation, immediately frozen in liquid nitrogen, and kept at -80 °C until further processing. Embryos were in ice-cold buffer (60 mM β-glycerophosphate pH 7.2, 15 mM p-nitrophenyl phosphate, 25 mM 4-morpholinepropanesulfonic acid (MOPS), 15 mM ethylene glycol tetraacetic acid (EGTA), 15 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 1 mM sodium orthovanadate, 1 mM sodium fluoride, 1 mM disodium phenylphosphate, 10 µg/mL soybean trypsin inhibitor (SBTI), 100 µM benzamidine, 10 µg/mL aprotinin, 10 µg/mL leupeptin, 0.1% Triton). The homogenates were clarified by centrifugation. The CDK1/cyclin B complex was isolated by incubating the supernatant in the presence of $p13^{suc1}$-Sepharose beads. The beads were collected by centrifugation, washed twice in ice-

cold bead buffer and once in ice-cold 50mMTris-HCl pH 7.5. Proteins were eluted by boiling for 3 min in electrophoresis sample buffer.

Western blot analysis

15 µl of the affinity-purified proteins were resolved by one-dimensional electrophoresis (SDS-polyacrylamide gel). Proteins were transferred to nitrocellulose, stained with Ponceau red and processed for Western blotting.

Phosphatase assays

The specific 3-*O*-methylfluorescein phosphate (OMFP) artificial substrate was used for the estimation of cdc25 activity. Pure human cdc25C was assayed. Embryo extracts were prepared as described for the CDK1/cyclin B purification procedure, except that the homogenization buffer was 80 mM β-glycerophosphate pH 7.3, 20 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 25 µg/mL aprotinin, 25 µg/mL leupeptin, 1mM benzamidine, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), 10 µg/mL soybean trypsin inhibitor (SBTI), 10 µg/mL pepstatin A. Supernatants used for phosphatase assays were from metaphase-stage embryos as judged by the chromatin state.

Phosphatase assays were performed at 30 °C for at least 1 h. The mixture contained either commercial recombinant cdc25C or embryo extracts in substrate buffer (500 µM 3-*O*-methylfluorescein phosphate (OMFP), 100 mM Tris-HCl pH 8.2, 40 mM NaCl, 1mMDTT, 10mMglutathione, 20% glycerol).

Determination of DNA synthesis in vivo

10 min post-fertilization, methyl-^{3}H-thymidine (10 µCi/ml final concentration), was added to the embryo suspension. Formulated glyphosate or aphidicolin (positive control, 10 µg/mL final) were added 10 min post-fertilization. At different times thereafter, embryos were rapidly packed by centrifugation, rinsed in Millipore-filtered seawater, and suspended in ice-cold 20% trichloroacetic acid (TCA). Aliquots were taken for the determination of total thymidine uptake. After precipitation over night at 4 °C, the pellets were collected by centrifugation, washed with 20% TCA and dissolved in 0.125MNaOH.

5. Observations/analyses:

Cytological observations:	Approximately 100 embryos were scored at short time intervals by phase contrast microscopy for cytokinesis. Chromatin state observation under fluorescence microscopy was used to determine metaphase stage timing.
Western blot analysis:	Anti-cyclin B antibody, anti-PSTAIR and anti p-Tyr were used. Membranes were incubated with peroxidase-coupled secondary antibodies (Biorad): goat anti-mouse IgG (H + L)/HRP conjugate for PSTAIR detection and goat anti-rabbit IgG (H + L)/HRP conjugate for cyclin B detection. When needed, membranes were stripped and processed as above with a new antibody. The antigen–antibody complexes were revealed by the chemiluminescence system according to the manufacturer's instructions (Amersham Biosciences).

Phosphatase assays:	Absorbance was measured at 477 nm in a Pharmacia Biotech Ultrospec 2000 spectrophotometer. Activity of pure cdc25 C was 0.12 OD units per hour.
Determination of DNA synthesis *in vivo*:	Radioactivity was measured on duplicate 500 µL aliquots of the dissolved samples in the presence of Optiphase Supermix scintillation liquid in a 1450 Wallac MicroBeta-Counter. The protein content was measured on the remaining sample using the BCA protein assay kit
Statistics:	Not reported

KLIMISCH EVALUATION

1. Reliability of study: **Not Reliable**

Comment: Non-standard, non-guideline study. Commonly used surfactants have previously shown the same effects in this model.

2. 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.

3. Klimisch code: **3**

Comments based on Monsanto web site response
http://www.monsanto.com/products/Documents/glyphosate-background-materials/Response_ISIS_apr_06.pdf

- Marc and her colleagues conducted *in vitro* studies using sea urchins. They have now published a number of articles based on the faulty premise that Roundup enhances the ability of glyphosate to get into cells to disrupt the cell cycle. While they measure a variety of cellular/molecular endpoints in these studies, the results are not reflective of cellular effects in real-life systems since non-specific changes in cell membrane function have been shown to occur due to surfactants and may also result from other changes in the culture medium such as effects on pH and calcium levels. Note that when the sea urchin embryos are placed back in normal medium they develop into normal sea urchins, indicating a lack of any permanent biological effect.
- When surfactants found in products such as bath gels and shampoos that humans tested in the same sea urchin model (Amouroux, 1999; ref. Doc L Table 3 and included in Doc K) sea urchin assay they produced the same results as Marc *et al.* did ...cell cycle delays.
    - http://www.ncbi.nlm.nih.gov/pubmed/9828259?dopt=Abstract
- Other researchers have found that caffeine also alters cell division in sea urchin embryos
    - http://www.ncbi.nlm.nih.gov/pubmed/9276510?dopt=Abstract
- The sea-urchin test system is not relevant to predicting adverse effects on human health.

The following two recent publications, by Heu et al. (2012a; 2012b) 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.	2012a	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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
Source of test items:	Not reported
Lot/Batch #:	Not specified
Purity:	Not specified

2. Vehicle and/or positive control: Not reported

3. Test system / cells:

Cell lines:	Immortalized human HaCaT cell line
Source:	ATCC, Teddington, UK
Culture conditions:	Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS; D. Dutscher, Brumath, France) and 80 mg/L of gentamicin in a humidified 5% CO_2 atmosphere at 37 ^{0}C. The keratinocytes were grown to confluence in 75 cm^2 culture flasks. The medium was removed

every 48 h, and cells were subcultured every 7 days at a ratio of 1:6.

4. Test methods:

Guideline: Non-guideline assays

GLP: No

Guideline deviations: Not applicable

Determination of IC50 (Inhibitory concentration 50%: The *in vitro* conditions to study the glyphosate-induced stress were determined by carrying out cytotoxicity assays on 96-well microtiter plate HaCaT cultures with different glyphosate concentrations. The cytotoxic profiles remained unchanged from 6 to 18 h of contact with glyphosate, showing a similar 50% inhibitory concentration (IC50) value, around 30 mM. We chose to test 30 mM to observe achieved events and 15 mM (80% cytotoxicity) for ongoing events. These concentrations approximate field spray dilutions of 2500 to 5000 ppm glyphosate), but without pH adjustment for the glyphosate acid.

Atomic Force Microscopy (AFM)

Conditions: HaCaT cells were seeded in 50 mm glass bottom Petri dishes (Willco Wells BV, Amsterdam, The Netherlands) at a density of 5 x 10^5 cells in 3 mL DMEM supplemented with 10% (v/v) FCS and 80 mg/L of gentamicin in a humidified 5% CO_2 atmosphere at 37 °C for 24 h, for cell attachment.

Treatment: Cultured cells were exposed to several concentrations of glyphosate in FCS-free media depending on different incubation periods: 40 and 53 for 0.5 h; 15 and 30 for 6 and 18 h. A 100 mM quercetin treatment was tested concomitantly to 18 h glyphosate conditions.
Following exposure the cells were washed with pre-warmed FCS-free media and imaged at ambient temperature which was found to be 32 °C and over a maximum time of 90 min to be sure that the cells were still healthy and adherent during the imaging.

Replicates per dose level: Not reported

5. Observations/analyses:

Measurements: Tensile modulus calculation and fit model

The YM (Young's modulus) or tensile modulus is obtained by dividing the tensile stress by the tensile strain and is related to the stiffness of the scanned material.
By default, the AFM's software (Nanoscope V8.2) uses a DMT fit to extract the Young's modulus from each force curve. The obtained value is an average of 300,000 force curves.
The data was also processed outside of the AFM software using the Sneddon model (Sneddon, 1965). Although, only one probe was used for the entire series of experiments, the Young's modulus calculated from the exported force curves is an average of 27,000 data points and Young's modulus values extracted using a DMT fit are superimposed to those of another serie of experiments carried out in the same conditions.

Analysis: AFM imaging was carried out on a Bioscope Catalyst™ (Bruker, Billerica, USA). AFM probes used in Peak Force Quantitative Nanomechanical Mapping (PFQNM) mode were ScanAsyst-Fluid (Bruker, Billerica, USA) probes. The Young's moduli were calculated by using a Sneddon fit (Sneddon, 1965).

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Not Relevant** (*in vitro* data on the effects on an immortalized epidetmal 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 in formulated glyphosate based products)

3. Klimisch code: **3**

Author(s)	Year	Study title
Heu, C., Elie-Caille, C., Mougey, V., Launay, S., Nicod, L.	2012b	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

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
Active substance(s):	Glyphosate
Source of test item:	Sigma-Aldrich, St. Louis, MO, USA
Lot/Batch #:	Not specified
Purity:	≥ 95% (powder)

2. Vehicle and/or positive control: Not reported

3. Test system / cells:

Cell lines:	Immortalized human HaCaT cell line (human keratinocytes)
Source:	ATCC, Teddington, UK
Culture conditions:	Cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal calf serum (FCS; D.Dutscher, Brumath, France) and 80 mg/L of gentamicin in a humidified 5% CO_2 atmosphere at 37 °C. The keratinocytes were grown to confluence in 75 cm^2 culture flasks. The medium was removed every 48 h, and cells were subcultured every 7 days at a ratio of 1:6.

4. Test methods:

Guideline:	Non-guideline assay (for all tests)
GLP:	No
Guideline deviations:	Not applicable (for all tests)
<u>Cytotoxicity assays</u>	
Conditions:	HaCaT cells were seeded at a density of 1×10^4 cells per well in 100 µL FCS-supplemented DMEM on 96 multiwell culture plates (Nunc, D. Dutscher) and incubated overnight for adherence. The following day, the medium was removed and cells were incubated in FCS-free medium containing increasing concentrations of glyphosate (for nine incubation times in 37 °C–5% CO_2 atmosphere.
Dose concentrations:	0, 5, 10, 20, 30, 40, 50, 60, and 70 mM glyphosate (approximately 850 – 11,800 ppm glyphosate acid)
Incubation (exposure) times:	0.5, 1, 2, 4, 6, 9, 12, 15 and 18 h
Replicates:	3 per concentration
<u>Flow cytometry</u>	
Conditions:	For all experiments of the flow cytometry study, HaCaT cells were seeded in 25 cm² culture flasks at a density of 5×10^5 cells/ DMEM supplemented with 10% (v/v) FCS and 80 mg/L of gentamicin in a humidified 5% CO_2 atmosphere at 37 °C for 24 h for cell attachment.
<u>Intracellular ROS (H_2O_2)</u>	2′-7′-Dichlorodihydrofluorescein diacetate (DCFDA) dye was used to study the intracellular hydrogen peroxide generation in HaCaT cells.
Conditions:	After 24 h of cell attachment, the cells were washed twice with 2 mL PBS and DCFDA dye was added at a concentration of 20 µM in 2 mL per flask. The flasks were placed in a humidified 5% CO_2 atmosphere at 37 °C for 30 min.

Treatment: Following incubation, the dye solution was removed, the cells were washed with 2 mL PBS and were treated with several concentrations of glyphosate in FCS-free media depending on different incubation periods: 30, 40, 53 and 70 mM for 0.5 h ; 15, 20, 30 and 45 mM for 6 and 18 h. To ensure the dye specificity, a positive control is carried out, a treatment with tert-Butyl hydroperoxide (tBHP) 15 µM for 18 h.

Apoptosis

Treatment: After 24 h of cell attachment, cultured cells were exposed to several concentrations of glyphosate in FCS-free media depending on different incubation periods: 30, 40, 53 and 70 mM for 0.5 h; 15, 20, 30 and 45 mM for 6 and 18 h.

Mitochondrial transmembrane potential

Treatment: After 24 h of cell attachment, keratinocytes were treated as for the apoptosis study.

5. Observations/analyses:

Measurements: Cell viability, flow cytometry, intracellular ROS (H_2O_2), apoptosis induction, cell morphology

Cytotoxicity assays: After the exposure periods, the reaction medium was removed, and the residual cell viability was measured in each well using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide procedure. Results were expressed as percentage of controls (100% viability) according to the glyphosate concentrations, and the 50% inhibition concentrations (IC50), i.e., the concentrations of glyphosate killing 50% of keratinocytes, were compared according to the four exposure times. Each experiment was done three times, and each determination was carried out in triplicate.

Flow cytometry: Flow cytometry analyses were performed on a FC500 cytometer (Beckman-Coulter, France).

Analyses were performed on no less than 10 000 cells, using the CXPTM software (Beckman-Coulter).

Intracellular ROS (H_2O_2): Following exposure, and after supernatant recovery, the cells were washed with pre-warmed PBS and were collected by trypsinization; whereafter, the trypsin was removed by centrifugation. Cells were resuspended in PBS before flow cytometry.

Apoptosis: Following exposure, and after supernatants recovery, the cells were washed with pre-warmed PBS and were collected by trypsinization; whereafter, the trypsin was removed by centrifugation. The cells were then stained with the FITC annexin V Apoptosis Detection Kit II (BD biosciences, Franklin Lakes, USA) as recommended in the procedure.
To ensure the dye specificity, a positive control with camptothecin and a negative control after a pretreatment with recombinant annexin V were carried out.

Mitochondrial transmembrane potential: The cells were then stained with the mitochondria staining kit (Sigma CS0390) as recommended by the procedure. To ensure the dye specificity, a negative positive control with

valinomycin was carried out.

