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-orthophosphate-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* genotoxicity 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 [redacted] 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 [redacted] 1995 (ALS)	Cytogenicity	CHL cells	- S9: 62.5 – 1000 µg/mL (+ S9: 255 – 2000 µg/mL (95.68%)	negative
	IIA 5.4.2/02 [redacted] 1996 (NUF)	Cytogenicity	CHL cells	+/- S9: 0 - 1250 µg/mL (95.3%)	negative
	IIA 5.4.2/03 [redacted] 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	[redacted]	1995	HR-001: In vitro cytogenicity test. [redacted] Data owner: Arysta LifeScience Study No.: [redacted] 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**
   - **Description:** Glyphosate technical
   - **Lot/Batch #:** HR-001
   - **Purity:** 940908-1
   - **Stability of test compound:** 95.68%
   - **Solvent used:** Not mentioned in the report
   - 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 1.0×10^5 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	108
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	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	3.0	0	0.5	50.5	49.0	+
	48	-	0.1	100	2.6	1.0	-	2.5	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	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	[redacted]	1996	Technical glyphosate: Chromosome aberration test in CHL cells in vitro [redacted] Data owner: Nufarm [redacted] 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

Negative/solvent control:	suspended in minimal essential culture media
Positive control:	Mitomycin C ([redacted] 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[redacted] was prepared in-house at [redacted] 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	[redacted]	1996	Glyphosate Acid: *In Vitro* Cytogenetic Assay In Human Lymphocytes [redacted] Data owner: Syngenta Report No.: [redacted]/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: 1995-05-25 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 400, 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. Both donors had a previously established low incidence of chromosomal aberrations in their peripheral blood lymphocytes.
	Chinese hamster ovary (CHO) cells

Media: RPMI-1640 (Dutch modification)				
Properly maintained?	X	Yes		No
Periodically checked for *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 (10mL) 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 ([redacted] ***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 [redacted] ***(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* genotoxicity 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 [redacted] 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 [redacted] 1983 (published MON study by [redacted] 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 [redacted] 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 [redacted] 1994 (FSG)	UDS assay	Primary rat (Sprague-Dawley) hepatocytes	0.13 – 111.69 mM (>98%)	negative
	Annex B.5.4.1.3 Glyphoate Monograph [redacted] 1983 (published MON study by [redacted] 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 [redacted] 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	[redacted]	1998	Glyphosate Acid: L5178Y TK+/- Mouse Lymphoma Gene Mutation Assay [redacted] Data owner: Syngenta Report No.: [redacted]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 1995End: 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	[redacted]	1995b	HR-001: DNA Repair Test (Rec-Assay). [redacted] Data owner: Arysta LifeScience Study No.: [redacted]04-0141 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 $MgCl_2$, 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 (-) Inhibitory zone* (mm) M45	S9 fraction (-) Inhibitory zone* (mm) H17	S9 fraction (-) Difference** (mm)	S9 fraction (+) Inhibitory zone* (mm) M45	S9 fraction (+) Inhibitory zone* (mm) H17	S9 fraction (+) Difference** (mm)
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], 2005). 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* genotoxicity 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 (95.4 %) 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:	[redacted] 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 [redacted] ([redacted] 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	31.46	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	1.2	0.06	0.06	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.: [redacted] 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:	[redacted]
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 [redacted] feeding was discontinued approx. 16 hours befor administration
Water:	Tap water, *ad libitum*
Housing:	Animals were kept in groups of 2 – 4 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.: 3393/2007-3.0[redacted] 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 micronuclei. 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:	[redacted] - SP)
Age:	09 – 10 weeks
Sex:	Male
Diet/Food:	Commercial food ([redacted]), *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 resuspension. 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 3000 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.: [redacted] - 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:	[redacted]
Age:	07 – 12 weeks

Sex:	Male and Female
Weight at dosing:	approx. 30 g
Diet/Food:	Commercial food ([redacted] Brsail, [redacted])
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	12.5**	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 tecnico Nufarm [redacted] Data owner: Nufarm Study No.: [redacted]-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/October/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:	[redacted]
Age:	7-12 weeks
Sex:	Male / Female
Weight at dosing:	30-22 gg
Acclimation period:	Not specified
Diet/Food:	commercial pelleted diet ([redacted])
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: [redacted] 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:** 35 mg/kg	**Route:** oral

Test Animals:

Species	Mouse
Strain	CD-1
Age/weight at dosing	6-7 weeks / 22.8-37.6 g
Source	[redacted] UK.
Housing	Up to 5/cage
Acclimatisation period	At least 5 days
Diet	CT1 (supplied by [redacted] 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	[redacted]
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 ([redacted] ([redacted]). Cover slips were mounted with EUKITT ([redacted]). 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 according 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 5.5-2. 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 1746 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$_f$SD	♂ 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, 88.7, 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$_f$SD	♂ 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.: [redacted]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 glyphosate 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:	[redacted] 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 ([redacted] 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.[redacted] 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:	[redacted] 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 [redacted] ([redacted] 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 masses) 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

Sex	Dose group (ppm) 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	15/29	16/24	16/25	16/32	8/21
Hepatocellular carcinoma	9/20	16/20	9/16	15/29	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.: [redacted]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 80 rats per sex received daily dietary doses of 0, 3000, 10000, and 30000 ppm HR-001 (equivalent to 0, 104, 351 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:	[redacted] Japan, [redacted]
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:	[redacted] 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-oxaracetic 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 (%)

Sex	Dose group (ppm)*			
	0	3000	10000	30000
Male	32/50 (64)	30/50 (60)	33/50 (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 increase 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	116	171	116
Glutamic pyruvic transaminase	Week 52	94	95	148	75	67*	66
Creatinine	Week 26	102	99	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	103	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 dosed 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.: [redacted]1111 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:APfSD)
Source:	[redacted] 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 ([redacted] 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	868	814
Week 79	963	854	993	796	950	813	935	855
Week 105	1015	780	980	783	988	752	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	132.9*	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	159.7	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	1.096	1.43	0.90	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	1.58	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	2.06	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	2.96	3.09	3.02	3.24	3.18	3.13	2.98	3.15
Interim Kill	4.74	4.69	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	62.5	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	43		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:	[redacted] 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:	[redacted] Expanded (Fine Ground) Rat and Mouse Maintenance Diet No. 1 ([redacted]), *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 oncogenicity 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	20/85	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	0.397	0.414	0.408
Week 78/79	0.405	0.382	0.386	0.375	0.415	0.385	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	21.4	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	21.4	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	22.1	22.0	20.8	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	7.1	12.3	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	12.8	11.7	10.0	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:	[redacted] 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 ([redacted]) *ad libitum*. Freshly prepared weekly
Water:	Mains automated water system ([redacted]), *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	10.50	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 test 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.: [redacted] 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:	[redacted] 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:	[redacted] certified Rodent Chow [redacted] ([redacted]), *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	48	34	36

C. CLINICAL OBSERVATIONS

There were no treatment-related clinical signs 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 $p \leq 0.01$ (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.: 1251 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 (parathyroids 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:	[redacted] 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 ([redacted] 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*

Sex	Dose group (ppm)			
	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	19.84	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	26.78	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 1540 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.: 2060-0019 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:	[redacted] 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 [redacted] Ground Diet No.1 [redacted] 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 95.9, 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

* $p < 0.05$; ** $p < 0.01$