LABORATORY of PHARMACOLOGY and TOXICOLOGY

LPT

LPT Report No. 23916

MUTAGENICITY STUDY OF GLYPHOSATE TC IN THE *SALMONELLA TYPHIMURIUM* REVERSE MUTATION ASSAY (*IN VITRO*)

- according to Council Regulation (EC) No. 440/2008 B.13/14, OPPTS Guideline 870.5100 and OECD Guideline 471 -

Sponsor:

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April 30, 2009

This report consists of 34 pages.

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STATEMENT OF COMPLIANCE

MUTAGENICITY STUDY OF
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- according to Council Regulation (EC) No. 440/2008 B.13/14, OPPTS Guideline 870.5100 and OECD Guideline 471 -

The study was performed in compliance with:

- 'Good Laboratory Practice' Regulations of the EC enacted in Germany in the 'Chemikaliengesetz' [Chemicals Act], current edition;
- 'OECD Principles of Good Laboratory Practice' Document Nos. 1 and 13 ENV/MC/CHEM (98) 17, ENV/JM/MONO (2002) 9, respectively.

The following regulations were considered:

- United States Food and Drug Administration Good Laboratory Practice Regulations - 21 Code of Federal Regulations, Part 58, current edition;
- Japanese Guidelines for Non-clinical Studies of Drugs Manual 1995; Guidelines for Toxicity Studies of Drugs. Japanese Ministry of Health and Welfare.

There were no deviations from the 'Good Laboratory Practice' Regulations. Raw data obtained during the performance of the study are accurately reflected.

[redacted]
Study Director

30/04/09
Date

QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the raw data for the study. Methods, procedures and observations are correctly and completely described in the report.

MUTAGENICITY STUDY OF
GLYPHOSATE TC
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REVERSE MUTATION ASSAY (*IN VITRO*)

- according to Council Regulation (EC) No. 440/2008 B.13/14, OPPTS Guideline 870.5100 and OECD Guideline 471 -

Study Plan dated January 28, 2009.

Date of control	Criteria	Date of report to the Study Director and the Management
20 Jan 2009 / 22 Jan 2009	General inspection of mutagenicity studies in the *Salmonella typhimurium* reverse mutation assay: administration of test item, preparation of dilutions for the plate incorporation test, placing of test components onto minimum agar, incubation, evaluation, raw data, SOPs	20 Jan 2009 / 22 Jan 2009
28 Jan 2009	Study Plan	28 Jan 2009
30 Apr 2009	Final Report	30 Apr 2009

Approved and submitted by:

[redacted]

Director of Quality Assurance Unit (QAU)

30.Apr.09

Date

1. SUMMARY

Glyphosate TC was examined in the 5 *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 in two independent experiments, each carried out without and with metabolic activation (a microsomal preparation derived from Aroclor 1254-induced rat liver). The first experiment was carried out as a plate incorporation test and the second as a preincubation test.

Glyphosate TC was dissolved in *aqua ad iniectabilia*.

Preliminary test

Glyphosate TC was examined in a preliminary cytotoxicity test without metabolic activation in test strain TA 100 employing a plate incorporation test. Ten concentrations ranging from 0.316 to 5000 µg/plate were tested. Cytotoxicity (scarce background lawn and/or reduction of the number of revertants by more than 50%) was noted at concentrations of 3160 and 5000 µg/plate.

Hence, 3160 µg/plate were chosen as the top concentration for the main study.

Main study

Five concentrations ranging from 31.6 to 3160 µg/plate were employed in independent experiments each carried out without and with metabolic activation.

Cytotoxicity

Cytotoxicity (scarce background lawn) was noted at the top concentration of 3160 µg/plate in the plate incorporation test and the preincubation test, each carried out without and with metabolic activation in any test strain.

Mutagenicity

No mutagenic effect (no increase in revertant colony numbers as compared with control counts) was observed for Glyphosate TC tested up to a cytotoxic concentration of 3160 µg/plate in any of the 5 test strains in two independent experiments without and with metabolic activation (plate incorporation and preincubation test, respectively).

In conclusion, under the present test conditions Glyphosate TC tested up to a cytotoxic concentration of 3160 µg/plate caused no mutagenic effect in the *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 neither in the plate incorporation test nor in the preincubation test each carried out without and with metabolic activation.

[redacted]

30/04/09

Date

2. GENERAL INFORMATION

2.1 Aim of experiment

The purpose of this study was to evaluate Glyphosate TC for mutagenic activity (gene mutation) in bacteria without and with the addition of a mammalian metabolic activation system as originally described by AMES et al. (1973, 1975) and revised by MARON and AMES (1983).

