RCC - CCR STUDY NUMBER 1061401

SALMONELLA TYPHIMURIUM
AND
ESCHERICHIA COLI
REVERSE MUTATION ASSAY

WITH

Glyphosate technical (NUP-05068)

FINAL REPORT

STUDY COMPLETION DATE:
MARCH 16, 2007

1 STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of it falling within the scope of FIFRA Sect. 10 (d)(1)(A), (B) or (C).

Company

Company Agent

Title

Signature

Date

These data are considered to be CONFIDENTIAL for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality, which may exist under any statue or in any other country.

The above statement supersedes any other markings of confidentially which may appear elsewhere in the report.

2 CONTENTS

3 PREFACE

3.1 General

Title:	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Glyphosate technical (NUP-05068)
Sponsor:	Nufarm Asia Sdn Bhd No. 9A & B, Jalan USJ 21/5 47630 Subang Jaya, Selangor, D.E. Malaysia
Study Monitor:	[redacted] Nufarm Asia Sdn Bhd
Test Facility:	R C C Cytotest Cell Research GmbH (RCC-CCR) In den Leppsteinswiesen 19 64380 Rossdorf Germany
Contracting Institute:	R C C Ltd 4452 Itingen Switzerland
Reference Number:	B02338

3.2 Responsibilities

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

3.3 Schedule

Date of the Study Plan:	January 03, 2007
Experimental Starting Date:	January 15, 2007
Experimental Completion Date:	January 25, 2007
Date of Draft Report:	February 19, 2007
Date of Final Report:	March 16, 2007

3.4 Project Staff Signatures

Study Director

Dipl. Biol. [redacted]

[redacted]

Date: March 16, 2007

Management

[redacted]

Dr. [redacted]

[redacted]

Date: March 16, 2007

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) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

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

These procedures are consistent with Good Laboratory Practice Regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHW, MAFF, and MITI).

3.6 Guidelines

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

The OECD Guidelines for Testing of Chemicals No. 471: "Bacterial Reverse Mutation Test", adopted July 21, 1997 referenced as Method B13/14 of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC).

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), Guidelines for Study Results, Reverse mutation studies. Guideline No.2-1-19-1. >Notification 12NohSan No. 8147, as partly revised in 16-Shouan-9260, on March 16, 2005. English translation by ACIS on October 17, 2005.

3.7 Archiving

RCC Cytotest Cell Research GmbH will archive the following data for 15 years:

Raw data, study plan, final report, and a sample of the test item.

No data will be discarded without the sponsor's consent.

3.8 Deviations to Study Plan

There were no deviations to study plan.

4 STATEMENT OF COMPLIANCE

Study Number:	1061401
Test Item:	Glyphosate technical (NUP-05068)
Study Director:	Dipl. Biol. [redacted]
Title:	Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Glyphosate technical (NUP-05068)

This study performed in the test facility of RCC Cytotest Cell Research GmbH was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) dated July 25, 1994 ("BGBl. I 1994", pp. 1703), last revision dated June 27, 2002.

"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

R C C - C C R
Dipl. Biol. [redacted]

[redacted] .....
Date: March 16, 2007

5 STATEMENT OF QUALITY ASSURANCE UNIT

Study Number: 1061401

Test Item: Glyphosate technical (NUP-05068)

Study Director: Dipl. Biol. [redacted]

Title: Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Glyphosate technical (NUP-05068)

The general facilities and activities of RCC Cytotest Cell Research GmbH 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 (Draft):	January 03, 2007	January 03, 2007
Study Plan:	January 04, 2007	- -
Study Inspection:	January 18, 2007	January 18, 2007
Draft Report:	March 01, 2007 March 08, 2007	March 01, 2007 March 08, 2007

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

Head of Quality Assurance Unit [redacted]

[redacted]

.....

Date: March 16, 2007

6 SUMMARY OF RESULTS

This study was performed to investigate the potential of Glyphosate technical (NUP-05068) 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:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation. Only in experiment II a minor reduction in the number of revertants, occurred in strain TA 1537 in the absence of metabolic activation at 5000 µg/plate.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

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

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, Glyphosate technical (NUP-05068) 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. Since a negative result was obtained in this experiment, 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 at least 6 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 RCC-CCR Test Item Number: S 693611

The test item and the information concerning the test item were provided by the sponsor.