In the data representation, a point represents any cell with its red fluorescence intensity in abscissa and its green fluorescence intensity in ordinate.
The changes in the mitochondrial membrane potential were monitored using JC-1 (5,5V,6,6V-tetrachloro-1,1V,3,3V-tetraethylbenzimidazolcarbocyanine iodide).

Statistics: Not reported

KLIMISCH EVALUATION

1. 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)

2. Relevance of study: **Not Relevant** (*in vitro* data on the effects on an immortalized epidetmal 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 in formulated glyphosate based products)

3. Klimisch code: **3**

GTF Comments on the two Heu et al. (2012a; 2012b) publications

- Doses evaluated appear to be in the range of spray dilutions of glyphosate formulations.
- 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 section 5.9.9, showing very 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; See Section 5.9.9 Dermal Penetration). 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
Acquavella, J.F. Alexander, B.H. Mandel, J.S. Gustin, C. Baker, B. Chapman,P. Bleeke, M.	2004	Glyphosate Biomonitoring for Farmers and Their Families: Results from the Farm Family Exposure Study Environmental Health Perspectives Volume: 112 Number: 3 Pages: 321-326

Abstract*

Glyphosate is the active ingredient in Roundup agricultural herbicides and other herbicide formulations that are widely used for agricultural, forestry, and residential weed control. As part of the Farm Family Exposure Study, we evaluated urinary glyphosate concentrations for 48 farmers, their spouses, and their 79 children (4–18 years of age). We evaluated 24-hr composite urine samples for each family member the day before, the day of, and for 3 days after a glyphosate application. Sixty percent of 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 was 0.004 mg/kg. Farmers who did not use rubber gloves had higher GM urinary concentrations than did other farmers (10 ppb vs. 2.0 ppb). For spouses, 4% had detectable levels in their urine on the day of application. Their maximum value was 3 ppb. For 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 was present during herbicide mixing, loading, or application. None of the systemic doses estimated in this study approached the U.S. Environmental Protection Agency reference dose for glyphosate of 2 mg/kg/day. Nonetheless, it is advisable to minimize exposure to pesticides, and this study did identify specific practices that could be modified to reduce the potential for exposure.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup® Ultra
Active substance(s):	Glyphosate
Source:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Number of test persons:	48 farmers, 48 spouses, 79 children
Age:	≥ 4 years (children); 11.5 yr (mean) Farmers: 45 yr (mean) Spouses: 42.2 yr (mean)
Sex:	Males and females
Inclusion criteria:	o Live on the farm; o farmers and spouses that have at least 1 child (4-18 yr) o Farm at least 10 acres within 1 mile of the family residence, to which they planned to apply one or a combination of the

pesticides included in the study: glyphosate, 2,4-D (2,4-dichlorophenoxyacetic acid), or chlorpyrifos. There were no restrictions on using these pesticides before or immediately after the planned on-study application.
- Family members had to be willing to collect all urine voids for five consecutive days: the day before, the day of, and 3 days after the planned pesticide application.
- Farmer and spouse had to be willing to fill out pre- and post-study questionnaires, thereby detailing family activities for the week before the study and the week of the study, and agree to have their on-study pesticide application observed by trained field staff.

4. Test system:

Type: Glyphosate Biomonitoring Study

Collection of data:
- Questionnaire + interview (incl. assessment of demographic and lifestyle factors)
- Field observation of application or related activities, application equipment, meteorological conditions, work practice (incl. protective measures), and family activity patterns
- - Urine analyses

Guideline: Non-guideline study

GLP / GCP: Yes

Exposure conditions: No information on application rates reported. Numbers of mixing/loading operations and areas treated are provided.

Sample collection: During the study period, defined as 24 hr before the start of on-study pesticide-related activities (day –1) and continuing for 4 consecutive 24-hr periods (days 0, 1, 2, and 3, respectively), participants collected individual urine voids in 500-mL high-density polyethylene wide-mouth containers.

Field research staff collected the samples daily, monitored compliance, logged each sample in a computer database, and created 24-h composite urine samples with amounts proportional to the volume of each individual urine sample. Samples were shipped frozen to analytical laboratory.

5. Observations / analyses:

Measurements: Urine samples were analyzed for glyphosate concentration using a previously published method (Cowell et al. 1986) modified for urine (FFES 2003). The method employs chelation ion exchange for the concentration and isolation of glyphosate, followed by quantitation using high-performance liquid chromatography with postcolumn reaction and fluorescence detection. The method has a limit of detection (LOD) of 1 μg/L (or 1 ppb) for a 100-mL urine sample. Results were corrected for laboratory analytical recovery and storage stability as determined by analysis of fortified filed samples prepared throughout the study.

Calculations: The systemic dose (= absorbed dose in mg/kg bw) was estimated for all farmers, spouses and children who had detectable urinary levels of glyphosate, by calculating the amount of glyphosate excreted during the study period, adjusting for incomplete excretion and pharmacokinetic

recovery, and dividing the total corrected excretion by each individual´s body weight.

Statistics: Geometric mean (GM) urinary concentrations were calculated for farmer-applicators as the antilog of the average of the natural log (ln)–transformed urinary concentrations (SAS, version 8.2 for Windows). The standard deviation (SD) was calculated as the antilog of the SD of the ln-transformed urinary concentrations. In these calculations, a value of 0.5 ppb (LOD/2) was assigned for concentrations that were below the LOD. GM concentrations for spouses and children were not calculated because too few of these subjects had detectable concentrations in their urine. *t*-tests and one-way analysis of variance were used to compare GMs for farmers who followed different application practices (SAS, version 8.2). Two-tailed significance tests were used consistent with the null hypothesis of no relationship between application practices and urinary pesticide concentration.

Glyphosate that was recovered in urine was devided with complete pharmacokinetic recovery by each individual's body weight to yield a systemic dose in milligrams per kilogram body weight.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable without restrictions**

Comment: Well documented GLP study, which meets requirements of exposure test guidelines. Study design is scientifically acceptable.

2. Relevance of study: **Relevant** (Provides information on real-life exposure to glyphosate in farmers and their families)

3. Klimisch code: **1**

GTF Comments

- The Acquavella et al. (2004) Farm Family Exposure Study (FFES) is depicted by Earth Open Source (EOS) as not reflective of real world exposures and suggest a long term exposure assessment study would be more appropriate.
- EOS references a letter to the Editor by Mage, stating the obvious; increased spatial and temporal monitoring offer greater insight than shorter duration exposure and monitoring studies.
- EOS suggests an alternative exposure monitoring study by Curwin (2007), which reports geometric mean systemic (maximum likely dose to farmers' children (0.11 ug/kg/day, or 0.00011 mg/kg/day) and non-farm children (0.13 ug/kg/day, or 0.00013 mg/kg/day). These systemic doses are comparable to the conservative FFES geometric mean systemic dose to farmers (0.0001 mg/kg/day). However, the FFES provides a great deal of detail on urinary concentrations for farmers with different pesticide handling practices or procedures.
- Curwin (2007) which reports on children biomonitoring data for farm children and non-farm children, taken once "a few days after" application and again one month later. Glyphosate systemic exposures were similar for farm and non-farm children, and all were well below the chronic reference dose or acceptable daily intake.

- The GLP compliant Acquavella et al. (2004) is one of few comprehensive published operator and family exposure monitoring studies for glyphosate available, with time-course measurements for glyphosate; the day before, the day of, and for 3 days after application, reporting human systemic exposures for 48 farmers, their spouses and 79 children.

Below is a summary of the Letter to the Editor by Mage.

Author(s)	Year	Study title
Mage, D.T.	2006	Suggested Corrections to the Farm Family Exposure Study Environmental Health Perspective Volume: 114 Number: 11 Page: A 633

Abstract

No abstract.

[The author of letter claims that the study conducted by Acquavella et al. contains methodological deficiencies. Thus, the author recommends that Acquavella et al. (2004) consider revising their analysis by correcting properly for incomplete urine collection, correcting for the initial condition of prior glyphosate exposure, and a adjusting for the experience of the applicator (lifetime number of application days) as an explanatory variable].

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup® Ultra
Active substance(s):	Glyphosate
Description:	Not reported
Source of test medium:	Not reported
Lot/Batch #:	Not reported
Concentration:	Not reported

2. Study addressed: Acquavella et al.(Environmental Health Perspectives (2004), 112, 321-326)

Glyphosate Biomonitoring Study:

- Questionnaire + interview (incl. assessment of demographic and lifestyle factors)
- Field observation of application or related activities, application equipment, meteorological conditions, work practice (incl. protective measures), and family activity patterns
- Urine analyses

KLIMISCH EVALUATION

1. Reliability of study: **Not applicable**

Comment: In this publication the article by Aquavella et al. (Environmental Health Perspective (2004), 114, A 633) is discussed in detail. The author of letter claims that the study conducted by Acquavella et al. contains methodological deficiencies regarding urine collection, initial condition of prior glyphosate exposure, and statistical analysis.

2. Relevance of study: **Relevant** (No original publication but letter to the editor regarding the article by Acquavella et al., 2004)

3. Klimisch code: **Not applicable**

Author(s)	Year	Study title
Anadon, A. Martinez-Larranaga, M.R. Martinez, M.A. Castellano, V.J. Martinez, M. Martin, M.T. Nozal, M.J. Bernal, J.L.	2009	Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats Toxicology Letters Volume: 190 Pages: 91-95

Abstract*

The toxicokinetics of glyphosate after single 100 $mgkg^{-1}$ intravenous (i.v.) and 400 $mgkg^{-1}$ oral doses were studied in rats. Serial blood samples were obtained after i.v. and oral administration. Plasma concentrations of glyphosate and its metabolite amiomethyl 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 $l\ h^{-1}kg^{-1}$. 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 μml^{-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 article

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate
CAS No:	107-83-6
Purity:	95% (w/w)
Source:	SIGMA CHEMICAL CO., St Louis, MO, USA

2. Vehicle: Corn oil for oral and glycerol formal for i.v. applications

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Charles River Inc., Margate, Kent, UK
Age of test animals at study initiation:	Adult
Sex:	Male
Body weight:	200-210 g
Acclimation period:	Not reported

Diet/Food:	A04 rodent diet, Panlab SL, *ad libitum*, except for 12 hours before dosing
Water:	Water, *ad libitum*
Housing::	Individually housed in polycarbonate cages with sawdust bedding
Environmental conditions:	Temperature: 22 ± 2°C
	Humidity: 50 ± 10%
	Air changes: Not reported
	12-hour light/dark cycle

4. Test system:

Study type:	Toxicokinetic study
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Animals per dose group	The rats were divided into two groups of 80 animals each, one group (Group 1) received glyphosate orally and the other group (Group 2) intravenously.
Group 1	
Dose level:	400 mg/kg bw
Administration:	Rats received a single oral administration of 400 mg/ kg bw gavage in a volume of 0.5 mL corn oil/rat
Group 2	
Dose level:	100 mg/kg bw
Administration:	Rats received a single i.v. injection of 100 mg/ kg bw (in 0.1ml glycerol formal/rat)into the lateral tail vein
Sacrifice:	8 rats per observation time point were killed by cervical dislocation and then exsanguinated.
Time points:	0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after oral and i.v. administration of glyphosate
Blood sampling and processing:	Blood was withdrawn at each time point and collected in heparinised tubes. Plasma was separated by centrifugation and stored frozen at -80°C until analysis.

5. Observations/analyses:

Clinical signs:	Assessed, but no details provided
Measurement:	Glyphosate and its metabolite AMPA concentrations in plasma were measured by HPLC.
Calculations and statistics:	The mean plasma concentration versus time data were sequentially fitted to 1-, 2- and multiple-compartment models. The 2-compartment model was used to establish toxicokinetic characteristics.
	Plasma curves of glyphosate after a single i.v. and oral administration and those of AMPA (the main metabolite in plasma) after a single oral administration of glyphosate were fitted to the exponential equations:
	$C = A_1 e^{-\alpha t} + A_2 e_{-\beta t}$ (i.v.)

$$C = A_1 e^{-\alpha t} + A_2 e_{-\beta t} - A3\ e^{-\kappa at}\ (p.o.)$$

The following parameters were also calculated:

- area under the concentration-time curve (AUC)
- total plasma clearance
- mean residence time (MRT) (only for i.v. administration)
- volume of distribution in the central compartment (V_1)
- apparent volume of distribution in the second compartment (V_2)
- volume of distribution at steady state (V_{ss}) (only for i.v. administration)
- maximum drug plasma concentration (C_{max}) after oral administration and the time at which C_{max} was achieved (T_{max}) was determined directly from the concentration versus time curve.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable with restrictions**

Comment: Study report meets basic scientific principles, and is comparable to actual kinetics guidelines (large number of test animals used, standard deviations and mass balance not reported)

2. Relevance of study: **Relevant** (additional information on blood plasma concentrations of glyphosate and AMPA, as well as elimination after oral and i.v. application of glyphosate.)

3. Klimisch code: **2**

GTF Comments

- EOS made one comment on this kinetics study

  "Because glyphosate and AMPA were cleared from blood more slowly after oral dosing, they could be distributed to body tissues to exert systemic toxic effects."
- Intravenous injection is more rapidly excreted than oral simply because of lag time for GI tract absorption and entry into the blood stream. That reported, half-lives for i.v (0.345 and 9.99 hours for α and β phases respectively) versus oral (4.17 and 14.38 hours for α and β phases respectively) are all very rapid elimination kinetics, emphasizing the low duration of any systemic exposure to glyphosate, irrespective of the route.

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

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

MATERIALS AND METHODS

1. Test material:

Test items: **Diazinon**, (o,o-diethyl o-(2-isopropyl-6-methylpyrimidin-4-yl) phosphothioate);
phosmet, (o,o-dimethyl S-phthalimidomethyl phosphorodimethioate);
pirimiphos methyl, (o-(2-diethylamino- 6-methylpyrimidin-4-yl) o,o-dimethyl phosphorothioate);
chlorpyrifos, (o,o-diethyl o-(3,5,6- trichloro-2-pyridyl) phosphorothioate);
glyphosate, (N-(phosphonomethyl) glycine)

Roundup Weed Killer, containing 0.72% (w/v) glyphosate acid as a 9.7 g/L IPA salt of glyphosate in a soluble concentrate;
Tough Weed Killer, containing 5% (w/v) glyphosate trimesium

Sources: Purified pesticides – Chemserve/Greyhound Chromatography, Birkenhead, UK.