2.2 Sponsor / Test Facility / Responsible personnel

Sponsor	Helm AG Nordkanalstraße 28 20097 Hamburg Germany
Monitor	[redacted] Phone: [redacted] E-Mail:
Test Facility	**LPT** Laboratory of Pharmacology and Toxicology GmbH & Co. KG Redderweg 8 21147 Hamburg Germany Phone: +49 - 40 - 70 20 20 E-mail: LPT-Hamburg@t-online.de
Study director / Study conduct	[redacted] **LPT**, Redderweg 8 21147 Hamburg, Germany
Deputy study director Management Statistics	[redacted]
Quality Assurance Unit (QAU)	Until January 31, 2009: [redacted] As of February 1, 2009: [redacted]
Code number of the study in the raw data	23916

2.3 Rules and regulations

The study was performed in compliance with:

- OPPTS Guideline 870.5100 Bacterial Reverse Mutation Test, EPA 712-C-98-247, August 1988
- Council Regulation (EC) No. 440/2008 B.13/14: Mutagenicity (*Salmonella typhimurium* - reverse mutation test using bacteria); dated May 31, 2008;
- OECD Guidelines for Testing of Chemicals, No. 471, 'Bacterial Reverse Mutation Test', adopted July 21, 1997;
- ICH Guideline S2A: Genotoxicity: 'Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (CPMP/ICH/141/95)';
- ICH Guideline S2B: Genotoxicity: 'A Standard Battery for Genotoxicity Testing of Pharmaceuticals (CPMP/ICH/174/95)'.

In addition, the 'Good Laboratory Practice' Regulations were considered (see the Statement of Compliance and the enclosed GLP Certificate of the Test Facility **LPT**).

Standard Operating Procedures (SOPs)
: All work was carried out according to Standard Operating Procedures which were followed for all stages of the study; they may be inspected in those divisions which were engaged in the study and in the Quality Assurance Unit (QAU).

Staff safety
: The standard safety precautions operating within the department were applied to this study.

2.4 Archives

Archives of raw data and specimens
: All specimens, raw data and other documents generated at **LPT** during the course of this study, together with a second print of the final report, are stored in the **LPT** archives as required by the German 'Chemikaliengesetz' [Chemicals Act]:

During the course of the study:
in the depot
LPT, Redderweg 8
21147 Hamburg
Germany

	After reporting: written raw data, specimens and a second print of the final report in the Archive 11 **LPT**, Redderweg 8 21147 Hamburg Germany The final report will be archived by the Sponsor.
Duration of storage	According to the periods laid down in the German 'Chemikaliengesetz' [Chemicals Act]; afterwards the Sponsor will decide on further use.

2.5 Study dates

Start of study

Date of Study Plan	January 28, 2009
Start of the experimental phase	February 4, 2009
Period of treatment	February 2009

Study termination

Termination of the experimental phase	February 27, 2009
Date of the final report	April 30, 2009

2.6 Study Plan deviations

The study was conducted in accordance with the Study Plan agreed upon. There were no major deviations from this Study Plan. However, the following minor deviation was noted:

- Personnel change in the head of Quality Assurance Unit:
  until January 31, 2009: Dipl. Biol [redacted]
  as of February 1, 2009: Dr. med. vet. [redacted]

This minor deviation from the Study Plan did not have any effect on the scientific outcome or the validity of the study.

3. TEST ITEM

3.1 Identification of the test item

After receipt at **LPT**, the test item was inspected. Batch number, amount and characteristics (colour, consistency and form) were determined and compared with information given by the Sponsor. An identification sheet was then filed with the raw data.

Test item	Parameter	LPT Identification	Sponsor Identification
Glyphosate TC	colour consistency form	white solid powder	white solid none

No further identification was performed by **LPT** for this study.

3.2 Description

Name	Glyphosate TC
Batch No.	20080801
Sponsor's Sample No.	37/064/08
Active Ingredient(s)/Content	Declared: 95% (w/w) Analysed: 97.52% (w/w) c.f. Manufacturer's Certificate of Analysis attached in Appendix 1 Authenticated: 98.8% (w/w) c.f. Certificate of Analysis issued by Ibacon attached in Appendix 1
Formulation Type	Technical grade active ingredient
Type	Herbicide
Physical State at RT	Solid
Colour	White
Stability (expiry date)	August 1, 2010
Date of production	August 1, 2008
Receipt No.	41639
Date of receipt/Condition at the receipt at the Test Facility	December 22, 2008 The test item was received in proper conditions.
Storage	At room temperature
Safety precautions	Routine laboratory hygienic procedures

Retention sample of the test item	Stored at **LPT** Laboratory of Pharmacology and Toxicology GmbH & Co. KG Archive 11 Redderweg 8 21147 Hamburg Germany

4. METHODS

4.1 Principle

The *Salmonella typhimurium* histidine (his) reversion system is a microbial assay which measures $\underline{his}^-$ → $\underline{his}^+$ reversion induced by chemicals which cause base changes or frameshift mutations in the genome of this organism.

