HARLAN CCR STUDY 1332300

SALMONELLA TYPHIMURIUM AND *ESCHERICHIA COLI* REVERSE MUTATION ASSAY

WITH

Solution of Glyphosate TC spiked with Glyphosine

REPORT

STUDY COMPLETION DATE:
APRIL 07, 2010

1 COPY OF GLP CERTIFICATE

[redacted]

HESSEN

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:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

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

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
6 Prüfungen zur Bestimmung von Rückständen
8 Analytische Prüfungen an biologischen Materialien

2 Toxicity studies
3 Mutagenicity studies
6 Residues
8 Analytical studies on biological materials

15.08. und 27. – 29.10.2008
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

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

The above mentioned test facility 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 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 is able to conduct the aforementioned studies in compliance with the Principles of GLP.

[redacted]

Referent, Wiesbaden, den **30. März 2009**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ 2

Hess. Ministerium für Umwelt, Energie, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority

2 CONTENTS

3 PREFACE

3.1 General

Title:	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine
Sponsor:	Helm AG Nordkanalstrasse 28 20097 Hamburg/Germany
Study Monitor:	Dr
Test Facility:	Harlan Cytotest Cell Research GmbH (Harlan CCR) In den Leppsteinswiesen 19 64380 Rossdorf/Germany
Contracting Institute:	Harlan Laboratories Ltd. 4452 Itingen/Switzerland
Reference No.:	C88226

3.2 Responsibilities

Study Director:	Dipl. Biol.
Deputy Study Director:	Dr.
Management:	Dr.
Head of Quality Assurance Unit:	

3.3 Schedule

Experimental Starting Date:	March 17, 2010
Experimental Completion Date:	March 22, 2010

3.4 Project Staff Signatures

Study Director Dipl. Biol. [redacted]

[redacted] ....

Date: April 07, 2010

3.5 Good Laboratory Practice

The study was performed in compliance with:

“Chemikaliengesetz” (Chemicals Act) of the Federal Republic of Germany, “Anhang 1” (Annex 1), in its currently valid version.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final]

3.6 Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997

"Commission Regulation (EC) No. 440/2008 B13/14", dated May 30, 2008

3.7 Archiving

Harlan CCR will archive:

Raw data, study plan, report, and specimens (if any) for at least 3 years at the test facility's archive. Thereafter, the material will be transferred to the GLP archive of Harlan Laboratories Ltd. in Füllinsdorf, Switzerland for archiving the remaining time up to a total archiving period of 15 years. No data will be discarded without the sponsor's written consent.

A sample of the test item will be archived two years after the expiration date provided by the sponsor. If no expiration date is given, the archiving period will be the required 15 years. Thereafter the samples will be discarded without further notice.

3.8 Deviations to Study Plan

1. Contracting Institute and Reference No. were added.
2. The second experiment was performed before the results of experiment I were available.

These deviations had no detrimental impact on the outcome of this study.

4 STATEMENT OF COMPLIANCE

Harlan CCR Study:	1332300
Test Item:	Solution of Glyphosate TC spiked with Glyphosine
Study Director:	Dipl. Biol [redacted]
Title:	*Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine

This study performed in the test facility of Harlan CCR was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), in its currently valid version.

"OECD Principles of Good Laboratory Practice", as revised in 1997 [C(97)186/Final].

There were no circumstances that may have affected the quality or integrity of the study.

Study Director [redacted]

Dipl. Biol. [redacted]

[redacted]......

Date: April 07, 2010

5 STATEMENT OF QUALITY ASSURANCE UNIT

Harlan CCR Study: 1332300

Test Item: Solution of Glyphosate TC spiked with Glyphosine

Study Director: Dipl. Biol. [redacted]

Title: *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay with Solution of Glyphosate TC spiked with Glyphosine

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were inspected periodically. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below.

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	March 16, 2010	March 16, 2010
Process Inspection Preparation for application:	March 18, 2010	March 18, 2010
Report:	April 06, 2010	April 06, 2010

This statement is to confirm that the present report reflects the raw data.

Head of Quality Assurance Unit [redacted]

[redacted]

Date: April 07, 2010 [redacted]

6 SUMMARY OF RESULTS

This study was performed to investigate the potential of Solution of Glyphosate TC spiked with Glyphosine to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100, and the *Escherichia coli* strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I and II: 3; 10; 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 substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of Glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

6.1 Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Solution of Glyphosate TC spiked with Glyphosine is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

7 OBJECTIVE

7.1 Aims of the Study

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Experiment II was performed as a pre-incubation assay.

7.2 Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria (3). They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The *Salmonella typhimurium* histidine (his) and the *E. coli* tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The *S. typhimurium* and *Escherichia coli* strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 uvrA) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect eight dose levels with adequately spaced intervals were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

8 MATERIALS AND METHODS

8.1 Test Item

Internal Test Item Number: S 11113 11

The test item was prepared at Harlan Laboratories Ltd./Switzerland (Harlan Laboratories Study C88237) by spiking an aliquot of Glyphosate TC (batch 2009051501), supplied by the sponsor, with Glyphosine (batch 1438405; purity 99%), supplied by Sigma Aldrich.

The test item was prepared by weighing a defined amount of Glyphosate TC (batch 2009051501) into a suitable container. Following application of a defined amount of Glyphosine (reference item), it was dissolved in water using a defined amount of water. The resulting test item had a concentration of about 5000 mg Glyphosate/L and approximately 32 mg Glyphosine/L.