Identity:	Glyphosate technical (NUP-05068)
Batch No.:	200609062
Aggregate state at room temperature:	Crystalline powder
Colour:	White
Purity:	95.1 %
Solubility	10.9 g/L in water
Stability in solvent :	Not indicated by the sponsor
Storage:	Room temperature
Expiration Date:	September 14, 2008

On the day of the experiment, the test item Glyphosate technical (NUP-05068) was dissolved in deionised water, the stock solution was neutralized with 5N sodium hydroxide. The solvent was chosen because of its solubility properties (5).

No precipitation of the test item occurred up to the highest investigated dose.

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
Lot Number:	14760
Catalogue No.:	30175
Purity:	at least 99 %
Expiration Date:	August 2007
Dissolved in:	aqua deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Lot Number:	416324/1
Catalogue No.:	N 9504
Purity:	> 99.9 %
Expiration Date:	April 2009
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
Lot Number:	074K3720
Catalogue No.:	820775
Purity:	> 99.0 %
Expiration Date:	October 2007
Dissolved in:	aqua deionised
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
Lot Number:	S11804-252
Catalogue No.:	A 1381
Purity:	97.5 %
Expiration Date:	November 2007
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 will be 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 RCC Cytotest Cell Research 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 Merck 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.

8.3.4 Selective Agar

The plates with the selective agar were obtained from [redacted] D-64293 Darmstadt (Catalogue No.:1.13496.00.1; Lot No.:62355).

8.3.5 Overlay Agar

The overlay agar contains per litre:

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

* (MERCK, D-64293 Darmstadt)

Sterilisations were 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 R C C - 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 Hanlbm rats, 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 38.1 mg/mL (lot no. R 031106) 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
5 mM NADP

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

During the experiment the S9 mix was stored in an ice bath. 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

In the pre-experiment the concentration range of the test item was 3 – 5000 µg/plate. The pre-experiment is reported as experiment I since no relevant toxic effects were observed and 5000 µg/plate were chosen as maximal concentration.

The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

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:

100 µL	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µL	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µL	Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µL	Overlay agar

In the pre-incubation assay 100 µL test solution, 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and shaken at 37° C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45° C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark (2).

8.8 Data Recording

The colonies were counted using the Petri Viewer Mk2 (Perceptive Instruments Ltd, Suffolk CB 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 contamination of a few plates these had to be 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 Glyphosate technical (NUP-05068) 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:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without metabolic activation in both independent experiments.

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation, with the exception of strain TA 1537, where a minor recuction in the number of revertants was observed at 5000 µg/plate without metabolic activation in experiment II.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Glyphosate technical (NUP-05068) at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

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

The laboratory's historical control range was slightly exceeded in the solvent control of strain WP2 uvrA with metabolic activation in experiment I. This minor deviation is judged to be based on biologically irrelevant fluctuations and has no impact on the outcome of the study.

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

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	2 × copy
Study Director	1 × original

12 SUMMARY OF RESULTS

12.1 Summary of Results Pre-Experiment/Experiment I

Study Name: 1061401 | Study Code: RCC-CCR 1061401
Experiment: 1061401 VV Plate | Date Plated: 15/01/2007
Assay Conditions: | Date Counted: 18/01/2007

