LABORATORY of PHARMACOLOGY and TOXICOLOGY

LPT

LPT Report No. 24880

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

- according to EC directive 2000/32/EC, Method B.13/14 and OECD Guideline 471 and OPPTS guideline 870.5100 -

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January 25, 2010

This report consists of 38 pages.

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STATEMENT OF COMPLIANCE

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

- according to EC directive 2000/32/EC, Method B.13/14
and OECD Guideline 471 and OPPTS guideline 870.5100 -

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

25/Jan/12
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
IN THE *SALMONELLA TYPHIMURIUM*
REVERSE MUTATION ASSAY (*IN VITRO*)

- according to EC directive 2000/32/EC, Method B.13/14
and OECD Guideline 471 and OPPTS guideline 870.5100 -

Study Plan dated October 5, 2009.

Date of control	Criteria	Date of report to the Study Director and the Management
22 Sep 2009 - 24 Sep 2009	General inspection of mutagenicity studies in the *Salmonella typhimurium* reverse mutation assay: raw data of preparation, incubation, evaluation, documentation, SOPs	22 Sep 2009 - 24 Sep 2009
05 Oct 2009	Study Plan	05 Oct 2009
25 Jan 2010	Final Report	25 Jan 2010

Approved and submitted by:

[redacted]

Director of Quality Assurance Unit (QAU)

[redacted] Dipl. Biol. [redacted]

25. Jan 2010
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. Pronounced cytotoxicity (scarce background lawn and/or reduction of the number of revertants by more than 50%) was noted starting at a concentration of 3160 µg/plate. Test item precipitation was noted at the top concentration of 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

In the plate incorporation test and in the preincubation test, each carried out without and with metabolic activation, cytotoxicity (scarce background lawn and reduction of the number of revertants) was noted at the top concentration of 3160 µg/plate in all test strains.

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]

Study Director

25/Jan/10

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@LPT-Hamburg.de
Study director / Study conduct	[redacted] **LPT**, Redderweg 8 21147 Hamburg, Germany
Deputy study director	[redacted]
Management	
Statistics	
Quality Assurance Unit (QAU)	[redacted]
Code number of the study in the raw data	24880

2.3 Rules and regulations

The study was performed in compliance with:

- EC directive 2000/32/EC, L 136/2000 part B.13/14: Methods for the Determination of Toxicity - Mutagenicity (*Salmonella typhimurium* - reverse mutation test using bacteria), dated May 19, 2000;
- 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)';
- OPPTS Guideline 870.5100, August 1998.

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	October 5, 2009
Start of the experimental phase	October 15, 2009
Period of treatment	November 2009

Study termination

Termination of the experimental phase	November 23, 2009
Date of the final report	January 25, 2010

2.6 Study Plan deviations

The study was conducted in accordance with the Study Plan agreed upon. There were no deviations from this Study Plan.

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 none powder

No further identification was performed by **LPT**.

3.2 Description

Name	Glyphosate TC
Batch No.	2009051501
Sponsor's Sample No.	37/206/09
Active Ingredient(s)/Content	Declared: Min. 95% (w/w) Analysed: 95.23% (w/w) (cf. Manufacturer's Certificate of Analysis attached in Appendix 1) Authenticated: 96.4% (w/w) (cf. Certificate of Analysis issued by Springborn Smithers attached in Appendix 1)
Formulation Type	Technical grade active ingredient
Type	Herbicide
Physical State at RT	Powder
Colour	White
Production Date	May 15, 2009
Stability (Expiry Date)	May 15, 2011
Date and No. of Receipt/Condition at the Receipt at the Test Facility	September 18, 2009 (43590) 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 $\underline{his}^{-} \rightarrow \underline{his}^{+}$ 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. A correction factor of 1.05 was used. 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. For non-cytotoxic test items that are not soluble at 5 mg/plate or 5 µL/plate, one or more concentrations tested should be insoluble in the final treatment mixture. 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). The administration volume was 1000 µL/plate.

[1] Batch no. 906833, DeltaSelect GmbH, 63303 Dreieich, Germany

The following chemicals served as positive control items:

a) without metabolic activation	
sodium azide[2] in *aqua ad iniectabilia*[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] (10 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.

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 26.6 mg/mL S9, cytochrome P-450: 0.21 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), 1 mL of test item solution (or 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 - Pre-incubation 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. 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. Pronounced cytotoxicity (scarce background lawn and/or reduction of the number of revertants by more than 50%) was noted starting at a concentration of 3160 µg/plate. Test item precipitation was noted at the top concentration of 5000 µg/plate (see table 1).