The test item was assigned the following data, according to sponsor information:

Description:	An aqueous solution of Glyphosate technical grade active ingredient (purity 97.16 % w/w), containing 0.63% (w/w) Glyphosine in the technical grade active ingredient, corresponding to the maximum proposed limit. Analysed under Harlan Laboratories Ltd. Study C88237 (with GLP, see Annex II)
Sponsor's Sample No.:	37/422/10
Date of preparation:	March 15, 2010 at Harlan Laboratories, Ltd.
Expiry Date:	Date of Preparation + 2 weeks
Storage Conditions:	Room temperature, light protected

Identification of Test Item Components:

Identification:	Glyphosate TC
Active Substance:	Glyphosate
Batch No.:	2009051501
Purity:	95.23% w/w (cf. Manufacturer's Certificate of Analysis attached in Annex II)
Production Date:	May 15, 2009
Expiry Date:	May 15, 2011

Identification:	Glyphosine
Chemical Name:	N,N-bis(phosphonomethyl)glycine
Batch Number:	1438405
Purity:	99% w/w (cf. Manufacturer's Certificate of Analysis attached in Annex II)
Expiry Date:	February 15, 2011

The test item was used at the maximum possible dose and further dilutions were prepared in deionised water.

The test item precipitated in the overlay agar on the incubated agar plates at 2500 µg/plate and 5000 µg/plate in experiment II with S9 mix. The undissolved particles had no influence on the data recording.

8.2 Controls

8.2.1 Negative Controls

Concurrent untreated and solvent controls were performed.

8.2.2 Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99%
Dissolved in:	deionised water
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9%
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99 %)
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strain:	WP2 uvrA
Name:	methyl methane sulfonate, MMS
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0%
Dissolved in:	deionised water
Concentration:	3 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5%
Dissolved in:	DMSO (MERCK, D-64293 Darmstadt; purity > 99%)
Concentration:	2.5 µg/plate (TA 1535, TA 1537, TA 98, TA 100), 10 µg/plate (WP2 uvrA)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range is sufficient evidence of biological stability.

8.3 Test System

8.3.1 Characterisation of the *Salmonella typhimurium* Strains and *E. coli* Strain

The histidine dependent strains are derived from *S. typhimurium* strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker (6).

Strain WP2 (4) and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the uvrA derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarised the mutations of the TA strains and the *E. coli* strain, used in this study can be described as follows:

	Salmonella typhimurium	
Strains	**Genotype**	**Type of mutations indicated**
TA 1537	his C 3076; rfa^-; $uvrB^-$	frame shift mutations
TA 98	his D 3052; rfa^-; $uvrB^-$; R-factor	" "
TA 1535	his G 46; rfa^-; $uvrB^-$	base-pair substitutions
TA 100	his G 46; rfa^-; $uvrB^-$; R-factor	" "
	Escherichia coli	
WP2 uvrA	trp^-; $uvrA^-$	base-pair substitutions and others

Regular checking of the properties of the strains regarding the membrane permeability and ampicillin resistance as well as spontaneous mutation rates is performed in Harlan CCR according to B. Ames et al. (1) and D. Maron and B. Ames (6). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

8.3.2 Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

8.3.3 Precultures

From the thawed ampoules of the strains 0.5 mL suspension was transferred into 250 mL Erlenmeyer flasks containing 20 mL nutrient medium. A solution of 20 µL ampicillin (25 µg/mL) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37° C. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8-10^9 cells/mL).

8.3.4 Selective Agar

The plates with the selective agar were obtained from [redacted], D-64293 Darmstadt.

8.3.5 Overlay Agar

The overlay agar contains per 0.5 litre:

for *Salmonella* strains:	for *Escherichia coli*:
6.0 g Agar Agar*	6.0 g Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-Histidin×HCl×H_2O*	2.5 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, D-64293 Darmstadt)

Sterilisations will be performed at 121° C in an autoclave.

8.4 Mammalian Microsomal Fraction S9 Mix

The bacteria used in this assay do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

8.4.1 S9 (Preparation by Harlan C C R)

Phenobarbital/β-Naphthoflavone induced rat liver S9 is used as the metabolic activation system. The S9 is prepared from 8 - 12 weeks old male Wistar rats (Hsd Cpb: WU, Harlan Laboratories GmbH, 33178 Borchen, Germany), weight approx. 220 - 320 g induced by applications of 80 mg/kg b.w. Phenobarbital i.p. (Desitin; D-22335 Hamburg) and β-Naphthoflavone p.o. (Aldrich, D-89555 Steinheim) each on three consecutive days. The livers are prepared 24 hours after the last treatment. The S9 fractions are produced by dilution of the liver homogenate with a KCl solution (1+3) followed by centrifugation at 9000 g. Aliquots of the supernatant are frozen and stored in ampoules at -80°C. Small numbers of the ampoules can be kept at -20°C for up to one week. Each batch of S9 mix is routinely tested with 2-aminoanthracene as well as benzo(a)pyrene.

The protein concentration in the S9 preparation was 34.3 mg/mL (lot no. R 220110) in both experiments.

8.4.2 S9 Mix

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
4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(1).

8.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with strains TA 1535, TA 1537, TA 98, TA 100, and WP2 uvrA. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).

Toxicity of the test item results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

The pre-experiment is reported as main experiment I, if the following criteria are met:

Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

8.6 Dose Selection

Since the second experiment was performed before the results of experiment I were available both experiments were performed with the following concentrations:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

8.7 Experimental Performance

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:

1000	µL	Test solution at each dose level (solvent 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),
1000	µL	Overlay agar

In the pre-incubation assay 1000 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 1.0 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 (2).