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD) TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Deionised water		23 ± 4	10 ± 2	21 ± 2	134 ± 8	73 ± 7
	Untreated		18 ± 8	13 ± 4	20 ± 8	147 ± 18	68 ± 9
	GlyphosateTechnical (NUP-05068)	3 µg	25 ± 3	11 ± 1	24 ± 4	127 ± 9	65 ± 9
		10 µg	16 ± 5	13 ± 5	24 ± 5	132 ± 14	79 ± 10
		33 µg	16 ± 6	11 ± 4	27 ± 2	127 ± 7	86 ± 5
		100 µg	15 ± 2	11 ± 4	26 ± 8	126 ± 14	70 ± 9
		333 µg	23 ± 3	9 ± 2	25 ± 5	145 ± 9	63 ± 3
		1000 µg	17 ± 4	12 ± 3	24 ± 2	140 ± 8	69 ± 10
		2500 µg	19 ± 4	11 ± 5	20 ± 9	110 ± 13	69 ± 5
		5000 µg	18 ± 1	14 ± 9	22 ± 6	106 ± 11	57 ± 2
	NaN3	10 µg	1885 ± 55			2060 ± 80	
	4-NOPD	10 µg			378 ± 10		
	4-NOPD	50 µg		100 ± 4			
	MMS	3.0 µL					1558 ± 86
With Activation	Deionised water		22 ± 5	20 ± 7	30 ± 7	163 ± 8	90 ± 11
	Untreated		24 ± 4	19 ± 6	31 ± 3	162 ± 7	81 ± 14
	GlyphosateTechnical (NUP-05068)	3 µg	27 ± 3	21 ± 3	35 ± 2	157 ± 6	90 ± 18
		10 µg	22 ± 6	20 ± 5	44 ± 4	153 ± 9	97 ± 5
		33 µg	19 ± 5	20 ± 3	30 ± 2	157 ± 3	93 ± 17
		100 µg	22 ± 1	20 ± 4	33 ± 9	142 ± 15	83 ± 9
		333 µg	22 ± 11 C N	15 ± 6	38 ± 5	147 ± 10	88 ± 19
		1000 µg	27 ± 2	20 ± 4	31 ± 7	151 ± 4	84 ± 9
		2500 µg	23 ± 3	15 ± 8	31 ± 2	149 ± 24	81 ± 10
		5000 µg	24 ± 2	15 ± 2	22 ± 8	129 ± 1	69 ± 2
	2-AA	2.5 µg	398 ± 21	301 ± 7	1172 ± 116	2778 ± 91	
	2-AA	10.0 µg					269 ± 15

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

C	Contaminated
N	Analysis not possible in plate three mean of two plates

12.2 Summary of Results Experiment II

Study Name: 1061401
Experiment: 1061401 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 22/01/2007
Date Counted: 25/01/2007

Metabolic Activation	Test Group	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Deionised water		21 ± 4	11 ± 4	24 ± 4	123 ± 3	52 ± 5
	Untreated		17 ± 4	13 ± 8	29 ± 9	143 ± 1	54 ± 11
	GlyphosateTechnical	33 µg	16 ± 2	14 ± 2	26 ± 4	133 ± 3	61 ± 4
	(NUP-05068)	100 µg	19 ± 6	10 ± 3	28 ± 11	140 ± 4	53 ± 1
		333 µg	19 ± 5	10 ± 4	26 ± 2	140 ± 11	44 ± 4
		1000 µg	19 ± 6	9 ± 3	28 ± 8	143 ± 13	50 ± 7
		2500 µg	17 ± 2	10 ± 5	26 ± 2	112 ± 4	48 ± 4
		5000 µg	11 ± 3	4 ± 3	20 ± 9	95 ± 10	25 ± 12
	NaN3	10 µg	1934 ± 82			1861 ± 100	
	4-NOPD	10 µg			530 ± 11		
	4-NOPD	50 µg		112 ± 7			
	MMS	3.0 µL					568 ± 27
With Activation	Deionised water		19 ± 4	11 ± 2	28 ± 9	186 ± 9	73 ± 7
	Untreated		20 ± 1	17 ± 6	30 ± 7	185 ± 7	68 ± 5
	GlyphosateTechnical	33 µg	15 ± 5	18 ± 3	31 ± 6	180 ± 9	81 ± 10
	(NUP-05068)	100 µg	23 ± 4	11 ± 2	29 ± 2	169 ± 37	66 ± 3
		333 µg	22 ± 6	15 ± 5	30 ± 2	191 ± 29	69 ± 5
		1000 µg	23 ± 6	15 ± 6	29 ± 6	192 ± 17	67 ± 9
		2500 µg	22 ± 6	11 ± 3	23 ± 8	163 ± 5	53 ± 3
		5000 µg	23 ± 9	11 ± 4	24 ± 2	126 ± 4	55 ± 12
	2-AA	2.5 µg	365 ± 17	179 ± 18	764 ± 77	2004 ± 334	
	2-AA	10.0 µg					261 ± 20