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

In the plate incorporation test and in the preincubation test, each carried out without and with metabolic activation, cytotoxicity (scarce background lawn and reduction of the number of revertants) was noted at the top concentration of 3160 µg/plate in all test strains.

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 tables 2 to 5 and individual values are listed in tables 6 to 9.

6. REFERENCES

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Proc. Nat. Acad. Sci. USA 70, 2281 - 2285 (1973)

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4. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL: Protein measurement with the folin phenol reagent.
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7. TABLES

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	Revertants per plate (TA 100) (cytotoxicity)
Glyphosate TC		plate 1 / plate 2
5000	test item precipitation	0 / 0
3160	scarce background lawn	33 / 25
1000	normal	112 / 112
316	normal	113 / 103
100	normal	106 / 112
31.6	normal	122 / 115
10.0	normal	133 / 136
3.16	normal	123 / 105
1.0	normal	127 / 122
0.316	normal	135 / 118
Solvent control 1000 µL/plate	normal	122 / 119

The preliminary test was carried out without metabolic activation

Summarized data

TABLE 2 Plate incorporation test
w i t h o u t metabolic activation

Test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	13.3#	65.3#	226.3#	9.7#	2.3#
	SD	1.5	11.8	6.0	2.1	1.5
1000	mean	31.0	136.3	257.0	19.0	2.7
	SD	4.6	21.2	17.3	3.6	0.6
316	mean	25.0	142.7	271.3	17.0	4.0
	SD	3.6	14.2	8.0	1.7	1.0
100	mean	27.7	148.0	275.7	17.3	4.3
	SD	6.4	7.5	9.3	2.1	1.2
31.6	mean	26.3	151.3	261.3	15.7	4.7
	SD	5.5	25.7	8.1	1.5	1.5
Negative reference item 1000 µL/plate						
	mean	30.7	160.0	270.3	21.7	5.0
	SD	3.2	19.3	3.2	2.5	1.0
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	352.0	894.7	1038.3	247.7	210.3
	SD	52.0	87.9	7.8	64.3	9.5

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

Summarized data

TABLE 3 Plate incorporation test
w i t h metabolic activation

Test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	8.3 #	106.0 #	265.7 #	13.0 #	2.0 #
	SD	2.5	4.0	1.5	1.7	1.0
1000	mean	21.3	136.0	271.0	16.0	5.0
	SD	3.2	18.5	11.8	1.0	1.7
316	mean	24.3	148.7	281.0	18.3	4.0
	SD	5.1	14.4	7.0	3.5	1.0
100	mean	29.3	148.3	274.3	15.7	5.3
	SD	5.8	7.1	14.5	3.5	0.6
31.6	mean	29.3	166.0	267.7	15.7	7.0
	SD	4.2	17.5	12.5	2.1	1.0
Negative reference item 1000 µL/plate						
	mean	31.0	141.7	283.3	19.7	7.0
	SD	8.7	15.9	20.0	2.1	1.0
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	333.3	909.3	1034.0	243.7	212.0
	SD	8.3	69.3	11.1	22.2	5.6

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

Summarized data

TABLE 4 Preincubation test
w i t h o u t metabolic activation

Test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	5.3#	50.7#	75.3#	8.3#	1.3#
	SD	2.5	5.1	14.5	1.5	0.6
1000	mean	30.7	148.3	248.3	20.3	5.0
	SD	4.5	11.2	3.1	3.2	1.7
316	mean	30.0	169.7	275.0	19.7	6.0
	SD	5.3	17.2	12.5	2.1	1.0
100	mean	30.7	151.7	276.0	20.3	5.7
	SD	7.6	24.6	10.1	2.1	1.5
31.6	mean	34.0	139.7	275.0	16.7	5.3
	SD	3.5	4.6	8.9	1.5	1.5
Negative reference item 1000 µL/plate						
	mean	35.0	171.0	282.0	24.0	8.0
	SD	4.0	24.0	24.2	3.6	1.7
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	397.7	934.0	1040.7	303.3	243.7
	SD	24.5	72.0	28.9	29.7	21.2

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

Summarized data

TABLE 5 Preincubation test
w i t h metabolic activation

Test item (µg/plate)		Number of reverted colonies				
		TA 98	TA 100	TA 102	TA 1535	TA 1537
		mean values ± SD				
Glyphosate TC						
3160	mean	6.3#	38.0#	74.7#	8.0#	1.7#
	SD	1.5	3.0	10.0	2.0	0.6
1000	mean	29.3	109.3	250.3	18.0	6.3
	SD	3.5	2.3	2.1	2.6	1.5
316	mean	32.7	120.3	285.0	19.0	7.7
	SD	4.0	8.3	7.0	2.6	0.6
100	mean	35.7	129.3	293.7	17.0	7.3
	SD	2.9	21.4	8.4	2.6	1.5
31.6	mean	35.0	132.0	283.7	20.7	8.3
	SD	3.0	14.0	9.0	1.5	1.5
Negative reference item 1000 µL/plate						
	mean	36.0	136.7	294.7	19.3	9.0
	SD	3.6	8.0	26.1	3.1	1.0
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	427.0	943.3	1078.3	362.3	235.3
	SD	28.5	51.2	28.6	40.1	12.0