8.8 Data Recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB9 7BN, UK) with the software program Ames Study Manager. The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to precipitation of the test item and air bubbles the revertant colonies were partly counted manually.

8.9 Acceptability of the Assay

The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce a significant increase in mutant colony frequencies

8.10 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 uvrA) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed (3).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (2).

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.

8.11 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

9 DISCUSSION OF RESULTS

The test item Solution of Glyphosate TC spiked with Glyphosine 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* strain WP2 uvrA.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I and II: 3; 10; 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 S9 mix in both experiments.

No toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in the test groups with and without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Solution of Glyphosate TC spiked with Glyphosine at any dose level, neither in the presence nor absence of metabolic activation (S9 mix).

Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

10 REFERENCES

1. Ames, B.N., J. McCann, and E. Yamasaki (1977)
   Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
   In: B.J. Kilbey et al. (Eds.)"Handbook of Mutagenicity Test Procedures" Elsevier, Amsterdam, 1-17

2. de Serres F.J. and M.D. Shelby (1979)
   Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
   Mutation Res. 64, 159-165

3. Hollstein,M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
   Short-term tests for carcinogens and mutagens
   Mutation Res. 65, 133-226

4. Green, M.H.L. and W.J. Muriel (1976)
   Mutagen Testing Using TRP^+ Reversion in Escherichia Coli
   Mutation. Res. 38, 3- 32

5. Maron D.M., J. Katzenellenbogen and B.N. Ames (1981)
   Compatibility of organic solvents with the Salmonella/Microsome Test
   Mutation Res. 88, 343-350

6. Maron D.M., Ames, B.N. (1983)
   Revised methods for the Salmonella mutagenicity test
   Mutation Res. 113, 173-215

11 DISTRIBUTION OF THE REPORT

Sponsor	3 × (2 x copy, 1 x electronic copy (pdf-file))
Study Director	1 × (original)

12 SUMMARY OF RESULTS

12.1 Summary of Results Pre-Experiment and Experiment I

Study Name: 1332300
Experiment: 1332300 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 17/03/2010
Date Counted: 22/03/2010

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without	Deionised water		16 ± 3	10 ± 3	33 ± 5	132 ± 9	61 ± 8
Activation	Untreated		14 ± 2	14 ± 1	36 ± 7	135 ± 8	58 ± 8
	Solution of	3 µg	14 ± 4	10 ± 3	34 ± 5	131 ± 6	57 ± 10
	Glyphosate TC	10 µg	14 ± 5	9 ± 3	31 ± 3	144 ± 17	65 ± 4
	spiked with	33 µg	15 ± 2	8 ± 1	33 ± 5	149 ± 6	59 ± 7
	Glyphosine	100 µg	17 ± 5	9 ± 1	35 ± 2	146 ± 17	69 ± 10
		333 µg	11 ± 3	11 ± 4	31 ± 3	131 ± 20	59 ± 9
		1000 µg	13 ± 4	13 ± 1	32 ± 9	140 ± 8	68 ± 1
		2500 µg	15 ± 1	8 ± 1	34 ± 6	129 ± 14	47 ± 7
		5000 µg	12 ± 0	9 ± 4	26 ± 2	98 ± 16	35 ± 7
	NaN3	10 µg	1926 ± 45			1886 ± 60	
	4-NOPD	10 µg			369 ± 20		
	4-NOPD	50 µg		105 ± 15			
	MMS	3.0 µL					1057 ± 57
With	Deionised water		17 ± 2	20 ± 6	44 ± 4	154 ± 14	72 ± 8
Activation	Untreated		21 ± 1	15 ± 2	41 ± 5	153 ± 7	70 ± 12
	Solution of	3 µg	21 ± 5	20 ± 4	47 ± 3	164 ± 10	70 ± 11
	Glyphosate TC	10 µg	15 ± 3	17 ± 3	39 ± 7	158 ± 9	72 ± 9
	spiked with	33 µg	16 ± 5	21 ± 2	45 ± 5	165 ± 8	70 ± 3
	Glyphosine	100 µg	19 ± 5	17 ± 2	43 ± 2	162 ± 9	75 ± 5
		333 µg	17 ± 2	20 ± 1	43 ± 7	166 ± 4	76 ± 5
		1000 µg	14 ± 1	14 ± 5	41 ± 7	169 ± 5	65 ± 4
		2500 µg	14 ± 1	11 ± 2	50 ± 1	155 ± 5	65 ± 8
		5000 µg	15 ± 4	11 ± 3	40 ± 6	92 ± 18	43 ± 15
	2-AA	2.5 µg	342 ± 8	437 ± 7	2072 ± 40	3249 ± 170	
	2-AA	10.0 µg					350 ± 13

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

12.2 Summary of Results Experiment II

Study Name: 1332300
Experiment: 1332300 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 18/03/2010
Date Counted: 22/03/2010