Key to Positive Controls

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

13 HISTORICAL CONTROL DATA

These data represent the laboratory's historical control data from May 2005 until June 2006 representing approx. 200 experiments (WP2 uvrA the historical data are based on approx. 100 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	20.8	4.7	9	35	24.7	5.9	7	43
	Negative control	20.4	4.4	11	31	24.2	5.5	10	38
	Positive control	1422.0	464.7	781	4900	332.0	95.3	107	695
TA1537	Solvent control	11.2	3.7	5	28	16.2	5.0	6	36
	Negative control	11.6	4.0	4	28	17.1	5.4	7	34
	Positive control	99.8	32.5	53	425	276.8	132.6	59	746
TA 98	Solvent control	28.1	6.1	15	49	37.9	7.4	20	57
	Negative control	30.2	6.6	16	60	39.0	7.5	18	64
	Positive control	439.0	155.2	176	1818	1839.4	898.6	407	4891
TA 100	Solvent control	130.7	20.8	87	197	147.0	25.5	84	255
	Negative control	138.2	21.6	86	216	150.1	24.2	96	214
	Positive control	2083.1	281.3	616	2872	2372.9	958.4	417	5230
WP2uvrA	Solvent control	55.8	7.2	31	74	63.9	9.1	34	84
	Negative control	55.9	8.6	36	76	65.6	10.4	33	91
	Positive control	991.0	522.9	249	1810	319.4	84.8	211	930

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: 1061401 VV Plate Incorporation (4 pages)

Experiment II: 1061401 Pre-Incubation (4 pages)

Study Name: 1061401
Experiment: 1061401 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 15/01/2007
Date Counted: 18/01/2007

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Glyphosate**	3 µg	*25.0*	*2.6*	*1.1*	24, 28, 23
	Technical	10 µg	*16.3*	*4.9*	*0.7*	13, 22, 14
	(NUP-05068)	33 µg	*15.7*	*6.1*	*0.7*	21, 9, 17
		100 µg	*15.3*	*2.1*	*0.7*	13, 17, 16
		333 µg	*22.7*	*3.1*	*1.0*	20, 22, 26
		1000 µg	*17.3*	*3.8*	*0.8*	20, 13, 19
		2500 µg	*19.3*	*4.2*	*0.9*	18, 16, 24
		5000 µg	*17.7*	*1.2*	*0.8*	17, 19, 17
	Deionised water		*22.7*	*4.0*		22, 27, 19
	Untreated Control		*18.3*	*8.1*		24, 9, 22
TA 1537	**Glyphosate**	3 µg	*11.3*	*1.2*	*1.1*	10, 12, 12
	Technical	10 µg	*13.3*	*4.7*	*1.3*	8, 17, 15
	(NUP-05068)	33 µg	*11.3*	*4.0*	*1.1*	7, 12, 15
		100 µg	*11.0*	*4.4*	*1.1*	16, 9, 8
		333 µg	*8.7*	*1.5*	*0.8*	10, 9, 7
		1000 µg	*12.3*	*3.1*	*1.2*	15, 13, 9
		2500 µg	*10.7*	*4.7*	*1.0*	7, 16, 9
		5000 µg	*13.7*	*9.1*	*1.3*	10, 24, 7
	Deionised water		*10.3*	*1.5*		12, 10, 9
	Untreated Control		*13.0*	*3.6*		10, 12, 17
TA 98	**Glyphosate**	3 µg	*23.7*	*3.5*	*1.1*	24, 20, 27
	Technical	10 µg	*24.3*	*4.9*	*1.2*	22, 30, 21
	(NUP-05068)	33 µg	*27.3*	*1.5*	*1.3*	27, 26, 29
		100 µg	*25.7*	*8.1*	*1.2*	17, 27, 33
		333 µg	*25.3*	*4.9*	*1.2*	31, 23, 22
		1000 µg	*23.7*	*2.1*	*1.1*	26, 22, 23
		2500 µg	*19.7*	*8.7*	*1.0*	27, 10, 22
		5000 µg	*22.0*	*6.2*	*1.1*	15, 27, 24
	Deionised water		*20.7*	*2.1*		20, 19, 23
	Untreated Control		*20.0*	*7.5*		13, 19, 28
TA 100	**Glyphosate**	3 µg	*127.3*	*9.0*	*1.0*	118, 128, 136
	Technical	10 µg	*132.3*	*14.2*	*1.0*	116, 140, 141
	(NUP-05068)	33 µg	*127.0*	*6.6*	*1.0*	120, 133, 128
		100 µg	*126.0*	*14.0*	*0.9*	112, 140, 126
		333 µg	*145.0*	*8.9*	*1.1*	138, 142, 155
		1000 µg	*139.7*	*7.6*	*1.0*	138, 133, 148
		2500 µg	*110.0*	*13.2*	*0.8*	115, 95, 120
		5000 µg	*106.3*	*11.2*	*0.8*	116, 109, 94
	Deionised water		*133.7*	*7.8*		125, 140, 136
	Untreated Control		*146.7*	*18.2*		150, 127, 163