Herbicide formulations – Roundup Weed Killer from Solaris, Garden Division of Monsanto, High Wycombe, UK, (Lot No. C8K2767);

	Tough Weed Killer from Miracle Gardencare, Godalming, UK, (coded Dec97294)
Purity:	≥ 99% for all pesticides except the formulations

2. Vehicle and/or positive control: Methanol (0.25 % w/v) or dimethyl sulphoxide (DMSO) or serum-containing medium (for non-induction of differentiation)

3. Test system/cells:

(in the following information is only provided for test with glyphosate or glyhosate-containing herbicides)

Cells:	Mouse NB2a neuroblastoma, cell line: 89121404
Source:	ECACC
Culture medium:	High glucose Dulbecco's Modified Eagle's Medium (DMEM) containing Glutamax- 1, and supplemented with 5% (v/v) horse serum, 5% (v/v) foetal calf serum, 100 U/ml penicillin plus 100 µg/ml streptomycin and 25 µg/ml gentamicin
Culture conditions:	Temperature: 37 C Atmosphere: 5% CO_2; humidified

4. Test method:

Study type:	Acute pesticide exposure on neuroblastoma cells after chronic exposure of organophosphate
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Pre-exposure to diazinon:	Diazinon-pre-exposed cells were maintained for either 24 h or 8 weeks before use in maintenance medium containing 25 µM diazinon; Control cells were maintained under exactly the same condition, but with addition of only the solvent (methanol at 0.25 %) in which the diazinon was dissolved
Exposure to test substances:	After the pre-exposure period, cells were added to wells of 48-well culture plates at a density of 9000 cells per cm^2 in normal diazinon-free culture medium. After 24 h the medium was removed and replaced with serum-free medium containing 1 mM dibutyryl cyclic AMP (without diazinon) to induce differentiation. (Differentation led to the growth of neurite-like structures). At the same time, the test compouns to be tested were added to the medium at a range of concentrations. Control cells were exposed to the vehicle of dilution only (either DMSO or methanol), and negative control cells continued to be grown in serum-containing medium, i.e. there were not induced to differentiate.
Exposure duration:	24 h
Dose levels:	For pre-exposure (8 weeks or 24 h): 25 µM diazinon For acute exposure: 50 – 1000 µM glyphosate not specified for glyphosate-containing formulations After exposure cells were fixed and stained with Comassie Blue.
Replicates per combination of substances:	4

5. Observations/analyses:

Measurements: ≥ 200 cells from six to 20 different fields were evaluated for each experiment microscopically.
An automated image analysis program performed a segmentation and skeletalisation of the images of the cells by serial erosion to single pixel width.
The average length of neuritis per cell for each different treatment was recorded. Toxixicty was measured in terms of a reduction in neurite length of treated differentiating cells compared with untreated differentiating controls, after subtraction of a baseline level on no neurite outgrowth as measured in the cells grown in serum-containing medium.
IC_{50} (the level at which 50% reduction in neurite outgrowth was achieved) was determined in separate experiments from the relationship between neurite outgrowth and the test substance concentration.
Effects of individual pesticides pre-exposed to diazinon were compared to non-pre-exposed cells.

Statistics. The significance of any difference was compared by Students' t -test of IC_{50} values, or of the neurite inhibition at single concentration points.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Incorrect chacacterization of glyphosate as an organophosphate pesticide. Inappropriate test system for formulations containing surfactant; cytoxic 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.

2. 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,)

3. Klimisch code: **3**

GTF Comment

- Inappropriate test system to evaluate test materials containing membrane damaging surfactants.
- See earlei discussion under *In Vitro* Glyphosate DART/ED Publications under this dossier point (literature review).

Author(s)	Year	Study title
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® Toxicology Letters Volume: 153 Pages: 227-232

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

MATERIALS AND METHODS

1. Test material:

Test item:	Glyphosate-Biocarb®
Active substance(s):	Glyphosate
Source:	Sao Jose dos Pinhais, Brazil
Purity:	360 g/L
Surfactant:	Polyoxyethyleneamine
Purity:	18% (w/v)

2. Vehicle: Distilled water

3. Test animals:

Species:	Rat
Strain:	Wistar
Source:	Departamento de Analises Clinicas – CSS – Universidade Federal de Santa Catarina, Florianopolis, SC, Brazil
Age of test animals at study initiation:	90 days
Sex:	Male
Body weight:	280-310 g
Acclimation period:	Not reported
Diet/Food:	Laboratory rat chow, *ad libitum*
Water:	Water, *ad libitum*
Housing::	Polyethylene (65 cm × 25 cm × 15 cm) home cages, with sawdust-covered floors. Number of rats per cage not reported.

Environmental conditions:	Temperature: 22 ± 2°C Humidity: Not reported Air changes: Not reported 12-hour light/dark cycle

4. Test system:

Study type:	Sub-chronic study
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	75 days
Dose levels:	0 (distilled water), 4.87, 48.7, or 487 mg/kg of Glyphosate-Biocarb® diluted in water
Animals per dose group:	Control group: n=16 Experimental groups: n=14 each group
Administration route:	Gavage
Administration volume:	0.5 ml/kg
Administration frequency:	Each 2 days
Blood sampling and processing:	Blood samples collected from all animals by percutaneous cardiac puncture before sacrifice. Blood samples were transferred to test tubes and allowed to stand for 30 min to clot before being centrifuged at 300×*g* for 10 min. Sera were obtained by centrifugation and stored at 4 C.
Tissue sampling and processing:	Liver samples were obtained from all animals by surgical processing. Sections of 2 µm were mounted in glass slides covered with saline.

5. Observations/analyses:

Measurements of liver enzymes

Mortality:	Not reported
Clinical signs:	Not reported
Body weight:	Not reported
Food- and water consumptions:	Not reported
Haematology:	Not reported
Clinical chemistry:	AST and ALT levels
Urine analysis	Not reported
Sacrifice/pathology:	Not reported
Organ weights:	Not reported
Histopathology:	Liver tissue samples were evaluated after haematoxylin–eosin staining. The liver samples were also assessed for the deposition of reticulin fibers by Gomory trichromic staining.
Statistics:	Parametric data, expressed as mean ± S.E.M., were analyzed by one-way variance Newman–Keuls for ALT and AST tests. Differences were considered to be statistically significant when

$p < 0.05$

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comments: Study report meets basic scientific principles. Study design and documentation is insufficient for assessment.

2. Relevance of study: **Not relevant** (Study design not sufficient for assessment. Toxicity attributable to high oral dosing of surfactant component. There are several reporting deficiencies.)

3. Klimisch code: **3**

GTF Comment

- Effects noted at the high dose of 487 mg/kg/day formulated product in this study are equivalent to approximately 87 mg/kg/day POEA surfactant.
- High dose surfactant effects are noted at doses well below those reported in the US EPA Alky Amine Polyalkoxylates Human Health Risk Assessment (http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064809b983b), in which a surfactant within the same class as POEA demonstrated a range of adverse effects, including mortality, at 30 mg/kg/day and a NOAEL of 15 mg/kg/day.
- Overt toxicity reported in Benedetti et al (2004) is consistent with the ingestion of an irritating surfactant and not glyphosate.

Author(s)	Year	Study title
Mesnage, R. Clair, E. Gress, S. Then, C. Szekacs, A. Seralini, G.E.	2012b	Cytotoxicity on human cells of Cry1Ab and Cry 1Ac *Bt* insecticidal toxins alone or with a glyphosate-based herbicide. Journal of Applied Toxicology doi: 10.1002/jat.2712. [Epub ahead of print]

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

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup® GT Plus
	Cry1Ab toxin
	Cry1Ac toxin
Active substance(s):	Glyphosate
	Cry1Ab toxin
	Cry1Ac toxin
Source of test items:	Roundup® GT Plus: Monsanto, Anvers, Belgium
	Cry1Ab toxin and Cry1Ac toxin: unspecified laboratories (described elsewhere)
Lot/Batch #:	Not specified
Purity:	Roundup® GT Plus: 450 g/L glyphosate acid (N-phosphonomethyl-glycine)
	Cry1Ab toxin and Cry1Ac toxin: unspecified
Homologation:	Roundup® GT Plus: 2020448
	Cry1Ab toxin and Cry1Ac toxin: unspecified

2. Vehicle and/or positive control: Not reported

3. Test system / cells:

Cell lines:	Human embryonic kidney (HEK) 293 cell line (ECACC 85120602)
Source:	Sigma-Aldrich (Saint-Quentin Fallavier, France)
Culture conditions:	Cells were grown in phenol red-free EMEM containing 2 mM glutamine, 1% nonessential amino acid, 100 U/mL of antibiotics (a mixture of penicillin, streptomycin and fungizone), 10 mg/mL of liquid kanamycin and 10% fetal bovine serum. Cells were grown at 37 °C (5% CO_2, 95% air) during 24 h to 80% confluence.

4. Test methods:

Mitochondrial respiration assay (MTT)	Assessment of cell viability
ToxiLight® assay	Bioluminescent assay for quantitative measurement of cell membrane damage
Caspase-Glo® 3/7 assay	Assessment of caspase activity or apoptosis induction
Guideline:	Non-guideline assays
GLP:	No
Guideline deviations:	Not applicable
Cell treatments for all tests:	Cells at 80% confluence in 48- or 96-well plates were washed with serum-free EMEM, in order to avoid other combined effects, and then exposed to various concentrations of *Bt* toxins or Roundup GT Plus in EMEM serum-free medium for 24 h.
Dose levels:	*Bt* toxins: 10 ppb - 100 ppm (in the range of GM plant production). Roundup: 1 - 20 000 ppm (the latter is the agricultural dilution). Combined effects: measured by mixing LC_{50} of Roundup with *Bt* toxin: 1, 10, 100 ppm.
Test methods:	For the methods used authors refer to: Benachour N, Séralini GE. 2009. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. *Chem. Res. Toxicol.* **22**: 97–105.
Replicates per dose level:	The experiments were repeated at least 3 times in different weeks on 3 independent cultures (n = 9).

5. Observations/analyses:

Measurements:	Cytotoxicity - mitochondrial respiration level, by succinate dehydrogenase (SD) activity assessment; Membrane damage - determination of adenylate kinase (AK) activity; Apoptosis induction – assessment of caspase 3 and 7 activities.
Mitochondrial respiration assay (MTT)	Analysis: Succinate dehydrogenase activity measurement. The optical density was measured at 570 nm using a Mithras LB 940 luminometer.

ToxiLight® assay: Analysis: Bioluminescent assay, membrane degradation measured by the intracellular AK release in the medium (necrosis marker). Luminescence measured using a Mithras LB 940 luminometer.

Caspase-Glo® 3/7 assay: Analysis: Luminescence measured using a Mithras LB 940 luminometer.

Statistics: LC_{50} values were calculated by a nonlinear regression using a sigmoid (five-parameter) equation with the GraphPad Prism 5 software. All data were presented as the means ± standard errors (SEs). Statistical differences were determined by Student t-test using significance levels at $P<0.01$ and $P<0.05$.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Non-guideline, non-GLP *in vitro* 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; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.

2. Relevance of study: Relevant with restrictions (Due to reliability. The assessed combinatory effects are of limited relevance)

3. Klimisch code: **3**

Monsanto Comments: Mesnage et al. (2012)

General Statement:

This publication presents no new findings relevant to a safety assessment of glyphosate-based herbicides or Cry proteins Cry1Ab or Cry1Ac. The experimental system placed high concentrations of herbicide and Cry proteins directly on living cells in culture, which is not viewed by public health experts as relevant methodology for evaluating risks to humans.

Animal data and human experience contradicts findings of Petri dish experiments with glyphosate-surfactant herbicides. Surfactants are routinely added to herbicide formulations to break down the waxy coating on plants and allow for efficient absorption. Surfactants also occur in soaps, shampoos, dishwashing detergents, and laundry products, which account for nearly all (over 99%) of consumer surfactant exposure.

Numerous studies have been conducted where animals were administered very high dosages of Cry proteins and suffered no ill effects. High-dose animal toxicity testing using Cry1Ab and Cry1Ac demonstrates no toxic effects at doses thousands of times higher than any potential human intake. Moreover, Cry proteins are destroyed by heating and digested in the gastro-intestinal tract. As a result, intact Cry protein is not detectible in the serum and tissues of animals that have ingested feed containing Cry proteins. As with glyphosate, the safety of Cry1Ab and Cry1Ac has been reviewed and confirmed by regulatory agencies around the globe.

Comments:

1) **Glyphosate has an excellent human health and environmental profile and a long history of safe use in more than 130 countries.** This has been a key factor in the acceptance of glyphosate products as among the most widely used herbicides in the world. When used according to label directions, these products present no unreasonable risk of adverse effects to human health or the environment. This is confirmed by the extensive studies as well by the first-hand experience of millions of farmers and home gardeners who have used this product.

   Glyphosate, the active ingredient in Roundup branded agricultural products and other glyphosate based herbicide formulations, inhibits an enzyme that is essential to plant growth; this enzyme is not found in humans or other animals, which explains the generally low acute toxicity of glyphosate in humans and animals (Franz et al., 1997). Comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (U.S. EPA, 1993; Williams et al., 2000; Williams et al., 2012); European Commission, 2002; JMPR/WHO, 2004; Mink et al., 2011). In fact, after a thorough review of all toxicology data available, the U.S. EPA concluded that glyphosate should be classified in Category E ("Evidence of Non-carcinogenicity in Humans"), the most favorable category possible (U.S. EPA, 1993).

2) **Artificial conditions.** 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.

3) **Glyphosate-surfactant levels tested are not relevant to real exposures.** Anadon et al. (2009) dosed rates 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) 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) following maximum allowable intake.