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without	Deionised water		17 ± 3	12 ± 3	34 ± 4	167 ± 14	58 ± 3
Activation	Untreated		16 ± 4	11 ± 3	30 ± 9	155 ± 9	54 ± 7
	Solution of	3 µg	17 ± 3	14 ± 2	35 ± 9	161 ± 10	53 ± 3
	Glyphosate TC	10 µg	18 ± 3	14 ± 4	34 ± 4	155 ± 17	55 ± 1
	spiked with	33 µg	18 ± 3	12 ± 3	36 ± 12	168 ± 19	59 ± 12
	Glyphosine	100 µg	18 ± 3	11 ± 3	35 ± 4	150 ± 2	55 ± 2
		333 µg	18 ± 3	12 ± 3	29 ± 1	170 ± 11	59 ± 8
		1000 µg	15 ± 2	14 ± 2	32 ± 3	157 ± 12	56 ± 9
		2500 µg	12 ± 4	15 ± 2	29 ± 4	138 ± 11	52 ± 7
		5000 µg	12 ± 2	10 ± 5	20 ± 6	129 ± 9	34 ± 3
	NaN3	10 µg	1688 ± 150			1985 ± 99	
	4-NOPD	10 µg			453 ± 10		
	4-NOPD	50 µg		123 ± 11			
	MMS	3.0 µL					691 ± 67
With	Deionised water		21 ± 1	18 ± 5	47 ± 3	166 ± 9	67 ± 8
Activation	Untreated		21 ± 3	16 ± 1	46 ± 4	169 ± 12	67 ± 8
	Solution of	3 µg	20 ± 4	18 ± 2	44 ± 7	171 ± 22	66 ± 3
	Glyphosate TC	10 µg	18 ± 4	19 ± 4	45 ± 8	157 ± 12	73 ± 12
	spiked with	33 µg	20 ± 4	21 ± 2	44 ± 3	168 ± 15	69 ± 9
	Glyphosine	100 µg	23 ± 3	20 ± 1	45 ± 4	175 ± 9	66 ± 12
		333 µg	18 ± 3	18 ± 5 U M	43 ± 10	171 ± 17	63 ± 13 U
		1000 µg	23 ± 2	18 ± 4 U M	49 ± 6	165 ± 2	64 ± 6 U
		2500 µg	24 ± 1 P	12 ± 2 U M P	45 ± 6 P	149 ± 12 P	59 ± 7 P U
		5000 µg	22 ± 2 P	11 ± 1 U M P	50 ± 1 P	100 ± 12 P	62 ± 5 P U
	2-AA	2.5 µg	431 ± 16	547 ± 31	2686 ± 742	3992 ± 38	
	2-AA	10.0 µg					378 ± 42

Key to Positive Controls	
NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes	
P	Precipitate
U	Air bubbles
M	Manual count

13 HISTORICAL CONTROL DATA

These data represent the laboratory´s historical control data from January 2009 until December 2009 representing approx. 550 experiments (WP2 uvrA the historical data are based on approx. 300 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	16	3.37	8	38	19	4.37	10	41
	Untreated control	15	3.41	7	36	18	4.69	8	55
	Positive control	1886	242.09	663	2690	304	154.47	134	2404
TA1537	Solvent control	12	2.84	6	27	15	3.59	7	33
	Untreated control	12	3.23	5	27	16	3.92	7	31
	Positive control	101	28.30	58	440	227	67.24	68	498
TA 98	Solvent control	30	5.26	15	52	38	6.58	16	59
	Untreated control	31	5.67	14	59	39	6.91	16	84
	Positive control	407	98.13	216	897	1586	454.52	198	3309
TA 100	Solvent control	132	23.21	94	218	144	25.42	94	241
	Untreated control	142	21.88	85	226	154	25.80	94	239
	Positive control	1954	426.94	563	2844	2032	569.62	594	3724
WP2uvrA	Solvent control	52	8.11	33	76	61	8.66	34	82
	Untreated control	53	8.10	34	80	62	8.80	32	87
	Positive control	808	434.27	168	2528	332	154.50	175	1718

Mean = mean value of revertants/plate
SD = standard deviation Min = minimal value/Max = maximal value

14 ANNEX I: TABLES OF RESULTS (8 PAGES)

Pre-Experiment and Experiment I: 1332300 VV Plate Incorporation (4 pages)

Experiment II: 1332300 HV2 Pre-Incubation (4 pages)

Study Name: 1332300
Experiment: 1332300 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 17/03/2010
Date Counted: 22/03/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Solution of**	3 µg	*14.3*	*3.8*	*0.9*	10, 16, 17
	Glyphosate TC	10 µg	*14.0*	*5.0*	*0.9*	19, 9, 14
	spiked with	33 µg	*15.3*	*2.1*	*1.0*	16, 17, 13
	Glyphosine	100 µg	*17.3*	*4.5*	*1.1*	17, 22, 13
		333 µg	*11.0*	*2.6*	*0.7*	9, 10, 14
		1000 µg	*12.7*	*3.5*	*0.8*	13, 9, 16
		2500 µg	*15.3*	*1.2*	*1.0*	16, 14, 16
		5000 µg	*12.0*	*0.0*	*0.8*	12, 12, 12
	Deionised water		*15.7*	*3.1*		15, 13, 19
	Untreated Control		*14.0*	*2.0*		16, 12, 14
TA 1537	**Solution of**	3 µg	*10.3*	*2.5*	*1.1*	8, 13, 10
	Glyphosate TC	10 µg	*9.0*	*3.0*	*0.9*	12, 6, 9
	spiked with	33 µg	*8.3*	*0.6*	*0.9*	8, 9, 8
	Glyphosine	100 µg	*8.7*	*1.2*	*0.9*	8, 8, 10
		333 µg	*11.3*	*4.2*	*1.2*	8, 16, 10
		1000 µg	*13.3*	*0.6*	*1.4*	13, 13, 14
		2500 µg	*8.3*	*0.6*	*0.9*	9, 8, 8
		5000 µg	*9.3*	*4.0*	*1.0*	14, 7, 7
	Deionised water		*9.7*	*3.1*		9, 7, 13
	Untreated Control		*14.0*	*1.0*		14, 15, 13
TA 98	**Solution of**	3 µg	*34.3*	*4.6*	*1.0*	37, 37, 29
	Glyphosate TC	10 µg	*30.7*	*2.9*	*0.9*	29, 29, 34
	spiked with	33 µg	*32.7*	*4.7*	*1.0*	38, 29, 31
	Glyphosine	100 µg	*34.7*	*2.1*	*1.0*	37, 34, 33
		333 µg	*31.0*	*2.6*	*0.9*	34, 29, 30
		1000 µg	*32.0*	*8.5*	*1.0*	23, 40, 33
		2500 µg	*34.3*	*6.1*	*1.0*	29, 41, 33
		5000 µg	*26.0*	*2.0*	*0.8*	24, 28, 26
	Deionised water		*33.3*	*4.6*		28, 36, 36
	Untreated Control		*35.7*	*7.4*		44, 33, 30