Upon a layer of histidine-free agar (minimum agar), a second layer containing test organisms and test item (top agar) is placed. A trace of histidine in the top agar allows the logarithmic division of the histidine-requiring bacteria in the presence of the test item and any of its metabolites generated by the S9 mix. This period of several generations of auxotrophic cell division is essential for the fixation of pro-mutagenic lesions in the DNA, and results in the formation of a lawn of histidine-requiring bacteria whose further division is prevented by exhaustion of histidine. Only that small fraction of bacteria which has reverted to histidine-independence (either spontaneously or by the action of the test chemical) will continue to divide to form discrete, randomly distributed visible colonies, each one of which consists of the progeny of a single mutant bacterium. The assay determines whether the addition of graded concentrations of the test item to a series of such plates induces a concentration-related increase in mutant colonies compared with plates treated only with the appropriate volume of the solvent.

Two independent experiments were carried out each without and with metabolic activation, each experiment consisted of 3 plates/concentration and strain.

The first experiment was carried out as the standard plate incorporation method whereas the second was carried out as the preincubation method.

4.2 Test strains

Strains	5 strains of *Salmonella typhimurium* (TA 98, TA 100, TA 102, TA 1535, TA 1537)
Concentrations	31.6, 100, 316, 1000 and 3160 µg/plate
Plates	3 per concentration and experiment
Data	2 independent experiments without and with metabolic activation

The following *Salmonella typhimurium* strains were used in this study - obtained from [redacted] -: TA 98 and TA 1537 which primarily respond to frameshift mutagens and TA 100, TA 102 and TA 1535 which respond to base-pair substitution mutagens. In addition to the mutation in the histidine operon, these strains contain several other mutations that greatly increase their ability to detect mutagens.

The growth requirements and the genetic identity of the strains, their sensitivity to UV-radiation and crystal violet and their resistance to ampicillin and tetracycline are regularly checked (see section 4.7). The strains used yield spontaneous revertants within the frequency ranges expected.

For the mutagenicity experiments, frozen permanent copies of the test strains were used.

Strain Designation	Histidine Gene Locus Affected	Additional Mutations			Type of Mutation Detected
		Repair	LPS	Plasmids	
TA 98	his D 3052	uvr B⁻	rfa⁻	pKM 101	Frameshift
TA 100	his G 46	uvr B⁻	rfa⁻	pKM 101	Base-pair substitution
TA 102	his G 428	wild-type	rfa⁻	pKM 101 / pAQ1	Base-pair substitution
TA 1535	his G 46	uvr B⁻	rfa⁻	-	Base-pair substitution
TA 1537	his C 3076	uvr B⁻	rfa⁻	-	Frameshift

rfa⁻: partial loss of lipopolysaccharide (LPS) barrier that causes increased permeability to macromolecules
uvr B⁻: loss of DNA excision repair system
pKM 101: R-factor plasmid, thought to cause an increased error-prone DNA repair
pAQ1: plasmid, carrier of tetracycline resistance

4.3 Dose levels / Solvents / Reference items

Glyphosate TC was dissolved in *aqua ad iniectabilia*[1] shortly before use. The vehicle served as the negative control.

Preliminary to the main test a cytotoxicity test was carried out as a plate incorporation test without metabolic activation using strain TA 100 and the procedure described in 4.6.

Toxicity is evidenced by a reduction in the number of spontaneous revertants, a clearing or diminution of the background lawn or by the degree of survival of the treated cultures. Insolubility could have been assessed as precipitation in the final mixture under the actual test conditions and evident to the unaided eye. The recommended maximum test concentration for soluble non-cytotoxic test items is 5 mg/plate or 5 µL/plate. Test items that are cytotoxic already below 5 mg/plate or 5 µL/plate were tested up to a cytotoxic concentration. The precipitate should not interfere with the scoring.

In the main study 5 different concentrations of the test item were tested, with half-log intervals between plates (31.6, 100, 316, 1000 and 3160 µg/plate).