4) **Animal data and human experience contradicts findings of Petri dish experiments with glyphosate-surfactant herbicides**. Glyphosate has been tested extensively in higher order animals (Giesy et al., 2000; Williams et al., 2000). There is no evidence for developmental or reproductive concerns in multiple species despite numerous high-dose tests by different manufacturers (Williams et al 2012, EU, 2002, JMPR/WHO 2004). Furthermore, studies with POEA have not demonstrated any target organ toxicity or effects on embryos, fetuses, or the placenta (Williams et al., 2000; Williams et al 2012).

5) **The surfactant effects are not surprising.** Levine et al., 2007 demonstrated that surfactants found in household and personal care products could alter mouse Leydig cell function. It should not be a surprise that a glyphosate-based formulation which contains surfactants similar to surfactants found in household and personal care products would have an effect on cellular membranes. The exposure of humans to surfactants is common from bath gels, hand soaps, shampoos, and laundry and dishwashing detergents to name a few. In addition human oral exposure to surfactants can originate from residues on eating utensils and dishes washed with dish washing detergents and from residues taken up via drinking water (HERA, 2003).

6) **Doses of Cry proteins are irrelevant to real life exposures.** High-dose animal toxicity testing via the oral routes using Cry1Ab and Cry1Ac demonstrates no toxic effects at doses thousands of times higher than any potential human intake. The only concentration of Cry protein demonstrating any effect on cellular function was 100 ppm, used in an otherwise protein free medium. The concentration

of Cry protein in grain is below 1 ppm (see Monsanto product safety data, link above), and these cry proteins are both degraded by cooking and are readily digestible.

Studies of meat, milk, and eggs have not demonstrated intact Cry protein detection in animals fed on GM crops containing these proteins. The studies of Aris and Leblanc, taken at face value, indicate Cry protein concentration in human blood up to about 0.2 parts per billion- or 500-fold LESS than the concentrations used by Mesnage et al.(2012). We would note, however, that the validity of the Aris and Leblanc (2011) publication has been seriously questioned by scientists and regulators. Regulatory opinions, original article, and associated correspondence at:
http://www.food.gov.uk/multimedia/pdfs/acnfp10308pest
http://www.foodstandards.gov.au/consumerinformation/gmfoods/fsanzresponsetostudy5185.cfm
http://www.sciencedirect.com/science/article/pii/S0890623811000566 .

7) **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) 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 we would not argue for any true in-vivo protective effects as 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-stabilization 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.

8) **The references cited regarding the in-vitro toxicity studies of other Bt derived proteins are largely irrelevant.** There are many different Bt varieties that produce many different kind of toxins, and some Bt toxins are known to be toxic to mammalian cells when in vitro.We utilize the Cry proteins that are closely related to the many kinds of proteins found in commercial Bt microbial pesticides that have been safely used in agriculture around the world for approximately 50 years. The Bt toxins used in GM planta have been subject to extensive safety assessment (Betz 2000, Federici and Siegel 2008, OECD 2007, WHO/IPCS 1999)The work of Ito et al reports the effect of a NON-insecticidal Bt-derived protein which is cytotoxic to some human cell lines. The work of Nagamatsu similarly reports on a non-insecticidal Bt protein. The work of Rani does involve a solubilized Cry protein from an insecticidal Bt strain. Oral toxicity is not demonstrated in any case but, more to the point, extensive toxicity studies of Cry1Ab and Cry1Ac in mammalian species indicate to toxic effect at relevant doses and by relevant routes.

9) **Caffeine metabolites, alcohol and nicotine can disrupt cell function.** It is important to note that a metabolite of caffeine inhibited the development of Leydig cells in Petri dish experiments. (Pollard et al., 2001). In addition, alcohol (Jang et al., 2002) and nicotine (Kim et al., 2005) activate specific intracellular death-related pathways, capsase -3, inducing apoptosis in mouse Leydig cells grown in Petri dishes similar to that reported in this abstract. *In vivo* and *in vitro* exposures demonstrate that alcohol can damage Sertoli cells (Shu et al 1997). These findings clearly put this experimental model into context. Caffeine, in its natural and added forms, is found in coffee, tea, cola beverages, energy drinks, chocolate and even some medicines. The average intake of caffeine in the US by children 5-18 years of age averages 1 mg/kg/day and adults 2.4 mg/kg/day (Mandel, 2002). A typical cup of coffee

can contain 150 mg of caffeine, a cup of blended tea 43 mg and a small portion of a milk chocolate candy bar contains about 7 mg of caffeine. (Health Canada 2010).

10) **Prior Publications.** Seralini and colleagues at the University of Caen in France have five prior publications on the results of exposing unprotected cells in culture to glyphosate, AMPA (aminomethylphosphonic acid, the primary environmental degradate of glyphosate), glyphosate- based formulations or a surfactant used in some formulated products.

- Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., and Seralini, G.-E. 2005. Differential effects of glyphosate and Roundup on human placental cells and aromatase. Environ. Health Perspect. 113:716-720. http://ehp03.niehs.nih.gov/article/fetchArticle.action?articleURI=info:doi/10.1289/ehp.7728

- Benachour, N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C., and Séralini, G. E. 2007. Time- and dose-dependent effects of Roundup on human embryonic and placental cells. Arch. Environ. Contam. Toxicol. 53:126-133. http://www.springerlink.com/content/d13171q7k8631446/

- Benachour, N., and Séralini, G. E. 2009 Glyphosate Formulations Induce Apoptosis and Necrosis in Human Umbilical, Embryonic, and Placental Cells. Nora Benachour and Gilles-Eric Seralini. Chem. Res. Toxicol., 22, 97–105. http://pubs.acs.org/doi/pdf/10.1021/tx800218n

- Gasnier, C., Dumont, C., Benachour, N., Clair, E., Chagnon, M., Gilles-Eric Seralini (2009). Glyphosate-based herbicides are toxic and endocrine disruptors in human cell lines. Toxicology; 262(3):184-91 http://www.sciencedirect.com/science/article/pii/S0300483X09003047

- Clair E, Mesnage R, Travert C, Séralini GE., 2012. A glyphosate-based herbicide induces necrosis and apoptosis in mature rat testicular cells in vitro, and testosterone decrease at lower levels. Toxicology in Vitro. http://www.sciencedirect.com/science/article/pii/S0887233311003341

The same group has published two publications suggesting that homeopathic remedies can protect cells against purported adverse effects of glyphosate. Co-authors are associated with the purveyor of these homeopathic products, although they claim no conflict of interest.

- Gasnier et al. Dig1 protects against cell death provoked by glyphosate-based herbicides in human liver cell lines. Journal of Occupational Medicine and Toxicology 2010, 5:29 http://www.occup-med.com/content/5/1/29

- Gasnier et al. Defined plant extracts can protect human cells against combined xenobiotic effects. Journal of Occupational Medicine and Toxicology 2011, 6:3 http://www.occup-med.com/content/6/1/3

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Anadon A, et al. (2009) Toxicokinetics of glyphosate and its metabolite aminomethyl phosphonic acid in rats. Toxicology Letters 190 91–95

Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P, Bleeke M, 2004, Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study, *Environmental Health Perspectives 112(3): 321-326.* doi:10.1289/ehp.6667

Betz FS, Hammond BG, Fuchs RL (2000) Safety and Advantages of Bacillus Thuringiensis-Protected Plants to Control Insect Pests. Regul Toxicol Pharmacol 32, 156-173.

EU Commission. (2002) Review report for the active substance glyphosate. http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf

Federici B, Siegel J (2008) Safety assessment of Bacillus thuringiensis and Bt crops used in insect control. in Food Safety of Proteins in Agricultural Biotechnology, B.G. Hammond, (ed.) CRC Press, Boca Raton, Florida.

Giesy JP, Dobson S, and Solomon KR. (2000) Ecological Risk Assessment for Roundup® Herbicide. *Reviews of Environmental Contamination and Toxicology*, 167: 35-120.

Health Canada. http://www.hc-sc.gc.ca/hl-vs/iyh-vsv/food-aliment/caffeine-eng.php

HERA. (2009) Human & environmental risk assessment on ingredients of European household cleaning products. Alcohol Ethoxylats, version 2.0. http://www.heraproject.com/files/34-F-09%20HERA%20AE%20Report%20Version%202%20-%203%20Sept%2009.pdf

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Author(s)	Year	Study title
Clair E., Linn, L., Travert, C., Amiel, C., Seralini, G.E	2012b	Effects of Roundup® and Glyphosate on Three Food Microorganisms: *Geotrichum candidum*, *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Current Microbiology Volume: 64 Number: 5 Pages: 486-491

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 microbiodiversity and microbial concentration observed in raw milk for many years.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup®R400 and R450
Active substance(s):	Glyphosate
Description:	Not reported
Source:	Roundup®R400 and R450:Monsanto, Anvers, Belgium Glyphosate: Sigma-Aldrich, France
Lot/Batch #:	Not reported
Purity:	400 g/L and 450 g/L glyphosate

2. Test system/cells:

Species:	*Geotrichum candidum* ATCC 204307
Test system:	Fungi
Source:	Not reported
Maintenance medium:	MSF, pH 5.6
Initial cell concentration:	$1.95 \times 10^4 \pm 0.36$ UFC/mL
Species:	*Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1

Test system:	Bacteria
Source:	Not reported
Maintenance medium:	M17 containing lactose, pH 7.1
Initial cell concentration:	$6.31 \times 10^5 \pm 2.03$ UFC/mL
Species:	*Lactobacillus lactis* subsp. *cremoris* ATCC 19257
Test system:	Bacteria
Source:	Not reported
Maintenance medium:	MRS, pH 6.4
Initial cell concentration:	$6.70 \times 10^8 \pm 2.52$ UFC/mL

3. Cell treatments:

Exposure: Roundup R400 (400 g/L of glyphosate) and R450 (450 g/L of glyphosate) were diluted in autoclaved culture media, pH adjusted to each medium and 0.2 µm filtered. A solution of glyphosate, equivalent in glyphosate concentration and pH to R450, was diluted in different media.

The minimal inhibitory concentration (MIC) was evaluated, after treatment, by turbidimetry measurement at 600 nm using a microplate reader. The minimal microbicide concentration (MMC) which corresponds to the minimal treatment leading to 99.99% of lethality was evaluated by colony counting, after plating the previously treated micro-organisms. Concentrations between the MIC value and the MMC value correspond to cells that do not growth but are not dead.

4. Observations:

Colonies observations: Each microorganism was plated on Petri dishes containing agar and Roundup, and then incubated during 48 h. Colonies were macro- and microscopically observed after coloration with cotton blue for *G. candidum* and methylene blue for the two bacteria.

Statistics: Data were expressed as mean ± SEM for three independent determinations (n = 9). Significant differences were determined by Student *t* test with P<0.05*, P<0.01** and P<0.001***.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Non-validated, non-guideline test with methodological and reporting deficiencies (e.g. dose concentrations in media not specified, positive controls or controls that show the validity of the test system /and concentration range tested). Inappropriate test system for formulations containing surfactant; cytoxic membrane disruption potential of surfactants are well known for in vitro test systems.

2. Relevance of study: Not relevant (Due to reliability)

3. Klimisch code: 3

GTF Comments

- Clair et al look at in vitro effects of Roundup and glyphosate on *Geotrichum candidum* (a yeast-like fungal species found in some ripening cheeses) and two lactobacilli - *Lactococcus lactis* and *Lactobacillus delbrueckii*.
- The authors fail to provide any evidence whatever of difficulties related to the presence/ absence/ population of these organisms in dairy production or in the environment.
- 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.
- At concentrations at or above 100 ppm glyphosate- which would be equivalent to surfactant concentrations around 30 ppm and up (approximate, as the precise formulations are not known), one sees growth inhibition of all 3 tested species.
- Surfactants are known to be bacteriostatic, with (for example) quaternary ammonium compounds typically being active in the 30-150 ppm range.
- A wide variety of disinfectants, including surfactants, are approved and recommended for use in food services, including milk processing. The surfactant based products typically provide concentrations (at use dilution) of 50-500 ppm.
- General recommendations for liquid milk processing facilities generally recommend daily cleaning using a disinfectant solution. Cleaning products generally contain surfactants as milk residues are fat-containing and thus difficult to remove with water alone.
- Glyphosate residues in milk are permitted- currently at 0.1 ppm in the United States. This is 100,000 times LESS than the only effective concentration tested (10,000 ppm) for glyphosate.
- The results of this study have no apparent applicability to effects of glyphosate-surfactant herbicides on soil microbial populations.
- In short, Clair et al demonstrate that surfactants are bacteriostatic for these 3 organisms at concentration ranges well within the range of concentrations generally found to be useful for sanitation purposes. Application dilutions (1%) of glyphosate were shown to inhibit a yeast-like organism, which is unsurprising. Surfactant solutions are routinely used to sanitize food processing equipment at concentrations at or above those tested by Clair et al. These concentrations are vastly higher than the concentrations of glyphosate or possible surfactant present (if any) in incoming milk. This paper is simply irrelevant to agricultural use of glyphosate.
- Surfactants kill micro-organisms. This research is a valuable reminder of the importance of washing our hands before we eat.