Study Name: 1332300
Experiment: 1332300 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 17/03/2010
Date Counted: 22/03/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Solution of**	3 µg	*131.3*	*6.0*	*1.0*	132, 125, 137
	Glyphosate TC	10 µg	*144.3*	*17.2*	*1.1*	129, 141, 163
	spiked with	33 µg	*148.7*	*6.0*	*1.1*	155, 143, 148
	Glyphosine	100 µg	*145.7*	*17.2*	*1.1*	143, 164, 130
		333 µg	*131.0*	*19.9*	*1.0*	142, 143, 108
		1000 µg	*140.3*	*7.6*	*1.1*	149, 137, 135
		2500 µg	*128.7*	*13.6*	*1.0*	116, 143, 127
		5000 µg	*98.3*	*16.0*	*0.7*	115, 97, 83
	Deionised water		*132.3*	*8.5*		142, 129, 126
	Untreated Control		*135.3*	*8.1*		128, 134, 144
WP2 uvrA	**Solution of**	3 µg	*57.0*	*10.4*	*0.9*	51, 69, 51
	Glyphosate TC	10 µg	*65.0*	*3.6*	*1.1*	69, 64, 62
	spiked with	33 µg	*59.3*	*7.2*	*1.0*	64, 51, 63
	Glyphosine	100 µg	*69.0*	*9.6*	*1.1*	65, 80, 62
		333 µg	*59.0*	*8.7*	*1.0*	49, 63, 65
		1000 µg	*68.3*	*1.2*	*1.1*	69, 69, 67
		2500 µg	*47.0*	*7.0*	*0.8*	54, 47, 40
		5000 µg	*34.7*	*7.0*	*0.6*	34, 28, 42
	Deionised water		*61.0*	*7.9*		58, 70, 55
	Untreated Control		*58.3*	*8.1*		49, 64, 62
TA 1535	**NaN3**	10 µg	*1926.0*	*44.9*	*122.9*	1976, 1913, 1889
TA 1537	**4-NOPD**	50 µg	*104.7*	*15.4*	*10.8*	112, 87, 115
TA 98	**4-NOPD**	10 µg	*369.3*	*19.7*	*11.1*	347, 377, 384
TA 100	**NaN3**	10 µg	*1886.3*	*59.5*	*14.3*	1935, 1904, 1820
WP2 uvrA	**MMS**	3.0 µL	*1057.0*	*56.8*	*17.3*	1117, 1050, 1004

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Study Name: 1332300
Experiment: 1332300 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 17/03/2010
Date Counted: 22/03/2010

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Solution of**	3 µg	*20.7*	*4.9*	*1.2*	23, 15, 24
	Glyphosate TC	10 µg	*15.3*	*2.9*	*0.9*	12, 17, 17
	spiked with	33 µg	*16.3*	*4.5*	*1.0*	16, 12, 21
	Glyphosine	100 µg	*19.3*	*4.7*	*1.1*	23, 21, 14
		333 µg	*17.0*	*1.7*	*1.0*	16, 16, 19
		1000 µg	*14.3*	*0.6*	*0.8*	14, 15, 14
		2500 µg	*13.7*	*0.6*	*0.8*	14, 14, 13
		5000 µg	*15.0*	*3.6*	*0.9*	19, 14, 12
	Deionised water		*17.0*	*2.0*		15, 17, 19
	Untreated Control		*20.7*	*1.2*		22, 20, 20
TA 1537	**Solution of**	3 µg	*19.7*	*4.0*	*1.0*	24, 19, 16
	Glyphosate TC	10 µg	*16.7*	*2.9*	*0.8*	20, 15, 15
	spiked with	33 µg	*20.7*	*1.5*	*1.0*	19, 22, 21
	Glyphosine	100 µg	*17.3*	*1.5*	*0.9*	17, 16, 19
		333 µg	*19.7*	*1.2*	*1.0*	19, 21, 19
		1000 µg	*14.3*	*4.5*	*0.7*	19, 10, 14
		2500 µg	*11.3*	*2.3*	*0.6*	14, 10, 10
		5000 µg	*11.0*	*2.6*	*0.6*	10, 14, 9
	Deionised water		*20.0*	*5.6*		26, 15, 19
	Untreated Control		*15.3*	*2.1*		16, 13, 17
TA 98	**Solution of**	3 µg	*46.7*	*3.2*	*1.1*	49, 43, 48
	Glyphosate TC	10 µg	*39.3*	*6.8*	*0.9*	47, 34, 37
	spiked with	33 µg	*44.7*	*4.6*	*1.0*	50, 42, 42
	Glyphosine	100 µg	*43.0*	*1.7*	*1.0*	41, 44, 44
		333 µg	*43.3*	*6.5*	*1.0*	50, 43, 37
		1000 µg	*41.3*	*6.7*	*0.9*	38, 37, 49
		2500 µg	*49.7*	*1.2*	*1.1*	51, 49, 49
		5000 µg	*40.0*	*6.1*	*0.9*	47, 37, 36
	Deionised water		*44.0*	*3.6*		43, 48, 41
	Untreated Control		*41.3*	*5.1*		47, 37, 40