Study Name: 1061401
Experiment: 1061401 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 15/01/2007
Date Counted: 18/01/2007

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**Glyphosate**	3 µg	*65.0*	*8.5*	*0.9*	66, 73, 56
	Technical	10 µg	*79.3*	*9.6*	*1.1*	88, 69, 81
	(NUP-05068)	33 µg	*86.0*	*4.6*	*1.2*	90, 81, 87
		100 µg	*69.7*	*9.3*	*1.0*	62, 80, 67
		333 µg	*63.3*	*3.2*	*0.9*	62, 61, 67
		1000 µg	*68.7*	*10.4*	*0.9*	57, 72, 77
		2500 µg	*68.7*	*4.9*	*0.9*	71, 63, 72
		5000 µg	*57.3*	*1.5*	*0.8*	59, 56, 57
	Deionised water		*72.7*	*6.7*		65, 76, 77
	Untreated Control		*68.3*	*8.6*		76, 70, 59
TA 1535	**NaN3**	10 µg	*1884.7*	*55.1*	*83.1*	1821, 1917, 1916
TA 1537	**4-NOPD**	50 µg	*100.0*	*4.4*	*9.7*	97, 98, 105
TA 98	**4-NOPD**	10 µg	*378.3*	*9.5*	*18.3*	369, 378, 388
TA 100	**NaN3**	10 µg	*2060.3*	*79.9*	*15.4*	2085, 1971, 2125
WP2 uvrA	**MMS**	3.0 µL	*1558.3*	*85.9*	*21.4*	1464, 1579, 1632

Key to Positive Controls

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

Study Name: 1061401
Experiment: 1061401 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 15/01/2007
Date Counted: 18/01/2007

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Glyphosate**	3 µg	*27.0*	*2.6*	*1.2*	29, 28, 24
	Technical	10 µg	*21.7*	*5.7*	*1.0*	20, 17, 28
	(NUP-05068)	33 µg	*18.7*	*5.1*	*0.9*	13, 20, 23
		100 µg	*22.3*	*1.2*	*1.0*	23, 21, 23
		333 µg	*22.0*	*11.3*	*1.0*	30 C, 14 C, N C
		1000 µg	*26.7*	*2.3*	*1.2*	28, 24, 28
		2500 µg	*23.3*	*2.5*	*1.1*	21, 23, 26
		5000 µg	*24.0*	*1.7*	*1.1*	23, 23, 26
	Deionised water		*21.7*	*4.6*		19, 27, 19
	Untreated Control		*23.7*	*3.5*		27, 24, 20
TA 1537	**Glyphosate**	3 µg	*20.7*	*3.2*	*1.1*	22, 23, 17
	Technical	10 µg	*20.3*	*4.6*	*1.0*	15, 23, 23
	(NUP-05068)	33 µg	*19.7*	*2.5*	*1.0*	22, 17, 20
		100 µg	*19.7*	*4.0*	*1.0*	24, 16, 19
		333 µg	*14.7*	*5.7*	*0.7*	21, 13, 10
		1000 µg	*20.0*	*4.4*	*1.0*	23, 22, 15
		2500 µg	*15.3*	*7.5*	*0.8*	8, 15, 23
		5000 µg	*15.3*	*2.1*	*0.8*	13, 17, 16
	Deionised water		*19.7*	*6.5*		26, 13, 20
	Untreated Control		*19.0*	*6.2*		26, 17, 14
TA 98	**Glyphosate**	3 µg	*34.7*	*1.5*	*1.1*	36, 33, 35
	Technical	10 µg	*44.3*	*3.5*	*1.5*	48, 44, 41
	(NUP-05068)	33 µg	*29.7*	*1.5*	*1.0*	28, 30, 31
		100 µg	*33.0*	*8.7*	*1.1*	29, 43, 27
		333 µg	*37.7*	*4.6*	*1.2*	35, 43, 35
		1000 µg	*30.7*	*6.5*	*1.0*	24, 31, 37
		2500 µg	*31.0*	*1.7*	*1.0*	33, 30, 30
		5000 µg	*22.3*	*7.5*	*0.7*	22, 30, 15
	Deionised water		*30.3*	*7.2*		35, 22, 34
	Untreated Control		*31.0*	*3.0*		34, 31, 28
TA 100	**Glyphosate**	3 µg	*157.0*	*6.0*	*1.0*	163, 151, 157
	Technical	10 µg	*152.7*	*9.2*	*0.9*	158, 158, 142
	(NUP-05068)	33 µg	*157.3*	*3.2*	*1.0*	161, 156, 155
		100 µg	*142.0*	*15.1*	*0.9*	125, 147, 154
		333 µg	*147.3*	*10.3*	*0.9*	150, 156, 136
		1000 µg	*150.7*	*4.0*	*0.9*	155, 147, 150
		2500 µg	*149.0*	*24.2*	*0.9*	127, 145, 175
		5000 µg	*128.7*	*1.2*	*0.8*	128, 130, 128
	Deionised water		*162.7*	*7.5*		155, 163, 170
	Untreated Control		*162.0*	*6.9*		158, 170, 158