[1] Batch no. 15810631; DeltaSelect GmbH, 63303 Dreieich, Germany

The following chemicals served as positive control items:

a) without metabolic activation	
sodium azide[2] in H_2O[3] (10 µg/plate)	TA 1535, TA 100
2-nitro-fluorene[4] in DMSO[5] (10 µg/plate)	TA 98
9-amino-acridine[2] in ethanol, abs.[6] (100 µg/plate)	TA 1537
methyl methane sulfonate[7] (MMS) in DMSO[5] (1300 µg/plate)	TA 102
b) with metabolic activation	
2-amino-anthracene[2] in DMSO[5] (2 µg/plate)	TA 98, TA 102, TA 1537
cyclophosphamide[2] in *aqua ad iniectabilia*[3] (1500 µg/plate)	TA 100, TA 1535

The solvent *aqua ad iniectabilia* was used as negative reference item (all test strains).

4.4 Procedure for growing cultures

Test strains in nutrient broth containing 8% dimethyl sulfoxide (DMSO) were kept as frozen permanents in liquid nitrogen. For the mutagenicity experiments, frozen permanent copies of the test strains were thawed at room temperature and then used for inoculating the overnight cultures.

Overnight cultures were grown in a gyrorotary incubator (10 h/37°C) in Oxoid 2[8] nutrient broth. The final cell density was approximately 10^8 - 10^9 cells/mL.

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2 SIGMA-ALDRICH Chemie GmbH, 82024 Taufkirchen, Germany

3 DeltaSelect GmbH, 63303 Dreieich, Germany

4 Riedel de Haën AG, 30926 Seelze, Germany

5 DMSO, spectrometric grade; E. MERCK, 64293 Darmstadt, Germany

6 Ethanol spectrometric grade; E. MERCK, 64293 Darmstadt, Germany

7 E. MERCK, 64293 Darmstadt, Germany

8 Oxoid 2, UNIPATH GmbH, 46467 Wesel, Germany

4.5 Metabolic activation system

Post-mitochondrial fraction (S9 fraction) from rats treated with Aroclor 1254 was prepared according to MARON and AMES (1983). S9 was collected from 20 - 30 rats.

The pooled fraction was tested for:

- protein content, according to LOWRY et al. (1951)
- P-450 content, according to MAZEL (1971)

The protein content of the S9 fraction was 31.55 mg/mL S9, cytochrome P-450: 0.41 nmol/mg protein.

The S9 fraction was stored in liquid nitrogen. The S9 mix was freshly prepared on the day of the test according to MARON and AMES (1983): containing 5% S9 and the following components (per 100 mL):

- 5.0 mL rat liver S9 (Aroclor 1254-induced)
- 2.0 mL 0.4 M $MgCl_2$ + 1.65 M KCl-salt solution (sterile stock solution)
- 141.0 mg glucose-6-phosphate
- 306.5 mg NADP
- 50.0 mL 0.2 M phosphate buffer, pH 7.4 (sterile stock solution)
- sterile *aqua ad iniectabilia* ad 100 mL

Afterwards, the S9 mix was filter-sterilised by using a 0.45 µm filter and then kept on ice.

4.6 Main test procedure

First independent experiment - Plate Incorporation Method

Sterile top agar containing 0.6% agar and 0.5% NaCl was molten on the day of the test. 10 mL of a sterile solution of 0.5 mM L-histidine HCl/0.5 mM biotin were added to 100 mL of molten agar. 2 mL of this top agar were distributed into culture tubes held at 45°C in a heating block. 0.1 mL of *Salmonella* cell suspension (containing approximately 10^8 viable cells in the late exponential or early stationary phase), 0.1 mL of test item solution (or 0.1 mL solvent or 0.1 mL positive control) and 0.5 mL of S9 mix were added to these culture tubes. In the assay without metabolic activation, the S9 mix was substituted with 0.5 mL phosphate buffer mentioned above.

The test components were mixed by vortexing the soft agar for 3 sec at low speed and then poured onto a coded 27.5 mL minimal glucose agar plate (Vogel-Bonner medium E). To achieve a uniform distribution of the top agar on the surface of the plate, the uncovered plate was quickly tilted and rotated and then placed on a level surface with the cover on and finally allowed to harden.

Immediately, the plates were inverted and placed in a dark 37°C incubator for 48 to 72 hours. The revertant colonies on the test plates and on the control plates were counted with a colony counter[9], and the presence of the background lawn on all plates was confirmed. A lawn that was thin compared with the lawn on the negative control plate was evidence of bacterial toxicity.

Routine examination of the background lawn of bacterial growth resulting from the trace of histidine added to the top agar can be an aid in determining the presence of toxic effects. If massive cell death has occurred, the background lawn on the test plates will be sparse compared with control plates.