Study Name: 1332300
Experiment: 1332300 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 17/03/2010
Date Counted: 22/03/2010

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Solution of**	3 µg	*164.3*	*10.1*	*1.1*	175, 163, 155
	Glyphosate TC	10 µg	*158.3*	*8.7*	*1.0*	151, 156, 168
	spiked with	33 µg	*164.7*	*7.6*	*1.1*	158, 163, 173
	Glyphosine	100 µg	*161.7*	*9.3*	*1.0*	166, 151, 168
		333 µg	*166.3*	*3.5*	*1.1*	166, 170, 163
		1000 µg	*169.0*	*4.6*	*1.1*	173, 164, 170
		2500 µg	*155.3*	*5.1*	*1.0*	151, 154, 161
		5000 µg	*92.0*	*18.3*	*0.6*	112, 88, 76
	Deionised water		*154.3*	*13.7*		170, 148, 145
	Untreated Control		*153.3*	*7.4*		159, 145, 156
WP2 uvrA	**Solution of**	3 µg	*70.0*	*11.4*	*1.0*	62, 65, 83
	Glyphosate TC	10 µg	*72.3*	*9.2*	*1.0*	67, 67, 83
	spiked with	33 µg	*69.7*	*2.5*	*1.0*	70, 67, 72
	Glyphosine	100 µg	*75.0*	*5.2*	*1.0*	78, 69, 78
		333 µg	*76.0*	*5.3*	*1.1*	80, 78, 70
		1000 µg	*65.3*	*4.0*	*0.9*	69, 66, 61
		2500 µg	*65.0*	*7.5*	*0.9*	66, 72, 57
		5000 µg	*42.7*	*14.6*	*0.6*	31, 59, 38
	Deionised water		*72.3*	*8.0*		73, 64, 80
	Untreated Control		*70.3*	*11.6*		57, 76, 78
TA 1535	**2-AA**	2.5 µg	*341.7*	*8.1*	*20.1*	333, 343, 349
TA 1537	**2-AA**	2.5 µg	*436.7*	*7.4*	*21.8*	445, 431, 434
TA 98	**2-AA**	2.5 µg	*2072.0*	*40.3*	*47.1*	2101, 2089, 2026
TA 100	**2-AA**	2.5 µg	*3249.3*	*170.4*	*21.1*	3062, 3291, 3395
WP2 uvrA	**2-AA**	10.0 µg	*350.0*	*13.0*	*4.8*	343, 365, 342

Key to Positive Controls

2-AA 2-aminoanthracene

Study Name: 1332300
Experiment: 1332300 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 18/03/2010
Date Counted: 22/03/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Solution of**	3 µg	*16.7*	*3.1*	*1.0*	14, 16, 20
	Glyphosate TC	10 µg	*17.7*	*2.9*	*1.0*	16, 16, 21
	spiked with	33 µg	*17.7*	*3.2*	*1.0*	14, 19, 20
	Glyphosine	100 µg	*18.3*	*2.9*	*1.1*	15, 20, 20
		333 µg	*18.0*	*3.5*	*1.0*	16, 22, 16
		1000 µg	*15.3*	*1.5*	*0.9*	14, 17, 15
		2500 µg	*12.0*	*4.4*	*0.7*	10, 9, 17
		5000 µg	*11.7*	*1.5*	*0.7*	13, 12, 10
	Deionised water		*17.3*	*2.5*		20, 17, 15
	Untreated Control		*16.3*	*3.5*		13, 20, 16
TA 1537	**Solution of**	3 µg	*13.7*	*2.1*	*1.1*	13, 16, 12
	Glyphosate TC	10 µg	*14.3*	*3.8*	*1.2*	16, 17, 10
	spiked with	33 µg	*12.0*	*2.6*	*1.0*	14, 9, 13
	Glyphosine	100 µg	*11.0*	*2.6*	*0.9*	14, 9, 10
		333 µg	*11.7*	*2.5*	*1.0*	12, 9, 14
		1000 µg	*13.7*	*1.5*	*1.1*	12, 15, 14
		2500 µg	*15.0*	*2.0*	*1.3*	17, 15, 13
		5000 µg	*10.0*	*5.2*	*0.8*	7, 7, 16
	Deionised water		*12.0*	*2.6*		14, 9, 13
	Untreated Control		*11.3*	*3.2*		10, 15, 9
TA 98	**Solution of**	3 µg	*34.7*	*8.6*	*1.0*	33, 27, 44
	Glyphosate TC	10 µg	*34.3*	*3.8*	*1.0*	36, 37, 30
	spiked with	33 µg	*35.7*	*11.6*	*1.1*	28, 30, 49
	Glyphosine	100 µg	*34.7*	*4.0*	*1.0*	30, 37, 37
		333 µg	*28.7*	*0.6*	*0.9*	29, 28, 29
		1000 µg	*31.7*	*3.1*	*0.9*	29, 31, 35
		2500 µg	*28.7*	*4.0*	*0.9*	31, 31, 24
		5000 µg	*20.3*	*5.8*	*0.6*	17, 17, 27
	Deionised water		*33.7*	*3.5*		30, 37, 34
	Untreated Control		*30.0*	*8.7*		35, 35, 20