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

Individual data

TABLE 6 Plate incorporation test
w i t h o u t metabolic activation

Test item (µg/plate)	Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
		individual counts			
Glyphosate TC					
3160	12 #	79 #	220 #	9 #	2 #
	15 #	59 #	232 #	12 #	4 #
	13 #	58 #	227 #	8 #	1 #
1000	36	117	268	20	3
	30	159	266	22	2
	27	133	237	15	3
316	29	133	279	18	5
	22	159	272	18	3
	24	136	263	15	4
100	35	141	273	18	5
	25	156	268	15	3
	23	147	286	19	5
31.6	21	136	270	16	3
	26	137	260	14	5
	32	181	254	17	6
Negative reference item					
1000 µL/plate	32	168	269	19	4
	33	174	274	22	6
	27	138	268	24	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
	324	987	1032	221	221
	412	885	1047	321	203
	320	812	1036	201	207

scarce background lawn

Individual data

TABLE 7

Plate incorporation test
w i t h metabolic activation

Test item (µg/plate)	Number of reverted colonies TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	6 #	106 #	264 #	15 #	2 #
	8 #	110 #	266 #	12 #	3 #
	11 #	102 #	267 #	12 #	1 #
1000	19	129	284	15	4
	20	157	268	16	7
	25	122	261	17	4
316	23	143	284	22	3
	20	138	286	15	4
	30	165	273	18	5
100	26	142	289	19	6
	26	147	260	12	5
	36	156	274	16	5
31.6	28	184	259	15	6
	26	149	282	14	8
	34	165	262	18	7
Negative reference item 1000 µL/plate	36	155	303	18	7
	36	124	263	22	8
	21	146	284	19	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
	324	987	1032	264	206
	340	887	1046	220	213
	336	854	1024	247	217

\# scarce background lawn

Individual data

TABLE 8 Preincubation test
w i t h o u t metabolic activation

Test item (µg/plate)	Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
		individual counts			
Glyphosate TC					
3160	8 #	45 #	90 #	10 #	2 #
	5 #	52 #	75 #	8 #	1 #
	3 #	55 #	61 #	7 #	1 #
1000	26	144	251	24	3
	35	161	245	18	6
	31	140	249	19	6
316	34	154	289	22	7
	32	167	265	18	6
	24	188	271	19	5
100	36	139	278	21	4
	22	136	285	22	6
	34	180	265	18	7
31.6	32	137	278	17	5
	38	145	282	15	4
	32	137	265	18	7
Negative reference item					
1000 µL/plate	31	147	296	25	10
	39	195	254	20	7
	35	171	296	27	7
Positive reference item	2-Nitro-fluorene	Sodium azide	Methyl-methane sulfonate	Sodium azide	9-Amino-acridine
Concentration µg/plate	10	10	1300	10	100
	423	987	1023	321	263
	396	963	1074	269	221
	374	852	1025	320	247

scarce background lawn

Individual data

TABLE 9 Preincubation test
w i t h metabolic activation

Test item (µg/plate)	Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
	individual counts				
Glyphosate TC					
3160	5 #	35 #	85 #	8 #	2 #
	8 #	38 #	65 #	10 #	2 #
	6 #	41 #	74 #	6 #	1 #
1000	26	108	248	20	8
	29	108	251	19	5
	33	112	252	15	6
316	29	127	282	17	8
	32	111	293	22	7
	37	123	280	18	8
100	39	154	284	14	7
	34	117	299	19	9
	34	117	298	18	6
31.6	32	126	278	19	8
	35	148	279	21	10
	38	122	294	22	7
Negative reference item 1000 µL/plate	35	136	286	20	8
	33	129	324	16	10
	40	145	274	22	9
Positive reference item	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene	Cyclo-phosphamide	2-Amino-anthracene
Concentration µg/plate	2	1500	2	1500	2
	452	987	1047	321	247
	433	887	1103	365	223
	396	956	1085	401	236

scarce background lawn

8. APPENDICES

APPENDIX 1

Certificates of Analysis

红太阳集团有限公司
RED SUN GROUP CORPORATION

CERTIFICATE OF ANALYSIS

Helm Sample No.:	37/206/09	*Helm Product No.:*	P-000481
Product:	Glyphosate TC	*Producer:*	Red Sun Gr. (Büro)
Spec No.:	GB12686-2004	*Recipe No.:*	
Batch No.:	2009051501	*Quantity:*	4000g
Date of Production:	15.05.2009	*Date of Expiry:*	15.05.2011
Date of Analysis:	15.05.2009	*Date of CoA Issuing:*	15.05.2009