In this case more histidine is available to the individual surviving bacteria and they undergo more cell divisions, consequently appearing as small colonies which can be mistaken for revertants if the absence of a normal background lawn is not noted.

[9] Biocount 2000, Biosys

Second independent experiment - Preincubation Method

The test item/test solution was preincubated with the test strain (containing approximately 10^8 viable cells in the late exponential or early stationary phase) and sterile buffer or the metabolic activation system (0.5 mL) for 20 minutes at 37°C prior to mixing with the overlay agar and pouring onto the surface of a minimal agar plate. 0.1 mL of the test item solution, 0.1 mL of bacteria, and 0.5 mL of S9 mix or sterile buffer, were mixed with 2 mL of overlay agar. Tubes were aerated during preincubation by using a shaker. The remaining steps were the same as described for the plate incorporation method.

4.7 Quality criteria

The genotypes of the test strains are regularly confirmed in the following way:

a) **Histidine and biotin requirement ((his⁻) (bio⁻)):**

Each of the five strains is streaked onto two Vogel-Bonner medium E plates in the following way:

1) with 0.1 mM L-histidine and 0.5 mM biotin (100 µL/each)
2) with 0.5 mM biotin (100 µL/each)

After incubation at 37°C for 24 hours, none of the strains should grow on plate 2; all strains should show excessive growth on plate 1.

b) **(rfa⁻) deep rough character:**

10 µL of 0.1% crystal violet applied with a paper disc should give zones of inhibition in all test strains after incubation at 37°C for 24 hours.

c) **UV-sensitivity (uvr B⁻):**

Plates are covered partly with black paper and placed under germicidal UV-irradiation. After incubation at 37°C for 24 hours, all strains except TA 102 should grow only under the covered portion of each plate. TA 102 should also grow under the uncovered area.

d) **Ampicillin-resistance (pKM 101):**

0.8 mg ampicillin/plate is placed onto plates seeded with bacteria: Absence of zones of inhibition around the discs indicate resistance to ampicillin (TA 98, TA 100 and TA 102), whereas strains TA 1535 and TA 1537 show zones of inhibition.

e) **Ampicillin- and tetracycline-resistance**

The pAQ1 strain (TA 102) is tested for both ampicillin and tetracycline resistance on ampicillin/tetracycline plates.

4.8 Evaluation

The statistical evaluation of the results of the AMES test is still under discussion. In our laboratory, a test item is considered to show a positive response if

- the number of revertants is significantly increased ($p \leq 0.05$, U-test according to MANN and WHITNEY, see section 6, reference 3) compared with the solvent control to at least 2-fold of the solvent control for TA 98, TA 100 and TA 102 and 3-fold of the solvent control for TA 1535 and TA 1537 in both independent experiments;
- in addition, a significant ($p \leq 0.05$) concentration (log value)-related effect (Spearman's rank correlation coefficient, see section 6, reference 3) is observed;
- positive results have to be reproducible and the histidine independence of the revertants has to be confirmed by streaking random samples on histidine-free agar plates.

The range of spontaneous reversion frequencies in our laboratory are generally

TA 98:	20 - 60
TA 100:	100 - 200
TA 102:	240 - 320
TA 1535:	10 - 35
TA 1537:	3 - 20

The numbers may be slightly different on plates with S9 and may vary slightly from experiment to experiment.

Cytotoxicity is defined as a reduction in the number of colonies by more than 50% compared with the solvent control and/or a scarce background lawn.

5. RESULTS

Preliminary test

Glyphosate TC was examined in a preliminary cytotoxicity test without metabolic activation in test strain TA 100 employing a plate incorporation test. Ten concentrations ranging from 0.316 to 5000 μg/plate were tested. Cytotoxicity (scarce background lawn and/or reduction of the number of revertants by more than 50%) was noted at concentrations of 3160 and 5000 μg/plate.

Hence, 3160 μg/plate were chosen as the top concentration for the main study.

Main study

Five concentrations ranging from 31.6 to 3160 μg/plate were employed in independent experiments each carried out without and with metabolic activation.

Cytotoxicity

Cytotoxicity (scarce background lawn) was noted at the top concentration of 3160 μg/plate in the plate incorporation test and the preincubation test, each carried out without and with metabolic activation in any test strain.

Mutagenicity

No mutagenic effect (no increase in revertant colony numbers as compared with control counts) was observed for Glyphosate TC tested up to a cytotoxic concentration of 3160 μg/plate in any of the 5 test strains in two independent experiments without and with metabolic activation (plate incorporation and preincubation test, respectively).

A summary of the results is given in table 2 and individual values are listed in table 3.