Key to Plate Postfix Codes

C Contaminated
N Analysis not possible

Study Name: 1061401
Experiment: 1061401 VV Plate
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 15/01/2007
Date Counted: 18/01/2007

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	**Glyphosate**	3 µg	*89.7*	*18.0*	*1.0*	98, 69, 102
	Technical	10 µg	*96.7*	*4.7*	*1.1*	95, 102, 93
	(NUP-05068)	33 µg	*92.7*	*17.2*	*1.0*	77, 90, 111
		100 µg	*83.3*	*9.5*	*0.9*	80, 76, 94
		333 µg	*88.0*	*19.1*	*1.0*	98, 100, 66
		1000 µg	*84.3*	*9.1*	*0.9*	83, 76, 94
		2500 µg	*81.3*	*9.7*	*0.9*	73, 79, 92
		5000 µg	*69.0*	*1.7*	*0.8*	70, 67, 70
	Deionised water		*90.0*	*10.5*		100, 91, 79
	Untreated Control		*80.7*	*13.8*		86, 65, 91
TA 1535	**2-AA**	2.5 µg	*397.7*	*21.4*	*18.4*	389, 422, 382
TA 1537	**2-AA**	2.5 µg	*300.7*	*6.8*	*15.3*	306, 293, 303
TA 98	**2-AA**	2.5 µg	*1172.0*	*115.7*	*38.6*	1291, 1060, 1165
TA 100	**2-AA**	2.5 µg	*2777.7*	*90.5*	*17.1*	2688, 2869, 2776
WP2 uvrA	**2-AA**	10.0 µg	*268.7*	*14.6*	*3.0*	255, 267, 284

Key to Positive Controls

2-AA 2-aminoanthracene

Study Name: 1061401
Experiment: 1061401 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 22/01/2007
Date Counted: 25/01/2007