Parameter	*Specification*	*Test Result*	*Test Method*
Appearance	White powder	White powder	
A.I	95% Min	95.23%	GB12686-2004
Formaldehyde	0.8g/kg Max	0.68g/kg	GB12686-2004
Insoluble in NaOH solution	0.2 g/kg Max	0.05 g/kg	GB12686-2004
Nitroso-Glyphosate mg/kg	1.0mg/kg Max	0.80mg/kg	GB12686-2004

红太阳集团有限公司
RED SUN GROUP CORPORATION

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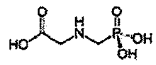
SPRINGBORN SMITHERS LABORATORIES (EUROPE)
Seestrasse 21 · Postfach 82 · CH-9326 Horn
Telephone +41 (0)71 844 69 70 · Fax +41 (0)71 844 86 39
E-Mail: ssre@springbornsmithers.ch · www.springbornsmithers.ch

CERTIFICATE OF ANALYSIS

Name	Glyphosate TC
IUPAC Name	N-(phosphonomethyl)glycine
CAS Registry Number	1071-83-6
Structure formula of active ingredient	
Supplier	Helm AG
Batch Number	2009051501
Declared Content of Active Ingredient	95.23 %
Analysed Content of Active Ingredient	96.4 % (w/w)
Date of Production	2009-05-15
Date of Expiry	2011-05-15
Storage conditions	In the dark at room temperature

Springborn Smithers Laboratories (Europe) Study Reference: 1041.027.898

The content of Glyphosate was above the declared minimum content of 95.0%.

The purity of Glyphosate was determined using a HPLC method:

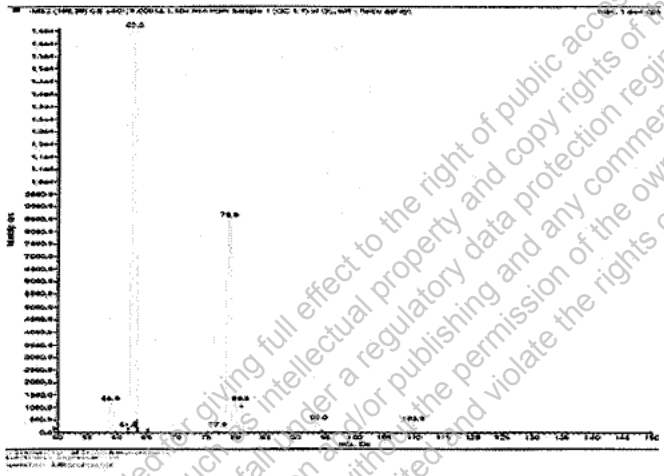
Column:	Phenomenex Intersil Sphere Clone 5 µm SAX, 250 x 4.6 mm
Mobile phase:	KH_2PO_4 + 4% methanol (pH 1.9)
Flow rate:	1.300 mL/minute
Injection volume:	50 µL
Temperature:	Room temperature
Retention time:	approximately 2.8 min
Acquisition time:	15 minutes
Wavelength:	195 nm

Quantification was done against a standard of Glyphosate (Glyphosate Pestanal®, Fluka Buchs Switzerland, Lot No. SZE8014X, Purity 99.7%). The standard used was dissolved before analysis in KH_2PO_4 + 4% methanol (pH 1.9).

Result:

The purity determined for this batch is 96.4%.

The identity of Glyphosate was confirmed by LC mass spectrometry.



Date of Analysis: 17 to 21 September 2009. The study was conducted under GLP.

SPRINGBORN SMITHERS LABORATORIES (EUROPE)

[redacted]

14. 10. 2009
Date

Study Director

APPENDIX 2

GLP Certificate of the Test Facility LPT

FREIE UND HANSESTADT HAMBURG

Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz

Gute Laborpraxis / Good Laboratory Practice

GLP – Bescheinigung / Statement of GLP Compliance

(gemäß/according to § 19b Abs.1 Chemikaliengesetz)

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 Directive 2004/9/ EEC at:

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

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

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

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)

25., 26., 27. und 28.11.2008

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 12.11.2009

Amt für Arbeitsschutz - Arbeitnehmerschutz * 1 * Hamburg

Amtsleiter

Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz
Marckmannstraße 129b, 20539 Hamburg