Study Name: 1332300
Experiment: 1332300 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 18/03/2010
Date Counted: 22/03/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Solution of**	3 µg	*160.7*	*9.5*	*1.0*	151, 161, 170
	Glyphosate TC	10 µg	*155.0*	*17.1*	*0.9*	157, 137, 171
	spiked with	33 µg	*168.0*	*19.1*	*1.0*	170, 148, 186
	Glyphosine	100 µg	*150.3*	*1.5*	*0.9*	149, 150, 152
		333 µg	*170.0*	*11.3*	*1.0*	177, 176, 157
		1000 µg	*157.0*	*12.2*	*0.9*	163, 165, 143
		2500 µg	*137.7*	*10.7*	*0.8*	140, 147, 126
		5000 µg	*129.0*	*8.5*	*0.8*	138, 121, 128
	Deionised water		*167.0*	*14.4*		151, 171, 179
	Untreated Control		*155.3*	*9.3*		166, 151, 149
WP2 uvrA	**Solution of**	3 µg	*53.0*	*3.5*	*0.9*	55, 55, 49
	Glyphosate TC	10 µg	*55.3*	*1.2*	*0.9*	56, 56, 54
	spiked with	33 µg	*59.3*	*11.6*	*1.0*	61, 47, 70
	Glyphosine	100 µg	*55.3*	*1.5*	*0.9*	55, 54, 57
		333 µg	*59.3*	*7.5*	*1.0*	52, 67, 59
		1000 µg	*56.0*	*8.7*	*1.0*	51, 66, 51
		2500 µg	*52.0*	*7.0*	*0.9*	59, 45, 52
		5000 µg	*33.7*	*3.1*	*0.6*	31, 37, 33
	Deionised water		*58.3*	*3.2*		62, 57, 56
	Untreated Control		*54.0*	*7.2*		62, 48, 52
TA 1535	**NaN3**	10 µg	*1688.3*	*149.5*	*97.4*	1728, 1814, 1523
TA 1537	**4-NOPD**	50 µg	*123.3*	*11.4*	*10.3*	120, 114, 136
TA 98	**4-NOPD**	10 µg	*452.7*	*10.2*	*13.4*	441, 460, 457
TA 100	**NaN3**	10 µg	*1985.3*	*98.5*	*11.9*	2099, 1925, 1932
WP2 uvrA	**MMS**	3.0 µL	*691.3*	*67.3*	*11.9*	748, 709, 617

Key to Positive Controls

NaN3 sodium azide
4-NOPD 4-nitro-o-phenylene-diamine
MMS methyl methane sulfonate

Study Name: 1332300
Experiment: 1332300 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 18/03/2010
Date Counted: 22/03/2010

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Solution of**	3 µg	*19.7*	*3.5*	*0.9*	20, 23, 16
	Glyphosate TC	10 µg	*18.3*	*4.2*	*0.9*	17, 15, 23
	spiked with	33 µg	*20.0*	*3.6*	*0.9*	21, 23, 16
	Glyphosine	100 µg	*22.7*	*3.1*	*1.1*	26, 22, 20
		333 µg	*18.0*	*2.6*	*0.8*	21, 17, 16
		1000 µg	*22.7*	*1.5*	*1.1*	24, 21, 23
		2500 µg	*23.7*	*0.6*	*1.1*	24 P, 24 P, 23 P
		5000 µg	*22.3*	*2.1*	*1.0*	23 P, 24 P, 20 P
	Deionised water		*21.3*	*0.6*		21, 21, 22
	Untreated Control		*21.0*	*2.6*		20, 24, 19
TA 1537	**Solution of**	3 µg	*17.7*	*2.1*	*1.0*	17, 16, 20
	Glyphosate TC	10 µg	*19.3*	*4.0*	*1.1*	17, 17, 24
	spiked with	33 µg	*21.3*	*2.1*	*1.2*	19, 22, 23
	Glyphosine	100 µg	*20.3*	*0.6*	*1.2*	20, 20, 21
		333 µg	*17.7*	*4.7*	*1.0*	23 U M, 14 U M, 16 U M
		1000 µg	*18.3*	*4.0*	*1.0*	23 U M, 16 U M, 16 U M
		2500 µg	*12.3*	*2.1*	*0.7*	13 U M P, 14 U M P, 10 U M P
		5000 µg	*11.0*	*1.0*	*0.6*	11 U M P, 12 U M P, 10 U M P
	Deionised water		*17.7*	*4.7*		23, 16, 14
	Untreated Control		*15.7*	*0.6*		15, 16, 16
TA 98	**Solution of**	3 µg	*43.7*	*6.5*	*0.9*	37, 44, 50
	Glyphosate TC	10 µg	*44.7*	*8.4*	*0.9*	49, 50, 35
	spiked with	33 µg	*44.3*	*3.1*	*0.9*	47, 45, 41
	Glyphosine	100 µg	*45.3*	*3.8*	*1.0*	48, 41, 47
		333 µg	*43.0*	*10.1*	*0.9*	41, 54, 34
		1000 µg	*48.7*	*6.4*	*1.0*	56, 45, 45
		2500 µg	*45.3*	*6.4*	*1.0*	50 P, 48 P, 38 P
		5000 µg	*50.0*	*1.0*	*1.1*	51 P, 49 P, 50 P
	Deionised water		*47.3*	*2.5*		50, 45, 47
	Untreated Control		*45.7*	*4.0*		41, 48, 48