Author(s)	Year	Study title
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 Volume: 31 Pages: 117-165

Abstract*

Reviews on the safety of glyphosate and Roundup herbicide that have been conducted by several regulatory agencies and scientific institutions worldwide have concluded that there is no indication of any human health concern. Nevertheless, questions regarding their safety are periodically raised. This review was undertaken to produce a current and comprehensive safety evaluation and risk assessment for humans. It includes assessments of glyphosate, its major breakdown product [aminomethylphosphonic acid (AMPA)], its Roundup formulations, and the predominant surfactant [polyethoxylated tallow amine (POEA)] used in Roundup formulations worldwide. The studies evaluated in this review included those performed for regulatory purposes as well as published research reports. The oral absorption of glyphosate and AMPA is low, and both materials are eliminated essentially unmetabolized. Dermal penetration studies with Roundup showed very low absorption. Experimental evidence has shown that neither glyphosate nor AMPA bioaccumulates in any animal tissue. No significant toxicity occurred in acute, subchronic, and chronic studies. Direct ocular exposure to the concentrated Roundup formulation can result in transient: irritation, while normal spray dilutions cause, at most, only minimal effects. The genotoxicity data for glyphosate and Roundup were assessed using a weight-of-evidence approach and standard evaluation criteria. There was no convincing evidence for direct DNA damage in vitro or in vivo and it was concluded that Roundup and its components do not pose a risk for the production of heritable/somatic mutations in humans. Multiple lifetime feeding studies have failed to demonstrate any tumorigenic potential for glyphosate. Accordingly, it was concluded that glyphosate is noncarcinogenic. Glyphosate, AMPA, and POEA were not teratogenic or developmentally toxic. There were no effects on fertility or reproductive parameters in two multigeneration reproduction studies with glyphosate. Likewise there were no adverse effects in reproductive tissues from animals treated with glyphosate, AMPA, or POEA in chronic and/or subchronic studies. Results from standard studies with these materials also failed to show any effects indicative of endocrine modulation. Therefore, it is concluded that the use of Roundup herbicide does not result in adverse effects on development, reproduction, or endocrine systems in humans and other mammals. For purposes of risk assessment, no-observed-adverse-effect levels (NOAELs) were identified for all subchronic, chronic, developmental, and reproduction studies with glyphosate, AMPA, and POEA. Margins-of-exposure for chronic risk were calculated for each compound by dividing the lowest applicable NOAEL by worst-case estimates of chronic exposure. Acute risks were assessed by comparison of oral LD50 values to estimated maximum acute human exposure. It was concluded that, under present and expected conditions of use, Roundup herbicide does not pose a health risk to humans.

* Quoted from article

1. Test materials addressed:

Test item: Roundup®
Active substance(s): Glyphosate (as isopropylamine salt)
Concentration:: 356 g glyphosate free acid equivalent/L (480 g/L salt)

Test item: Glyphosate
Purity: 96 %

Test item: POEA (polyethoxylated tallow amine)

Test item: AMPA (aminomethylphosphonic acid)

2. Studies addressed:

Metabolism and pharmacokinetics: With Glyphosate, AMPA, and Roundup

Glyphosate oral dosage studies in rats

- Absorption;
- Tissue distribution;
- Biotransformation/Excretion;

AMPA single oral dose study in rats

Glyphosate/AMPA oral studies in non-rodents

Glyphosate and Roundup – Dermal penetration

Toxicology studies: With Glyphosate and AMPA

Acute toxicity and irritation studies

Subchronic toxicity studies (glyphosate – mouse, rat, dog; AMPA – rat and dog)

Chronic toxicity/Oncogenicity studies (glyphosate – mouse and rat; AMPA – rat)

Reproduction/Developmental toxicology studies

Toxicology studies: With POEA and Roundup

Acute toxicity and irritation studies

Subchronic toxicity studies (POEA – rat and dog; Roundup – rat)

Reproduction/Developmental toxicology studies

Genetic toxicology studies: With Glyphosate, formulations, and AMPA

Gene mutation studies (bacteria, mammalian cells, *D. melanogaster*)

Chromosomal aberration studies

In vitro sister chromatid exchange

In vivo micronucleus assay

In vivo mutation (dominant lethal test)

DNA damage/reactivity (UDS, rec-assay, DNA adducts, alkaline elution)

Mutation studies with AMPA

Evaluation of potential specific organ/system effects:

Salivary gland changes

Potential for endocrine modulation (*in vitro*; *in vivo*)

Potential for Neurotoxicity

Potential for synergistic interactions

Human experience:

Irritation studies

Occupational exposure

Effects observed after ingestion

Exposure assessment:

Dietary exposure to residues in food (glyphosate, AMPA, and POEA)

Occupational dermal and inhalation exposure during application (AMPA and POEA)

Non-occupational exposure during application

Consumption of water (glyphosate, AMPA, and POEA)

Reentry into treated areas (glyphosate, AMPA, and POEA)

Bystander exposure during application

Possible inadvertent exposures derived from specific activities

Aggregate exposure estimates

Risk characterization: For Glyphosate, AMPA, and POEA

Identification of LD_{50}s, NOAELs

Derivation of the MOE, estimation of risks to Humans from acute or chronic exposure

KLIMISCH EVALUATION

1. Reliability of study: **Not applicable**

Comment: The publication represents an expert consideration on safety evaluation and risk assessment of the compounds, performed according to current scientific principles.

Available toxicity studies are summarized, but the generation of original data is not subject of the present publication.

2. Relevance of study: **Review of major relevance for the safety evaluation of glyphosate.**

3. Klimisch code: **Not applicable**

IIA 5.11 Summary of mammalian toxicity and overall evaluation

Toxicokinetics and metabolism

Absorption, distribution and elimination

The 2001 EU evaluation of glyphosate concluded that following oral administration, glyphosate is rapidly absorbed from the gastrointestinal tract but only to a limited extent of approximately 30 - 40 %. Data on the extent of biliary excretion in bile-cannulated rats, that was not available for the 2001 EU glyphosate evaluation, confirms the systemically available glyphosate is excreted exclusively in the urine ([redacted] 1996). Elimination of ingested glyphosate via faeces and systemic glyphosate via urine is rapid and is nearly complete within 48 hours. The pulmonary route of elimination is negligible (< 0.2%, [redacted] 1995 and [redacted] 1996). Faeces contain unabsorbed glyphosate.

Distribution into the organs and tissues after an oral dose is rapid but limited with generally low residues found in organs and tissues at termination. After a period of 3 to 7 days following oral administration, total body burden accounted for less than 1% of the applied radioactivity. There is no evidence of a potential for accumulation in animals based on residue analysis in organs and tissues after 72h -168 h. Elimination from bone is slower than from other tissues. However, the amount of radiolabel in bone after 168h after a single oral dose was relatively low at 0.02 – 0.03% of the applied dose ([redacted] 1995). The highest residues were measured in bone, followed by kidney and liver. This pattern of absorption, distribution and elimination was not significantly changed either by single high doses administered or by repeated administration of low doses. Similarly, the sex of the test animals did not affect the results. The pattern of distribution of radioactivity in whole-body autoradiograms showed the greatest intensity of radioactivity to be in bone and gastrointestinal tract at up to 24 hours after dosing which was reduced to negligible amounts within 48 hours ([redacted] 1996). Peak plasma levels were observed within 4 – 6 h and elimination from blood and plasma was rapid with no evidence of accumulation in blood cells. A biphasic pattern of elimination of radiolabel in plasma has been suggested from the plasma radiolabel in a range of studies and terminal half lives have been estimated at 8 – 10h. Radiolabel in plasma was negligible after 24 h and not detected at 168 h.

Metabolism

Metabolism of glyphosate is very limited. Most of the parent glyphosate is eliminated unchanged and a small amount, just under 0.5% of the applied dose is eliminated as aminomethylphosphonic acid (AMPA). While glyphosate is known to be the major metabolite of glyphosate in plants, metabolism in mammals has been shown to be very limited.

Acute toxicity

The 2001 EU evaluation of glyphosate concluded that glyphosate acid and its salts exhibit a low acute toxicity in laboratory animals by the oral and dermal route with LD_{50} values greater than 2000 mg/kg bw in previously conducted studies. These results were confirmed in other and new studies recently performed since the last review. Given all LD_{50} values exceed the highest dose tested and differences between the EU and GHS classification criteria, the acute oral and dermal toxicity endpoints should be amended to greater than 5000 mg/kg bw/day.

Glyphosate acid is of low acute inhalation toxicity with LC_{50} values above the limit test dose of 5 mg/L air per 4 hours obtained for the acid and the isopropylammonium salt (IPA). The ammonium salt was tested up to the maximum attaninable concentration of 1.9 mg/L with comparable clinical signs but no deaths occurring. Achieving inhalation doses up to the limit dose of 5 mg/L were often a technical challenge, based on the physic-chemical properties the test materials.

Regarding primary irritation, glyphosate acid and the salts were found to be non-irritant to intact skin and only slightly irritant to abraded skin. Studies conducted since the last EU review have confirmed that glyphosate acid is either non-irritating or only slightly irritating to the skin and that no classification is required.

Glyphosate acid was found to be strongly irritating to rabbit eyes requiring classification; previously as R41 – **'Risk of serious damage to eyes'** and now **'Irreversible effects on the eye/serious damage to eyes (Category 1)'** under GHS. Recently performed studies on the eye irritating potential of glyphosate acid supported the previous findings and classification. There was markedly less eye irritation observed

with the salts which are used in formulated products, presumably due to the salts having a more neutral pH than glyphosate acid.
Glyphosate acid has been tested for skin sensitisation by the stringent Magnusson-Kligman test method and the Buehler test method in guinea pigs. Glyphosate acid has also been tested for its skin sensitising properties in the local lymph node assay in mice. In all study types glyphosate acid was unequivocally negative for skin sensitisation potential. In addition, the IPA salt tested negative for skin sensitisation potential in a Magnusson-Kligman test in guinea pigs.

Short-term toxicity
Sub-acute and sub-chronic toxicity studies note a low oral toxicity of glyphosate and its salts in rats, mice and dogs.

Rodents:
In rats, the previous 2001 EU glyphosate evaluation concluded that the lowest NOEL was about 100 mg/kg bw/day in 90-day feeding studies in rats with the first effects occurring in the range 250-300 mg/kg bw/day, however in most studies higher NOAELs were established. Liver effects were observed indicated by clinical chemistry and organ weight changes in rats. Soft stools and diarrhoea, together with occasionally reduced bodyweight gain and food consumption, suggest irritation of the gastrointestinal tract at high dose levels. In some oral rat studies cellular alterations in salivary glands were observed upon histopathological examination. The glyphosate taskforce believes these salivary gland findings are a non-adverse adaptive response to treatment with a low pH diet. Overall the mouse is less sensitive than the rat with only effects observed on body weight at very high dose levels. Additional studies included in this submission that have not been previously reviewed also demonstrate that glyphosate is of low oral toxicity. The NOAELs ranged between 79-765 mg/kg bw/day and the lowest LOAEL observed in rats was 569 mg/kg bw/day ([redacted] 1995). Consistent with the previously reviewed studies effects were observed on clinical chemistry parameters (often non-specific markes of mild toxicity), bodyweight gain and food consumption at high dose levels. Additionally, caecum distension and an increase in caecum weight were observed in both a 13 week rat study ([redacted] 1995) and a 13 week mouse study ([redacted] 1995) . This effect appeared to be dose related at very high dose levels in both species but was not assocated with any corollary histopathological changes and is therefore of uncertain toxicological relevance. In contrast, in another 13 week dietary rat study ([redacted] 1996) mucosal atrophy of the caecum was observed where there was no associated weight change. Another finding observed in male mice that had been previously reported was cystitis of the urinary bladder in animals dosed at 6295 mg/kg bw/d.
Overall the NOEL/NOAEL levels established in the 90-day dietary studies in rats varied between approximately 80 and 1600 mg/kg bw/d. The lowest effect dose was in the range of 550 mg/kg bw day ([redacted] 1995). Mice appeared to be less sensitive, with substantially higher NOAEL values, the lowest of which was 600 mg/kg bw/d.

Dogs:
In oral sub-chronic toxicity studies in the dog previously evaluated in the 2001 EU glyphosate evaluation, only unspecific signs of toxicity (decrease in body weight gain and food consumption) were observed at high dose levels. In two dietary dog studies performed at the same laboratory, liver effects of equivocal toxicological significance were observed at low doses (8-29 mg/kg bw/d). However, the previous evaluation found that because these findings were not confirmed in more recent studies using much higher dose levels they were not considered to be compound-related. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid and the IPA salt.
This NOAEL is supported by four recently conducted studies ([redacted] 2007, [redacted] 1999, [redacted] 1996 [redacted] 1996). In the [redacted] (1999), [redacted] (1996) and [redacted] (1996) studies only minor effects on bodyweight and clinical chemistry parameters were observed at 1000 mg/kg bw/day. In the [redacted] (2007) study at 1000 mg/kg bw/day the test item administration induced marked clinical signs and mortality leading to the early termination of the group at week 11, however, the 300 mg/kg bw/d was the NOAEL. Overall the lowest NOAEL observed was 253 mg/kg bw/d and the highest was 1000 mg/kg bw/day. The lowest effect level was 1000 mg/kg bw/d in 90 day dog studies.

Oral one year toxicity in the dog was previously evaluated in the 2001 EU glyphosate review. The [redacted] (1991) and and [redacted] (1985) studies have been previously evaluated and like the 90-day studies only non-specific signs of toxicity (slight effect body weight and an increase in clinical signs of soft, liquid stools) were observed at limit dose. The previous review concluded the lowest relevant NOAEL was 300 mg/kg bw/d for glyphosate acid. These previous conclusions were confirmed by 3 additional one year dog studies. Again non-specific effects of toxicity were observed at doses at, or close to limit dose. These effects were characterised as reduction in body weight gain, reduction in urinary pH and minor effects on clinical pathology parameters. The lowest dose level where treatment related effects were observed was 926 mg/kg bw/day in the [redacted] (1996) study. The most relevant one year oral dog NOAEL for glyphosate technical is 500 mg/kg bw/day.

One study on sub-acute inhalative toxicity (14 days) in rodents ([redacted] 1985: non-GLP (pre-GLP) study) was reviewed in the 2001 EU evaluation. The previous review concluded no treatment-related effects were observed and the NOEL was 3.8 mg/L. No further studies have been conducted.

The short-term percutaneous toxicity of glyphosate has been investigated in the rat and rabbit. In both Sprague-Dawley (SD) ([redacted] 1993) and Wistar derived ([redacted] 1996) rats no signs of systemic toxicity were noted following dosing for 21 days at 1000 mg/kg bw/day, the limit dose for this study type. Three studies were conducted in New Zealand White rabbits ([redacted]) and doses ranged from 1000 mg/kg bw/day to 5000 mg/kg bw/day. In both the rat and the rabbit no signs of systemic toxicity were evident following repeated application of glyphosate to the skin. The NOAEL for short term percutaneous toxicity was 1000 mg/kg bw/day in the rat and 5000 mg/kg bw/day in the rabbit as previously concluded inthe 2001 EU glyphosate evaluation.