6. REFERENCES

1. AMES, B. N, W. E. DURSTON, E. YAMASAKI and F. D. LEE: Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection.
Proc. Nat. Acad. Sci. USA 70, 2281 - 2285 (1973)

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3. COLQUHOUN, D.: Lectures on Biostatistics, Clarendon Press, Oxford (1971)

4. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL: Protein measurement with the folin phenol reagent.
J. Biol. Chem. 193, 265 - 275 (1951)

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Mutation Res. 113, 173 - 215 (1983)

6. MAZEL, P.: Determination of cytochrome P-450 in Ladu, Mandel and Way (Ed.): Fundamentals of drug metabolism and drug disposition. Williams & Wilkins Co (1971) Baltimore, p. 573 – 574.

7. WEBER, E.: Grundriss der biologischen Statistik, Gustav Fischer Verlag, Stuttgart (1967)

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 1 Preliminary cytotoxicity test

Test item (µg/plate)	Plate incorporation test: Background lawn	Plate incorporation test: Revertants per plate (TA 100) (cytotoxicity)
Glyphosate TC		plate 1 / plate 2
5000	scarce background lawn	60 / 51
3160	scarce background lawn	95 / 106
1000	normal	106 / 144
316	normal	177 / 133
100	normal	168 / 119
31.6	normal	162 / 155
10.0	normal	106 / 138
3.16	normal	149 / 104
1.0	normal	125 / 122
0.316	normal	114 / 146
Solvent control 100 µL/plate	normal	156 / 123

The preliminary test was carried out without metabolic activation

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 2 Summarized data

Test item (µg/plate)		Plate incorporation test *w i t h o u t* metabolic activation Number of reverted colonies TA 98	TA 100	TA 102	TA 1535	TA 1537
				mean values ± SD		
Glyphosate TC						
3160	mean	36.3#	109.0#	259.0#	28.7#	4.3#
	SD	2.1	3.6	3.6	2.5	0.6
1000	mean	30.0	124.0	266.0	30.3	4.7
	SD	1.7	13.1	7.0	0.6	1.5
316	mean	30.7	143.0	259.0	28.7	4.7
	SD	1.5	4.6	3.6	3.2	0.6
100	mean	33.3	143.0	274.0	25.3	6.7
	SD	0.6	12.5	4.0	4.5	1.2
31.6	mean	39.7	126.0	268.3	25.7	6.7
	SD	7.6	7.0	2.5	2.5	1.2
Negative reference item 100 µL/plate						
	mean	42.7	138.7	269.7	30.7	6.7
	SD	11.4	11.7	4.7	4.9	0.6
Positive reference item		2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate		10	10	1300	10	100
	mean	393.7	720.3	1045.0	366.7	364.0
	SD	28.6	31.2	19.2	8.1	21.0

\# scarce background lawn
SD standard deviation
mean (n = 3)

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 2 Summarized data

Test item (µg/plate)		Plate incorporation test *w i t h* metabolic activation Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	26.3 #	107.3 #	256.0 #	28.7 #	4.0 #
	SD	4.0	2.1	4.4	3.2	1.0
1000	mean	32.3	128.7	272.0	32.7	5.7
	SD	11.0	9.5	5.3	3.2	1.5
316	mean	42.0	132.7	274.7	28.3	6.0
	SD	7.0	2.1	5.5	3.8	1.0
100	mean	32.0	134.3	269.7	29.0	7.3
	SD	1.0	7.2	4.5	2.6	0.6
31.6	mean	41.7	150.7	279.3	28.7	5.0
	SD	8.3	32.7	5.7	1.5	1.0
Negative reference item 100 µL/plate						
	mean	42.0	158.3	274.0	30.0	6.3
	SD	2.0	16.0	5.6	3.0	0.6
Positive reference item		2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene
Concentration µg/plate		2	1500	2	1500	2
	mean	387.0	730.7	1159.3	375.0	375.3
	SD	10.5	9.3	31.4	12.5	24.6

\# scarce background lawn
SD standard deviation
mean (n = 3)