Key to Plate Postfix Codes

P	Precipitate
U	Air bubbles
M	Manual count

Study Name: 1332300
Experiment: 1332300 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1332300
Date Plated: 18/03/2010
Date Counted: 22/03/2010

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Solution of**	3 µg	*170.7*	*21.6*	*1.0*	191, 148, 173
	Glyphosate TC	10 µg	*157.3*	*12.0*	*0.9*	158, 145, 169
	spiked with	33 µg	*168.0*	*15.1*	*1.0*	156, 185, 163
	Glyphosine	100 µg	*175.3*	*9.0*	*1.1*	184, 176, 166
		333 µg	*171.0*	*16.8*	*1.0*	177, 184, 152
		1000 µg	*165.0*	*1.7*	*1.0*	166, 166, 163
		2500 µg	*148.7*	*11.7*	*0.9*	162 P, 144 P, 140 P
		5000 µg	*100.3*	*12.0*	*0.6*	112 P, 101 P, 88 P
	Deionised water		*166.3*	*9.1*		158, 165, 176
	Untreated Control		*169.3*	*11.9*		179, 173, 156
WP2 uvrA	**Solution of**	3 µg	*66.3*	*2.5*	*1.0*	66, 64, 69
	Glyphosate TC	10 µg	*72.7*	*11.6*	*1.1*	86, 67, 65
	spiked with	33 µg	*69.3*	*9.5*	*1.0*	66, 80, 62
	Glyphosine	100 µg	*66.3*	*11.9*	*1.0*	80, 61, 58
		333 µg	*62.7*	*12.5*	*0.9*	77 U, 57 U, 54 U
		1000 µg	*63.7*	*5.7*	*1.0*	62 U, 70 U, 59 U
		2500 µg	*59.3*	*7.4*	*0.9*	62 P U, 65 P U, 51 P U
		5000 µg	*62.3*	*5.0*	*0.9*	57 P U, 67 P U, 63 P U
	Deionised water		*67.0*	*7.8*		62, 63, 76
	Untreated Control		*67.3*	*8.1*		58, 73, 71
TA 1535	**2-AA**	2.5 µg	*431.0*	*15.6*	*20.2*	439, 441, 413
TA 1537	**2-AA**	2.5 µg	*547.3*	*30.5*	*31.0*	549, 577, 516
TA 98	**2-AA**	2.5 µg	*2685.7*	*741.6*	*56.7*	2408, 3526, 2123
TA 100	**2-AA**	2.5 µg	*3992.3*	*38.0*	*24.0*	3974, 4036, 3967
WP2 uvrA	**2-AA**	10.0 µg	*378.0*	*41.7*	*5.6*	351, 357, 426

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

P Precipitate
U Air bubbles
M Manual count

15 ANNEX II: CERTIFICATES OF ANALYSIS

CERTIFICATE OF ANALYSIS

Harlan Laboratories Study :	C88237
Sponsor:	**Helm AG** Nordkanalstrasse 28 20097 Hamburg / Germany
Test Facility:	**Harlan Laboratories Ltd.** Zelgliweg 1 4452 Itingen Switzerland

Data of Test Item as prepared at the test facility:

Identity:	Solution of Glyphosate TC spiked with Glyphosine
Batch:	37/422/10
Expiration date:	Date of preparation + two weeks (29-Mar-2010)
Storage:	Room temperature, in the dark

Results:

Date of Analysis by Harlan Laboratories Ltd.:	15-Mar-2010 and 16-Mar-2010
Purity/ Content of a.i :	Content Glyphosine: 0.63 % w/w
	Purity Glyphosate TC: 97.16 % w/w

The results described in this certificate were achieved in compliance with the Swiss Ordinance relating to GLP, based on the OECD Principles of Good Laboratory Practice.

Issued by:

[redacted]
(Study Director)

[redacted]
Date: April 6, 2010

[redacted]
(Quality Assurance)

[redacted]
Date: April 09, 2010

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

地址：中国海洛市汉中路89号 金鹰国际商城19楼
电话：+86-25-84785989
传真：+86-25-84785826
邮编：210029

Add: 19th Floor, Golden Eagle International Plaza
89 Hanzhong Road Nanjing 210029 China
Tel: +86-25-84785989
FAX: +86-25-84785826
http://www.chinaredsun.com

SIGMA-ALDRICH

ALDRICH

Industriestrasse 25, CH-9471 Buchs (SG), Switzerland
Tel +41 81 755 2511 Fax +41 81 755 5449

Product Name:	*N,N*-BIS(PHOSPHONOMETHYL)GLYCINE purum, >= 98 0 % T
Product Number:	15149
Product Brand:	Aldrich
Molecular Formula:	$C_4H_{11}NO_8P_2$
Molecular Mass:	263 08
CAS Number:	2439-99-8

TEST	SPECIFICATION	LOT 1438405 RESULTS
APPEARANCE (COLOR)	WHITE TO ALMOST WHITE	WHITE
APPEARANCE (FORM)	POWDER TO POWDER WITH LUMPS	POWDER WITH LUMPS
TITRATION (T) NAOH 0.1M	98 0 - 102 0 %	99 0 %
CARBON CONTENT	18 26 % (THEORY)	18 24 %
HYDROGEN CONTENT	4 21 % (THEORY)	3 83 %
NITROGEN CONTENT	5 32 % (THEORY)	5 54 %
PROTON NMR SPECTRUM	CONFORMS TO STRUCTURE	CONFORMS

QC RELEASE DATE 27/MAY/09

[redacted] Manager
Quality Control
Buchs, Switzerland