Genotoxicity

In the 2001 EU evaluation glyphosate was examined for mutagenicity and clastogenicity in a wide range of test systems covering all relevant endpoints *in vitro*. Additional studies have been conducted on glyphosate since the last EU review, however, all these studies were negative and are considered confirmatory data. Glyphosate has clearly been proved to have no genotoxcity potential a wide range of regulatory studies *in vitro*. During the 2001 EU glyphosate evaluation, a number of *in vivo* cytogenicity studies and bone marrow micronucleus tests in rats and mice have been evaluated. The last review concluded that glyphosate is not clastogenic *in vivo*. Since the last review the ability of glyphosate to cause chromosomal aberrations has been further investigated in the *in vivo* micronucleus test ([redacted] 2009b, [redacted] 2007, [redacted] 2008, [redacted] 1999, [redacted] 2006, [redacted] 1996 [redacted] 2008). All new studies were considered negative. Glyphosate has been tested in a wide array of *in vitro* and *in vivo* genotoxicity assays. Overall, in the vast majority of studies performed, glyphosate proved clearly negative and it can be concluded that the active ingredient does not exhibit a genotoxic risk to humans.

Chronic toxicity and Carcinogenicity

The long-term toxicity and carcinogenic potential of glyphosate has been assessed in rats and mice. The 2001 EU glyphosate evaluation concluded that in long-term studies in rats and mice there was no evidence of carcinogenicity. It also concluded that in rats, there was no adverse effects on survival or clinical signs. A reduction in body weight gain, increases in alkaline phosphatase and liver weight changes, an increase in incidence of cataracts, inflammation of the gastric mucosa and histopathological changes in the salivary glands were observed sporadically across the studies previously reviewed. In the mouse the previous 2001 review concluded that non-neoplastic treatment related effects were limited to high dose males in the [redacted] (1983) study and comprised of a reduction in body weight gain, hepatocyte hypertrophy and bladder epithelial hyperplasia.

Five additional long term studies have been conducted in the rat and 3 in the mouse that have not been previously reviewed at the EU level. There was no evidence that glyphosate acid is carcinogenic in any of these studies that have not been previously submitted.

Rat:
A 1-year toxicity study ([redacted] 1996) was performed in rats with dietary doses of 0, 2000, 8000 and 20000 ppm glyphosate acid. Based on body weight and salivary gland effects at 20000 ppm, the NOAEL for toxicity for glyphosate acid was 560 mg/kg bw/day in males and 671 mg/kg bw/day in females.
In 2-year dietary rat study, by [redacted] (1997), rats received diets providing 0, 3000, 10000 or 30000 ppm glyphosate. The NOAEL for toxicity is 104 and 115 mg/kg bw/day for males and females, respectively, based on histopathological and clinical effects of the caecum together with follicular hyperkeratosis and/or folliculitis/follicular abscess in the mid and high dose groups.
In another combined chronic toxicity and carcinogenicity study ([redacted]2001) which was performed with glyphosate technical in rats receiving diets providing 0, 2000, 6000 or 20000 ppm glyphosate acid, the NOAEL was set at 361 and 437 mg/kg bw/day for males and females, respectively. It was based on liver and kidney effects, prostatitis, periodontal inflammation, urinary acidosis and haematuria, which may be attributed to the acidity of the test substance.
The 2 year dietary rat study conducted by [redacted] (1997) concluded that there were no adverse treatment related effects and the NOAEL was 1290/1740 mg/kg bw/day in males and females respectively.
The most recent rat dietary carcinogenicity study was conducted in 2009 by [redacted], again there were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 1230 mg/kg bw/day.
In the previous review salivary glands have been suggested as possible target organ. Histological changes described as "cellular alteration" in the parotid and mandibular salivary glands and a higher organ weight of these glands were noted at 100 mg/kg bw/day and higher ([redacted] 1993). These findings determined the lowest NOAEL in the previous review from the long-term studies. In addition similar changes have been observed in subchronic rat studies. In contrast, there are several chronic studies where no effects on the salivary glands were reported. These differences may be more or less pronounced depending on the rodent strain used or methodological differences.Additional studies were conducted to examine species sensitivity, reversibility of the effects and the hypothesis previously suggested in the WHO/FAO 2004 evaluation of glyphosate; that local irritation of the oral cavity by the organic acid mixed into diet may result in an adaptive salivary gland response (IIA 5.10). Based on the outcome of these examinations the treatment-related pathological findings (increased salivary gland size and flow) can be considered as adaptive responses due to oral irritation from the ingestion of glyphosate acid in the diet. When the salivary glands are viewed in perspective as an adaptive change, the lowest effect level in the long-term rat studies is 354/393 mg/kg bw/day in males and females respectively ([redacted] 1997). Overall the NOEL/NOAEL levels established in the long term studies in rats varied between approximately 31 mg/kg/day (300 ppm in diet, the highest dose tested in this pre-guideline study, considered a supplementary study in the EU monograph) and 1740 mg/kg bw/day.

Mouse:
A combined toxicity and carcinogenicity study in mice ([redacted] 2001, 5.5.3/01) demonstrated a slightly higher mortality in the high dose group. Mortality was within the upper end of the the historical control range. However, treatment with glyphosate might slightly have affected the mortality at the highest dose of 10000 ppm, and because a relationship to treatment was unclear a conservative NOAEL for toxicity at the mid dose of 1000 ppm (150.5 mg/kg bw/day for combined sexes) was set for this study. The number of malignant lymphoma, the most common tumour in the mouse, was slightly elevated in the high dose group compared to control, but this was considered as incidental background variation based on historical control data and was not considered to be related to treatment. However it should be noted that the high dose group received a daily achieved dose of 1460 mg/kg bw/day which is in excess of the limit dose recommended by most current international guidelines.
In the study by [redacted] 1997 the low effect level was 8000 ppm (equivalent to 787 mg/kg bw/day) in females only based on a reduction in body weight gain. At the top dose of 40000 ppm (equivalent to 4348/4116 mg/kg bw/day in males and females respectively) signs of toxicity included loose stools, reduced body weight gain, food consumption and food utilisation, caecum distention and increased absolute and relative caecum weight (without corollary histopathological findings), increased incidence of anal prolapsed consitent with histopathological erosion/ulceration of the anus.

The most recent 80-Week dietary mouse study was conducted by [redacted] 2009. There were no adverse treatment related effects at the highest dose tested. The NOAEL for this study was 810/1081 mg/kg bw/day in males and females respectively.
Overall the lowest effect level observed in the long-term mouse studies was 787 mg/kg bw/day in females in the [redacted] (1997) study and the NOEL/NOAELs ranged from 151 – 1081 mg/kg bw/day.

There was no evidence for a carcinogenic potential of glyphosate noted in any of the studies performed in rats and mice.

Reproductive Toxicity
In the 2001 EU glyphosate evaluation a number of multigeneration studies were reviewed. It was concluded that glyphosate acid did not indicate a specific hazard for reproduction. It concluded that weak effects on the offspring as evidenced by reduced pup weight were confined to high dose levels where compound related effects were observed in the parent animals. Since the last review three additional studies have been conducted. Study summaries are available for these new studies below.
In the first additional study by [redacted] (1997) parental toxicity was evident at doses of 30000 ppm and consisted of reduced body weight, soft stool and distension of the caecum which was consistent with findings in the sub-chronic and chronic rats studies conducted at this laboratory. In this study, effects in offspring consisted mainly of reduced body weight and distension of the caecum at 30000 ppm only.
In the [redacted] (2000) study the only effect of treatment was a reduction in the bodyweight of the F1A pups in the 10000 ppm group (1063/1634 mg/kg bw/day in males and females respectively) with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the mating period. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected by treatment.
In the most modern study by [redacted]. (2007) there was no treatment-related effects on reproductive performance, parents or offspring.
In the previously reviewed study ([redacted] 1992) there were minimal histopathological changes on the salivary glands in parental and offspring animals noted at the highest dose (i.e., 10000 ppm) and to a lower extent at the mid-dose (i.e., 3000 ppm). This observation was also noted in other repeated dose studies with glyphosate but is considered an adaptive response to high dietary doses of glyphosate, which is a strong organic acid, and can therefore cause irritation of the oral cavity leading to increased salivary excretion (see chapter IIA 5.10). Overall the lowest effect level for parental toxicity was 668-771 & 752-841 mg/kg bw/day in males and females respectively based on slightly reduced body weight in F1 males, increased food and water consumption F1 females in the [redacted] (1992) study The relevant parental NOEL/NOAELs ranged from 197-1063 from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females.
There were no effects on reproduction (reproductive performance, fertility, parturition, lactation, sperm parameters and oestrus cycle) noted in any of the dose groups in any of the studies.
The lowest effect level for the offspring was 1063/1634 mg/kg bw/day in males and females respectively based on reduced body weight of first generation pups during lactation. The relevant NOEL/NOAELs for reproductive toxicity ranged from 197-1063 mg/kg bw/day for males and 226-1634 mg/kg bw/day for females.

Developmental Toxicity
The previous 2001 EU glyphosate review concluded that in the rat the lowest relevant NOEL for both maternal and developmental effects was 300 mg/kg bw/day and the lowest effect level was 1000 mg/kg bw/day. The evaluation found there was no evidence of teratogenicity. Two additional tetratogenicity studies have been performed in rats that have not been previously reviewed in the 2001 EU glyphosate evaluation. These studies are considered to be confirmatory data. Overall the lowest effect level for maternal and foetotoxicity was 1000 mg/kg bw/day and the appropriate overall NOAEL was 500 mg/kg bw/day for both the dams and the foetuses based on the [redacted] 1996.

In rabbits the previous 2001 EU glyphosate review concluded that the NOEL for developmental effects was 350 mg/kg bw/day ([redacted] 1980) and that effects on the foetuses were only observed in the presence of marked maternal toxicity. Overall the previous evaluation determined that glyphosate was not

teratogenic in rabbits. Three additional studies have been included in this submission. The results from these studies are consistent with the data that has been previously reviewed, the pattern of maternal toxicity is consistent and effects on the foetuses were only observed in the presence of maternal toxicity.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity in dams consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality. Overall maternal toxicity was observed at dose levels of 150 mg/kg bw/day and above. The highest relevant NOAEL for maternal toxicity was 100 mg/kg bw/day.

Foetotoxicity/developmental toxicity occurred at doses that were above (or rarely at the same dose as) the dose that caused maternal toxicity. Most indications of developmental toxicity were reduced ossifications of skull, phalangeal and sternebral bones, which are typically seen in the litters of pregnant animals that do not eat well and lose weight during pregnancy. The importance of this observation should not be misconstrued to mean that maternal toxicity in those cases was the proximate agent that injured the fetus, but rather that if exposures to the causative agent are kept below the doses that cause maternal toxicity, the developing offspring are protected. The lowest observed effects on the foetuses occurred at 300 mg/kg bw/day and were characterized by delayed ossification and decreased foetal weights ([redacted] 1996). The relevant NOAEL for foetotoxicity is 250 mg/kg bw/day.

A report from an independent source ([redacted] 2011) has claimed that congenital malformations, especially of the cardiovascular system, were caused by glyphosate exposure in this same series of studies. A variety of malformation was reported across the database of glyphosate studies; these included:

- Dilated aorta/narrow pulmonary artery
- Narrow aorta/dilated pulmonary artery
- Interventricular septal defect
- Cardiomegaly
- Single ventricle
- Retro-esophageal right subclavian artery
- Interrupted aorta
- Right subclavian artery arising from aortic arch
- "Seal-shaped" heart

If glyphosate does cause congenital heart defects, it would be anticipated that the prevalence of congenital heart defects would be increased and one would expect the malformation rate to increase with increasing dose until the pregnant does would become intoxicated or the fetuses would die. The malformations occurred at a low incidence across all dose groups; they did not exhibit a positive dose-response; and often clusters of the malformations occurred in the same fetuses.

The incidence of aorticopulmonary septum-related defects in the combined control groups was 1/879 (0.1%); in the combined glyphosate-treated groups the incidence was 12/2250 (0.5%). One half of the malformed fetuses was found in litters exposed to the highest doses (450 and 500 mg/kg/day), which also experienced severe maternal toxicity including maternal deaths, abortions, and weight loss. If these groups are not considered because of the potential confounding factor introduced by maternal health issues, the incidence of the defects is 6/2049 (0.3%). These data show that the overall incidence of aorticopulmonary septum-related defects in offspring from mothers exposed to glyphosate at doses below those that cause severe maternal toxicity is similar to that seen in non-exposed rabbits.

The other prominent cardiovascular malformation is dilated heart. All observations of this finding (among both control and treated groups) occurred in a study conducted in a single laboratory ([redacted] 1993). This study has several weaknesses including a small number of litters available for examination due to low pregnancy rates and maternal deaths in the mid- and high-dose groups. None of the other six studies reported dilated hearts, although there was a single case of cardiomegaly reported in the mid dose group of 100 mg/kg/day in the [redacted] (1993) study (this was considered not to be related to treatment). Neither the criteria used to diagnose dilated heart nor measurements of the hearts were provided, so it is not possible to directly compare the dilated heart findings to the hearts of the more than 2800 fetuses in the other

studies. It is possible that the observation of dilated hearts is due to overly stringent inspection compared to criteria used by other laboratories.

Taken together, overall data regarding potential cardiovascular malformations in the seven rabbit developmental toxicology studies do not support the contention that there is a clear compound related effect on the foetal heart.

Endocrine disruption potential

Glyphosate has been tested in a full battery of regulatory tests, including a number of rat reproduction (multigeneration), rat and rabbit developmental (teratology), one-year dog and lifetime rat studies. Such studies allow for the examination of toxicological effects following repeated exposure of a range of species to glyphosate.