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 2 Summarized data

Test item (µg/plate)		Preincubation test *w i t h o u t* metabolic activation Number of reverted colonies TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	36.3 #	140.0 #	264.3 #	19.0 #	5.0 #
	SD	0.6	8.7	5.7	5.6	1.0
1000	mean	43.0	146.0	260.3	24.0	6.0
	SD	6.1	10.6	3.2	5.6	1.0
316	mean	39.0	161.3	278.3	17.0	6.3
	SD	7.0	10.1	5.0	3.6	1.5
100	mean	44.3	145.3	275.0	16.3	6.3
	SD	5.7	24.6	9.0	2.3	1.2
31.6	mean	43.3	150.0	277.7	22.0	5.7
	SD	6.7	6.6	7.0	3.6	2.5
Negative reference item 100 µL/plate						
	mean	40.3	150.0	267.0	18.7	4.3
	SD	6.8	6.2	12.5	6.1	0.6
Positive reference item		2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate		10	10	1300	10	100
	mean	468.7	691.7	1178.3	366.7	463.7
	SD	12.6	26.6	15.3	24.9	18.0

\# scarce background lawn
SD standard deviation
mean (n = 3)

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 2 Summarized data

Test item (µg/plate)		Preincubation test *w i t h* metabolic activation Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	44.7#	161.0#	262.0#	18.0#	5.7#
	SD	0.6	5.3	3.6	2.6	0.6
1000	mean	41.3	157.0	264.7	21.3	7.0
	SD	0.6	20.1	8.6	8.1	1.0
316	mean	51.0	139.7	271.0	21.0	6.7
	SD	11.5	4.0	6.1	6.1	1.2
100	mean	50.7	164.0	268.7	19.7	6.3
	SD	1.5	1.0	2.5	5.5	0.6
31.6	mean	47.7	166.0	281.3	20.0	6.7
	SD	3.1	13.7	8.5	2.0	2.3
Negative reference item 100 µL/plate						
	mean	52.0	152.3	274.3	19.7	6.7
	SD	12.2	9.7	16.9	3.5	0.6
Positive reference item		2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene	Cyclophos-phamide	2-Amino-anthracene
Concentration µg/plate		2	1500	2	1500	2
	mean	482.3	700.7	1160.0	384.0	439.7
	SD	5.0	11.7	29.5	12.1	15.7

\# scarce background lawn
SD standard deviation
mean (n = 3)

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 3 Individual data

Test item (µg/plate)	Plate incorporation test *without* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	38 #	110 #	256 #	31 #	5 #
	34 #	105 #	258 #	29 #	4 #
	37 #	112 #	263 #	26 #	4 #
1000	32	133	269	31	5
	29	130	271	30	3
	29	109	258	30	6
316	31	144	255	25	4
	29	147	260	31	5
	32	138	262	30	5
100	34	133	274	21	6
	33	139	270	30	6
	33	157	278	25	8
31.6	45	133	266	26	8
	31	126	268	23	6
	43	119	271	28	6
Negative reference item					
100 µL/plate	30	126	268	33	7
	46	141	266	34	7
	52	149	275	25	6
Positive reference item	2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate	10	10	1300	10	100
	425	685	1058	368	387
	387	744	1023	374	346
	369	732	1054	358	359

scarce background lawn

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 3 Individual data

Test item (µg/plate)	Plate incorporation test *w i t h* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	30 #	105 #	254 #	31 #	4 #
	27 #	109 #	253 #	30 #	3 #
	22 #	108 #	261 #	25 #	5 #
1000	25	129	266	29	7
	45	138	274	35	6
	27	119	276	34	4
316	50	135	269	30	7
	37	131	280	24	5
	39	132	275	31	6
100	31	126	274	26	8
	32	138	265	31	7
	33	139	270	30	7
31.6	35	113	284	27	4
	51	172	281	30	6
	39	167	273	29	5
Negative reference item					
100 µL/plate	44	159	268	27	7
	42	142	275	33	6
	40	174	279	30	6
Positive reference item	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene
Concentration µg/plate	2	1500	2	1500	2
	388	723	1147	388	388
	376	741	1195	374	391
	397	728	1136	363	347

scarce background lawn

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 3 Individual data

Test item (µg/plate)	Preincubation test *w i t h o u t* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	36 #	146 #	258 #	24 #	5 #
	36 #	144 #	266 #	13 #	4 #
	37 #	130 #	269 #	20 #	6 #
1000	46	134	258	19	7
	47	154	259	23	6
	36	150	264	30	5
316	31	172	273	16	6
	42	152	283	14	8
	44	160	279	21	5
100	38	161	284	15	7
	49	158	275	15	5
	46	117	266	19	7
31.6	51	157	277	26	6
	40	144	271	21	3
	39	149	285	19	8
Negative reference item					
100 µL/plate	38	157	281	24	4
	35	145	257	20	4
	48	148	263	12	5
Positive reference item	2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate	10	10	1300	10	100
	482	685	1175	357	482
	467	721	1195	395	463
	457	669	1165	348	446

scarce background lawn

Mutagenicity study of
Glyphosate TC
in the Salmonella typhimurium reverse mutation assay (in vitro)