In the reproduction studies, animals were exposed to the compound at all stages of their development; including adults prior to mating, developing animals in utero, offspring to maturity and repeated through a second generation. It is noteworthy that the most recent rat multigeneration study conformed to the 1998 US EPA and the new OECD 416 guidelines, and included a rigorous investigation of endocrine sensitive endpoints. These mammalian studies can be used to evaluate the ability of a test material to cause significant adverse effects through endocrine disruption.

The toxicological profile for glyphosate does not exhibit effects indicating endocrine disruption in mammals. No endocrine effect was noted in reproductive and developmental toxicity studies in rats or rabbits. Likewise, no effects on the endocrine system were observed in subchronic or chronic studies in rat, mouse and dog.

Neurotoxcity

The previous 2001 glyphosate evaluation concluded that there was no evidence of neurotoxicity in acute, subchronic or chronic studies in rodents and dogs. An acute neurotoxicity in rats was performed by [redacted] (1996a) that was not previously reviewed during the 2001 glyphosate evaluation. Administration of glyphosate acid produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 hours after dosing on day 1 in 3/10 females, only, which received 2000 mg/kg. One of these females was subsequently found dead on day 2. Quantitative assessment of landing foot splay, sensory perception, muscle weakness and locomotor activity revealed no changes indicative of neurotoxic potential. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving 2000 mg/kg. The no-observed effect level (NOEL) for neurotoxicity, following single oral administration of glyphosate acid was 2000 mg/kg.

In addition a sub-chronic neurotoxicity study was also performed by [redacted] (1996b). In this study administration of glyphosate acid produced no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. In addition, there were no treatment-related changes in brain weight, length or width. Comprehensive histopathological evaluation of the peripheral and central nervous system revealed no evidence of any changes 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 (equivalent to 1547/1631 mg/kg bw/day in males and females respectively).

Other/special studies

From classical short- and long-term toxicity studies it was indicated that glyphosate possibly affects the salivary glands in rodents, which was described as increased basophilic staining and enlargement of cytoplasm especially in the parotid salivary glands. The toxicological significance of this effect was unexplained but assumed to be due to the low pH of the test substance blended into rodent diet. Therefore, a repeated feeding study in rats with citric acid was performed to evaluate the potential effects of the low pH on the salivary glands. Stastically significant higher parotid salivary gland weights and a stastically significant increase in severity of background cytoplasmic alterations in the parotid salivary glands were observed. In the absence of cytotoxicity and hyperplasia the noted effects were considered as 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 as similarly observed in studies with glyphosate

(Haas, 2010). Furthermore, rat strain differences for this effect was investigated by Allen (1996). Microscopic examination of the salivary glands showed the most pronounced effect occurred in the F344 rat 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. Based on the weight of evidence across the studies presented by the glyphosate taskforce it is proposed that the changes observed in the salivary gland (basophilia of the parotid acinar cells) are a non-adverse adaptive response to treatment with a low pH diet.

Pharmacological activity of the test substance was investigated *in vivo* with rats. No haematological, electrographic or behavioural/functional changes were observed (Wood, 1996). In the same study, *ex vivo* investigations with isolated guinea pig ileum and isolated rat gastrocnemius muscle were performed. Glyphosate technical caused a contractile response similar to that seen with known parasympathomimetic agents on the isolated guinea pig ileum but did not cause any neuromuscular blocking activity on the innervated muscle.
In an immunotoxicity assessment 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.

Regarding the metabolite AMPA, studies conducted to evaluate the acute and sub-chronic toxicity, as well as mutagenicity and teratogenícty, demonstrate that AMPA possesses a lower toxicity than glyphosate.

Acute Reference Dose (ARfD)
Due to the low acute toxicity profile of glyphosate the derivation of an ARfD for glyphosate is not necessary for the following reasons:

- The mechanism of action for glyphosate herbicidal activity in green plants is not relevant to humans.
- Glyphosate is not acutely toxic; it did not produce mortality, overt clinical signs, changes in behaviour or relevant pathological lesions after a single dose up to 1000 mg/kg bw.
- No significant changes in clinical signs, behaviour, body weight and food consumption were observed in repeated-dose toxicity studies during the first few days with doses up to and above 500 mg/kg bw/day.

Acceptable daily Intake (ADI)
In the previous evaluation a chronic study was considered the most appropriate to derive the ADI. Since the rat proved the most sensitive species upon long-term exposure, it was suggested the ADI for glyphosate be based on the chronic toxicity data obtained in rats. The ADI was based on a NOAEL of 31/34 mg/kg bw/day (males/females) derived from a two year rat study. This was the highest dose tested in this study and animals at this dose showed no signs of treatement related toxicity. Since then, further chronic toxicity studies have been performed that indicate the appropriate NOAEL in long-term toxicity studies in rats is appreciably higher. An overview of the NOAELs and LOAELs observed in all chronic rat and mouse studies are presented in the following Table 5.11-1 and Figure 5.11-1 below.

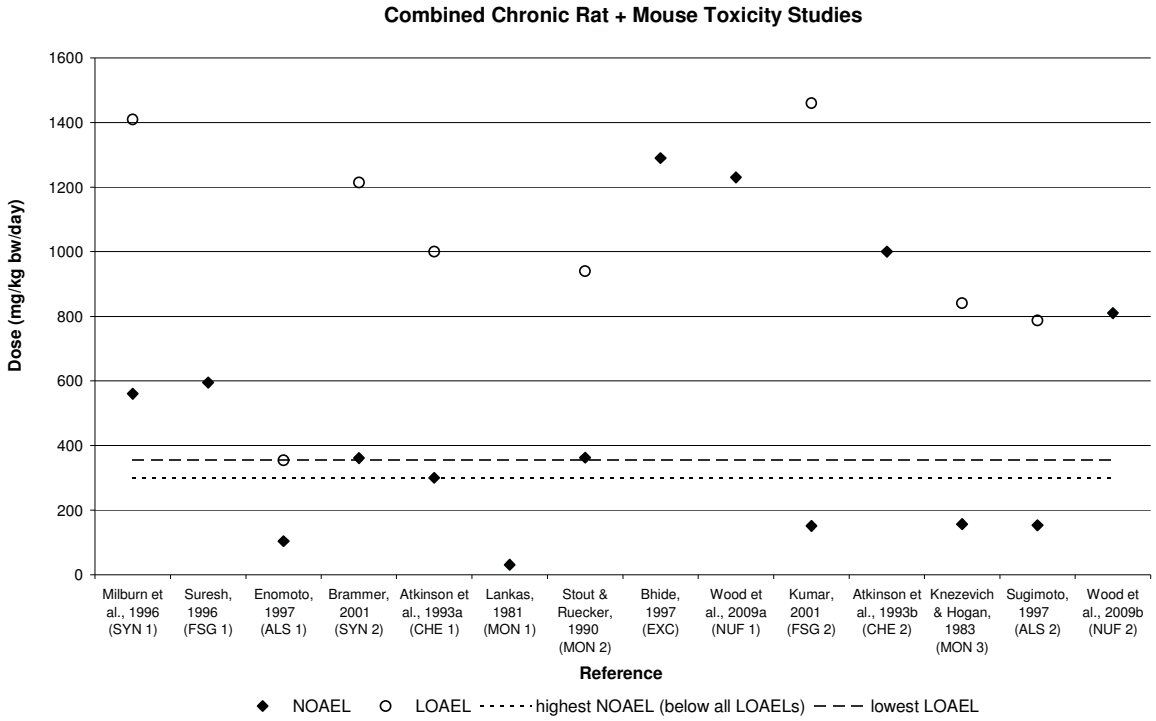
Table 5.11-1: Summary of long-term toxicity and carcinogenicity studies in rats and mice

Reference (Owner**)		Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Study not reviewed in the 2001 evaluation	IIA 5.5.1/01 [redacted] 1996 (SYN 1)	1-year, oral diet Rat, Wistar Alpk: APfSD	♂ 0, 141, 560, 1409 ♀ 0, 167, 671, 1664	560/671 ♂/♀ (1409/1664)	1409/1664 Salivary glands, body weight reduction
Studies from the 2001 evaluation	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/01 [redacted] 1996 (FSG 1)	2-year, oral diet Rat, Wistar	0, 7.4, 74, 741 ♂ 0, 6.3, 59.4, 595 ♀ 0, 8.6, 88.5, 886	595/886 ♂/♀ 741 ♂+♀ (741 ♂+♀)	> 595/886 Only mild effects on clinical chemistry (liver enzymes) without histopathological changes
Studies not reviewed in the 2001 evaluation	IIA 5.5.2/02 [redacted] 1997 (ALS 1)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 104, 354, 1127 ♀ 0, 115, 393, 1247	104/115 (1127/1247)	354/393 Caecum weight increased, distension of caecum, loose stool, follicular hyperkeratosis and/or folliculitis/ follicular abscess, reduced body weight
	[redacted] 1997 (EXC)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 150, 780, 1290 ♀ 0, 210, 1060, 1740	1290/1740 (1290/1740)	> 1290/1740 Only mild toxic effects without histopathological changes
	IIA 5.5.2/03 [redacted] 2001 (SYN 2)	2-year, oral diet Rat, Wistar Alpk: APfSD	♂ 0, 121, 361, 1214 ♀ 0, 145, 437, 1498	361/437 (1214/1498)	1214/1498 Kidney and liver findings. Increased survival due to bw reduction, reduced food consumption, prostatitis, periodontal inflammation
Studies from the 2001 evaluation	Annex B.5.5.1 Glyphosate Monograph IIA 5.5.2/04 [redacted] 1993a (CHE 1)	2-year, oral diet Rat, Sprague-Dawley	0, 10, 100, 300, 1000	300 (1000)	1000 Decreased body weights, decreased urinary pH, salivary glands (histopatho-logy at terminal and interim kill, organ weight ↑ at interim kill); evidence of weak liver toxicity (clinical chemistry AP ↑, organ weight ↓)
	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/05 [redacted] 1981 (MON 1)	26-month, oral diet Rat, Sprague-Dawley	♂ 0, 3, 10, 31 ♀ 0, 3.4, 11, 34	31/34	No treatment-related effects

Reference (Owner**)		Type of study / Species	Dose levels (mg/kg bw/day)	NOAEL (NOAEL)* (mg/kg bw/day)	LOAEL (mg/kg bw/day) Targets / Main effects
Studies from the 2001 evaluation	Annex B.5.5.1.2 Glyphosate Monograph IIA 5.5.2/06 [redacted] 1990 (MON 2)	2-year, oral diet Rat, Sprague-Dawley	♂ 0, 89, 362, 940 ♀ 0, 113, 457, 1183	362/457 (940/1183)	940/1183 Systemic effects: cataracts ♂, reduced body weight in ♀, increased liver weight. Local effects: inflammation of gastric mucosa
Studies not reviewed in the 2001 evaluation	[redacted] 2009a (NUF 1)	2-year, oral diet Rat, Wistar	0, 95, 317, 1230	1230 (1230)	> 1230 No treatment-related effects
	IIA 5.5.3/01 [redacted] 2001 (FSG 2)	18-month, oral diet Mouse, Swiss albino	0, 15, 151, 1460	151 (1460)	1460 Increased mortality
Studies from the 2001 evaluation	Annex B.5.5.2.2 Glyphosate Monograph [redacted] 1993b (CHE 2)	2-year, oral diet Mouse, CD-1	0, 100, 300, 1000	1000 (1000)	> 1000 Not clearly identified
	Annex B.5.5.2.1 Glyphosate Monograph [redacted] 1983 (MON 3)	2-year, oral diet Mouse, CD-1	♂ 0, 157, 814, 4841 ♀ 0, 190, 955, 5874	157/190 (4841/5874)	841/955 Decreased body weight, histological changes in liver and urinary bladder (epithelial hyperplasia)
Studies not reviewed in the 2001 evaluation	[redacted] 1997 (ALS 2)	18-month, oral diet Mouse, ICR	0, 1600, 8000, 40000 ppm	8000 / 1600 ppm (= 838/153 mg/kg bw/day (♂/♀) (4348/4116 (♂/♀))	8000 ppm (≅ 787 mg/kg bw/day) (♀ only): retarded growth 40000 ppm: pale-coloured skin ♂, loose stool, retarded growth, reduced food consumption and food efficiency, caecum distension and increased absolute and relative caecum weight without histopathological findings increased incidence of anal prolapse in , consistent with histopathological erosion/ulcer of the anus
	[redacted] 2009b (NUF 2)	18-month, oral diet Mouse, CD-1	0, 500, 1500, 5000 ppm	810/1081 (♂/♀) 946 (♂+♀) (810/1081 (♂/♀)	No treatment-related effects

* NOAEL for carcinogenicity
** Number refers to the data presented in Figure 5.11-1.
↓ = decreased; ↑ = increased

Figure 5.11-1: NOAELs and LOAELs observed in chronic rat and mouse studies with glyphosate



The 2004 JMPR review of glyphosate established an ADI for glyphosate of 1.0 mg/kg bw/day on the basis of the NOAEL of 100 mg/kg bw/day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. At that time the JMPR review of glyphosate concluded that this treatment-related effect was of unknown toxicological significance. In addition, it has to be noted that in the previous EU glyphosate evaluation the NOAEL for some 2-year rat studies were lower than presented in this review based on salivary gland effects. However, the cellular alterations observed in the salivary glands are considered to be an adaptive response to the acidic diet from glyphosate technical acid and are of no adverse consequence because:

- The effect is observed with other organic acids with a similar pH-dilution curve to glyphosate.
- The effect is only observed following treatment in the diet. The same effect has not been observed across an extensive database following other exposure routes. The ADME radiolabel studies indicate glyphosate does not accumulate in the salivary gland.
- The effect, seen primarily in the rat, is variable in severity and has not been observed consistently across sex, dose or strain.
- From a histopathological perspective across an extensive database, there is no accompanying evidence of cytotoxicity leading to necrosis or apoptosis, no evidence of inflammation or change in function and the cellular alterations do not progress with time to preneoplastic or neoplastic lesions (but in fact decrease in incidence and severity or disappear all together with time).
- The effect is reversible upon cessation of treatment with a low pH diet.

Based on sub-chronic toxicity studies performed in rats and mice, the mouse seems to be the least sensitive species. Due to the dose-spacing chosen in the long-term toxicity studies, the lowest LOAELs observed in mice were equal or greater than 841 mg/kg bw/day, with NOAELs of about 150 mg/kg bw/day and higher. The LOAELs observed in chronic rat studies were lower as compared to mice. Thus the rat is considered the most appropriate species for ADI derivation.

The highest relevant NOAEL observed in chronic toxicity studies is the **NOAEL of 300 mg/kg bw/day** from a 2-year rat study ([redacted] 1993). This value is supported by two other studies in rats ([redacted] 2001; [redacted] 1990) with slightly higher NOAELs of 361 mg/kg bw/day and 362 mg/kg bw/day, respectively.

Applying a safety factor (SF) of 100 the **ADI** is considered to be **3 mg/kg bw/day** (i.e. 300 mg/kg bw/day/ 100 (SF)).

Acceptable Operator exposure Level (AOEL)

In the previous 2001 EU glyphosate evaluation the AOEL based on maternal effects observed in rabbit developmental toxicity studies. The relevant NOAEL was 75 mg/kg bw/day. In addition to the multiple rabbit developmental toxicity studies reviewed in the initial Annex I inclusion of glyphosate, three more developmental toxicity studies in rabbits ([redacted] 1995, [redacted] 1996, and [redacted] 1996) have confirmed that adult rabbits are sensitive to oral gavage dosing with glyphosate.

In rabbits, glyphosate exposure via oral gavage led to clinical signs of toxicity consistent with gastro-intestinal disturbances. Rabbits were more sensitive to oral gavage dosing than other species. Clinical signs observed included diarrhoea/soft faeces, reduced faecal output, reduced body weights, reduced food consumption and increased mortality, all consistent with gastro-intestinal stasis (ileus). Rabbits (caecotrophs) are particularly sensitive to disruption of the gastro-intestinal tract. Stress and other environmental factors such as pain can lead to the normal muscular contractions of the stomach and intestines being greatly diminished which in turn leads to disruption of the normal intestinal/ceacum bacterial flora. It is likely that the mucosal membrane of the rabbit gastro-intestinal tract is irritated by bolus administration of glyphosate acid and that the associated stress or pain leads to gastro-intestinal stasis. The gross necropsy signs observed in maternal animals in the studies by [redacted] (1995), [redacted] (1996) and [redacted] (1996), such as hair like boluses in the stomach, fluid filled large intestines and gas distension in the lower gastrointestinal tract are indicative of gastro-intestinal stasis. The severity of this finding appears to be more relevant to hindgut fermenters as both the rat and the dog appear better adapted to tolerate the irritation potential of an oral bolus dose administration of glyphosate acid.

Further evidence, that these findings are related to gastro-intestinal disturbance comes from the [redacted] (2012) study that measured dermal absorption *in vitro* through rabbit skin. Based on the results of this study, 2.66% of the dermally applied dose in the [redacted] 21-day dermal toxicity study in the rabbit (1982), where there was no evidence of gastro-intestinal tract effects, was systemically available. Thus, the NOAEL for systemic effects after dermal application of 5000 mg/kg bw in the Johnson study was 133 mg/kg bw. Since lower systemic doses resulted in significant gastro-intestinal toxicity in dams from the rabbit developmental toxicity studies, such effects were likely attributable to a route specific toxicity rather than systemic toxicity.

In general the AOEL is derived from the highest dose at which no adverse effects are observed in relevant studies in the most sensitive species. Since operators are normally not exposed over long time periods to plant protection products, relevant studies for the AOEL derivation are sub-chronic (i.e. 90-day) or developmental toxicity studies.

The consistent delayed onset of symptoms in rabbit developmental toxicity studies, suggests that the effects may be due to repeated dosing of a low pH organic acid via oral gavage to the rabbit, causing local irritation of the gastric mucosa. These effects are not representative of glyphosate-related systemic toxicity, but due to gastrointestinal tract (GIT) disturbances caused by a large bolus dose of acidic material.

After dietary administration (e.g. as in the sub-chronic toxicity studies) effects observed at the LOAELs consisted mainly on systemic effects (e.g. changes in clinical chemistry parameters, decrease of urinary pH). Some effects caused by GIT disturbances (e.g. diarrhoea) were also present. However, these effects occurred at higher dose levels as compared to effects observed after gavage dosing in the rabbit.

Regarding the facts outlined above the rabbit developmental toxicity studies are considered inappropriate for derivation of the AOEL, since the observed effects are not representative of systemic toxicity.

This is substantiated by the rationale given in the draft guidance document for AOEL derivation[11]. According to the draft guidance the dependency of the observed toxicity on the exposure route is essential for the determination of the most appropriate study for AOEL setting (see 3.14 of the guidance document).

Therefore, the sub-chronic toxicity studies performed in rodents by dietary administration of glyphosate are used for AOEL derivation, since the observed effect levels based on systemic toxicity.
The relevant NOAELs/LOAELs from 90-day toxicity studies are summarised in Table 5.11-2 and Figure 5.11-2 below.

Table 5.11-2: Relevant NOAELs and main effects for AOEL derivation

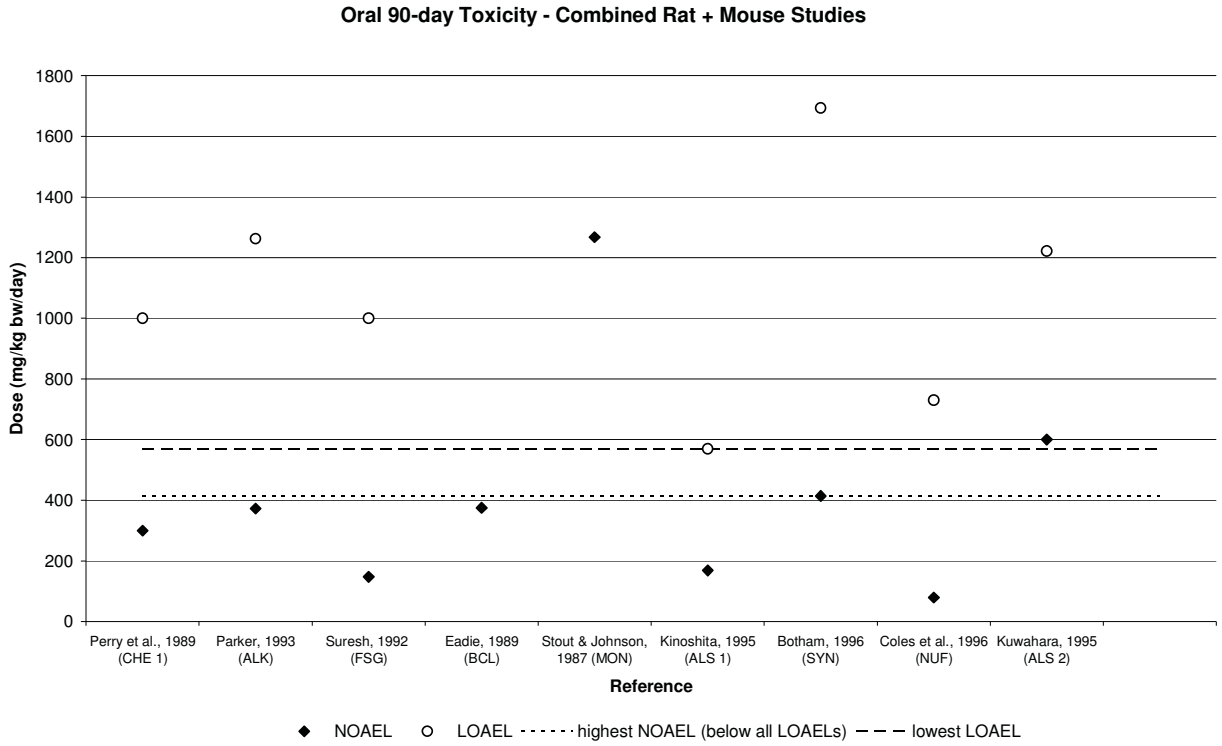
	Reference (data owner)*	Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
Studies from the 2001 evaluation	[redacted] 1989 (CHE 1)	90-day, oral diet Rat, Sprague-Dawley	0, 30, 300, 1000 mg/kg bw/day	NOAEL: 300 mg/kg bw/day	1000 mg/kg bw/day: Clinical chemistry, cellular alterations in paratoid salivary glands, decreased urinary pH (♂ only)
	[redacted] 1993 (Alkaloida, ALK)	13-week, oral diet Rat, Sprague-Dawley	0, 2000, 6000, 20000 ppm (≅ 0, 125.2/156.3, 371.9/481.2, 1262/1686.5 mg/kg bw/day (♂/♀)	NOAEL = 6000 ppm (≅ 379.1/481.2 mg/kg bw/day (♂/♀)	1262/1686.5 mg/kg bw/day (♂/♀) (calculated values): Diarrhea, blood in urine, organ weight changes
	[redacted] 1992 (FSG)	90-day, oral diet (+ 2-week recovery) Rat, Wistar	0, 200, 2000, 20000 ppm	2000 ppm (147/196 mg/kg bw/day (♂/♀))	1000 mg/kg bw/day (estimated value): Clinical chemistry, reduced body weight gain
Studies from the 2001 evaluation	[redacted] 1989 (Barclay, BCL)	13-week, oral diet (+ 5-week recovery) Rat, CD	0, 2000, 3000, 5000, 7500 ppm	NOEL: 7500 ppm (ca. 375 mg/kg bw/day)	No treatment-related effects
	[redacted] 1987 (MON)	90-day, oral diet Rat, Sprague-Dawley	0, 1000, 5000, 20000 ppm	NOEL: 20000 ppm (1267/1623 mg/kg bw/day (♂/♀))	No treatment-related effects
Studies not reviewed in the 2001 evaluation	[redacted] 1995 (ALS 1)	13-week, oral, diet Rat, Sprague-Dawley	0, 3000, 10000, 30000 ppm (≅ 168/195, 569/637, 1262/1686.5 mg/kg bw/day (♂/♀))	NOAEL: 3000 ppm (168/195 mg/kg bw/day ♂/♀)	10000 ppm (≅ 569/637 mg/kg bw/day (1262/1686.5 mg/kg bw/day (♂/♀)): caecum distension; caecum weight increased without histopathological findings 30000 ppm (≅ 1735/1892 mg/kg bw/day (1262/1686.5 mg/kg bw/day (♂/♀)): caecum distension; caecum weight increased without histopathological findings, reduced body weight and lower food efficiency; increased AP activity in ♀
	[redacted], 1996	90-day, oral,	0. 1000, 5000,	NOEL: 5000	1612/1821 mg/kg bw/day:

[11] Working Document – Draft Guidance for the setting and application of acceptable operator exposure levels (AOELs); SANCO 7531 – rev. 10; European Commission, Health & Consumer Protection Directorate-General, 2006-07-07

Reference (data owner)*		Type of study Species	Dose levels	NOEL / NOAEL	Targets / Main effects
	(1st revision of study) (SYN)	diet Rat, Alpk:AP$_f$SD	20000 ppm	ppm (414/447 mg/kg bw/day ♂/♀	Reduced body weight, food consumption and utilisation reduced in ♂ only, clinical chemistry changes (↑ ALP, ALT)
	[redacted] 1996 (NUF)	90-day, oral, diet Rat, Sprague-Dawley	0, 1000, 10000, 50000 ppm (≅ 0, 79/90, 730/844, 3706/4188 mg/kg bw/day (♂/♀))	1000 ppm (79/90 mg/kg bw/day ♂/♀)	730/884 mg/kg bw/day: Clinical chemistry changes, mucosal atrophy of the caecum
	[redacted] 1995 (ALS 2)	13-week, oral, diet Mouse, ICR	0, 5000, 10000, 50000 ppm (≅ 0, 600/765, 1221/1486, 6295/7435 mg/kg bw/day (♂/♀))	NOAEL: 5000 ppm (600/765 mg/kg bw/day ♂/♀)	10000 ppm (1221 / 1486 mg/kg bw/day ♂/♀): caecum distension ♀, increased absolute and relative caecum weight 50000 ppm 6295 / 7435 mg/kg bw/day ♂/♀):: reduced bodyweight and food consumption, decreased food efficiency ♀, haematological changes in ♀, blood chemistry changes, caecum distension and increased absolute and relative caecum weight in both sexes without histopathological changes in the caecum; cystitis of the urinary bladder in ♂
Studies from the 2001 evaluation	[redacted] 1991 (CHE 2)	13-week, oral, diet, Mouse, CD-1	0, 200, 1000, 4500 mg/kg bw/day	NOEL: 4500 mg/kg bw/d	No treatment-related effects

*: Number refers to data presented in Figure 5.11-2.
↓ = decreased; ↑ = increased;

Figure 5.11-2: NOAEL / LOAELs from 90-day toxicity studies in rodents



*The NOAEL of 4500 mg/kg bw/day derived from from [redacted] 1991 (CHE 2) was not considered in this Figure

As can be seen from the graph and table above, the 90-day rat study conducted by [redacted] 1996 (SYN) provides the most sensitive endpoint. The observed **NOAEL was 414 mg/kg bw/day**.

With a safety factor of 100, as well as a correction for 30% oral absorption, the resulting AOEL **is 1.2 mg/kg bw/day**.

Maximum Acceptable Concentration in drinking water (MAC_{DW})

Glyphosate is an organic herbicide and is thus a pesticide as defined by the Drinking Water Directive 98/83/EC. Thus, based on the arbitrarily mandated non-risk based value for single pesticides, the **limit concentration** of glyphosate in drinking water is considered **0.1 µg/L**.

In addition to the regulatory defined limit concentration, the drinking water limit can be scientifically derived using the ADI as starting point. Allowing 10 % of the ADI to be contributed by drinking water and assuming that an adult person of 60 kg bodyweight consumes 2 L water per day, the **MAC_{DW}** is calculated to be **9 mg/L** (i.e. MAC_{DW} = (ADI x 0.1 x 60 kg bw/2 L)).