TABLE 3 Individual data

Test item (µg/plate)	Preincubation test *w i t h* metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	44 #	163 #	258 #	21 #	6 #
	45 #	165 #	263 #	17 #	5 #
	45 #	155 #	265 #	16 #	6 #
1000	41	176	274	25	7
	41	159	263	12	8
	42	136	257	27	6
316	38	144	275	25	8
	60	136	274	24	6
	55	139	264	14	6
100	49	165	269	14	7
	51	163	271	25	6
	52	164	266	20	6
31.6	51	154	275	18	8
	47	163	278	22	4
	45	181	291	20	8
Negative reference item					
100 µL/plate	38	144	255	23	7
	60	163	286	16	6
	58	150	282	20	7
Positive reference item	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene
Concentration µg/plate	2	1500	2	1500	2
	477	688	1154	386	422
	483	711	1192	371	445
	487	703	1134	395	452

scarce background lawn

APPENDIX 1

Certificates of Analysis

江苏好收成韦恩农化股份有限公司
JIANGSU GOOD HARVEST-WEIEN AGROCHEMICAL CO. LTD.

Certificate of Analysis of Glyphosate Tech.

Name of product: Glyphosate Tech.
Manufacturer:
JIANGSU GOOD HARVEST-WEIEN AGROCHEMICAL CO.,LTD.
Batch No: 20080801
Quantity: 4000g (250g / bag , 16 bags)
Date of production: Aug.1, 2008
Date of Test: Aug.2 , 2008
Date of Expiry: Aug.1, 2010

Parameter	*Value*
Appearance :	*white crystal powder*
Content of glyphosate (g/g) :	*97.52%*
Formaldehyde (g/kg) :	*0.32*
N-Nitrosoglyphosate(mg/kg):	*0.43*
Insolubles in 1M NAOH (g/kg):	*0.11*
Loss on Drying(g/g) :	*0.21%*

JIANGSU GOOD HARVEST-WEIEN AGROCHEMICAL CO.,LTD.
(Stamp or Seal) 江苏好收成韦恩农化股份有限公司 JIANGSU GOOD HARVEST-WEIEN AGROCHEMICAL CO.,LTD.
QUALITY :
(signature)

1/1

GLP Test Facility IBACON GmbH
Arheilger Weg 17
64380 Roßdorf

IBACON
Institut für Biologische Analytik und Consulting IBACON GmbH

Certificate of Analysis

Glyphosate Tech.

IBACON Project Number 48166103

Name of the Test Item:	**Glyphosate Tech.**
Batch No.:	20080801
Sponsor's (HELM AG) Sample No.:	37/064/08
Date of Production:	August 01, 2008
Date of Expiry:	August 01, 2010

Chemical Analysis:

- Identity of the Active Ingredient: The identity of the active ingredient of the test item was established by comparison of the retention time and by comparison of the UV-spectra obtained from solutions of the test item and certified reference material.
- Analysed Content of the Active Ingredient: 98.8 % w/w
  Method of Analysis: IBACON 48166103
- Date of Analysis: January 07 to 08, 2009

Physical Analysis:

- Appearance: Solid, white

Stability:

- Storage Conditions: At room temperature, in the dark (as given by the sponsor)

The product complied with the pertinent FAO Specification 1/TC/S/F 1992.

[redacted]
(Study Director)

March 24, 2009
Date

APPENDIX 2

GLP Certificate of the Test Facility LPT

FREIE UND HANSESTADT HAMBURG

Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz

GLP – Bescheinigung / Statement of GLP Compliance

(gemäß/according to § 19b Abs.1 und Anhang 2 des Chemikaliengesetzes in der Neufassung vom 20. Juni 2002 (BGBl. I S. 2090) in der geltenden Fassung)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP - Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive2004/9/EC at:

[X] Prüfeinrichtung/Test facility [ ] Prüfstandort/ Test site

Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address:

LPT Laboratory of Pharmacology and Toxicology GmbH & Co. KG
Redderweg 8
21147 Hamburg

Prüfungen nach Kategorien/ Areas of Expertise (gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

Kategorie 2, 3, 4 und 9 (Sicherheitspharmakologie und Auftragsarchiv)

Datum der Inspektion/ Date of Inspection:
(Tag.Monat.Jahr/day.month.year)

23., 24. und 25.11.2004

Die/Der genannte Prüfeinrichtung /Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung / diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility/ test site is able to conduct the aforementioned studies in compliance with the Principles of GLP

Hamburg, den 20.4.2007

Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz 33 Hamburg

[redacted]

Amtsleiter