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Glyphosate**	33 µg	*15.7*	*2.3*	*0.7*	13, 17, 17
	Technical	100 µg	*18.7*	*5.9*	*0.9*	12, 23, 21
	(NUP-05068)	333 µg	*19.3*	*4.5*	*0.9*	24, 19, 15
		1000 µg	*19.3*	*5.7*	*0.9*	13, 24, 21
		2500 µg	*17.3*	*2.3*	*0.8*	16, 16, 20
		5000 µg	*11.0*	*2.6*	*0.5*	14, 9, 10
	Deionised water		*21.3*	*3.8*		23, 17, 24
	Untreated Control		*16.7*	*3.8*		14, 21, 15
TA 1537	**Glyphosate**	33 µg	*14.3*	*1.5*	*1.3*	14, 13, 16
	Technical	100 µg	*10.0*	*3.5*	*0.9*	12, 12, 6
	(NUP-05068)	333 µg	*9.7*	*4.0*	*0.9*	6, 9, 14
		1000 µg	*8.7*	*3.1*	*0.8*	8, 12, 6
		2500 µg	*10.0*	*5.3*	*0.9*	8, 16, 6
		5000 µg	*4.3*	*3.1*	*0.4*	5, 1, 7
	Deionised water		*11.3*	*4.2*		8, 16, 10
	Untreated Control		*13.0*	*7.8*		8, 22, 9
TA 98	**Glyphosate**	33 µg	*25.7*	*3.8*	*1.1*	24, 23, 30
	Technical	100 µg	*28.0*	*11.3*	*1.2*	15, 34, 35
	(NUP-05068)	333 µg	*25.7*	*1.5*	*1.1*	27, 24, 26
		1000 µg	*27.7*	*7.6*	*1.2*	31, 33, 19
		2500 µg	*25.7*	*1.5*	*1.1*	24, 26, 27
		5000 µg	*19.7*	*9.0*	*0.8*	15, 14, 30
	Deionised water		*24.0*	*3.6*		23, 28, 21
	Untreated Control		*28.7*	*8.5*		37, 29, 20
TA 100	**Glyphosate**	33 µg	*132.7*	*3.2*	*1.1*	134, 129, 135
	Technical	100 µg	*140.3*	*4.0*	*1.1*	141, 136, 144
	(NUP-05068)	333 µg	*139.7*	*10.8*	*1.1*	135, 152, 132
		1000 µg	*142.7*	*13.3*	*1.2*	158, 134, 136
		2500 µg	*111.7*	*3.5*	*0.9*	108, 115, 112
		5000 µg	*95.0*	*10.0*	*0.8*	95, 105, 85
	Deionised water		*123.3*	*2.9*		120, 125, 125
	Untreated Control		*142.7*	*1.2*		142, 142, 144
WP2 uvrA	**Glyphosate**	33 µg	*60.7*	*3.5*	*1.2*	57, 64, 61
	Technical	100 µg	*52.7*	*1.2*	*1.0*	52, 52, 54
	(NUP-05068)	333 µg	*44.3*	*3.5*	*0.9*	48, 44, 41
		1000 µg	*50.0*	*7.2*	*1.0*	48, 58, 44
		2500 µg	*47.7*	*4.0*	*0.9*	52, 47, 44
		5000 µg	*25.3*	*11.7*	*0.5*	15, 38, 23
	Deionised water		*52.0*	*5.3*		505, 58, 48
	Untreated Control		*54.3*	*10.5*		44, 65, 54

Study Name: 1061401
Experiment: 1061401 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 22/01/2007
Date Counted: 25/01/2007

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*1934.3*	*81.7*	*90.7*	1840, 1983, 1980
TA 1537	**4-NOPD**	50 µg	*111.7*	*7.1*	*9.9*	118, 104, 113
TA 98	**4-NOPD**	10 µg	*529.7*	*11.1*	*22.1*	531, 540, 518
TA 100	**NaN3**	10 µg	*1861.0*	*99.9*	*15.1*	1836, 1776, 1971
WP2 uvrA	**MMS**	3.0 µL	*568.3*	*27.2*	*10.9*	559, 599, 547

Key to Positive Controls

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

Study Name: 1061401
Experiment: 1061401 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 22/01/2007
Date Counted: 25/01/2007

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**Glyphosate**	33 µg	*15.0*	*4.6*	*0.8*	10, 16, 19
	Technical	100 µg	*23.3*	*4.0*	*1.2*	24, 19, 27
	(NUP-05068)	333 µg	*21.7*	*6.0*	*1.2*	16, 28, 21
		1000 µg	*22.7*	*6.0*	*1.2*	22, 29, 17
		2500 µg	*22.0*	*6.2*	*1.2*	27, 24, 15
		5000 µg	*22.7*	*8.5*	*1.2*	31, 14, 23
	Deionised water		*18.7*	*4.0*		14, 21, 21
	Untreated Control		*20.3*	*0.6*		21, 20, 20
TA 1537	**Glyphosate**	33 µg	*18.3*	*3.1*	*1.6*	21, 15, 19
	Technical	100 µg	*11.3*	*2.1*	*1.0*	9, 12, 13
	(NUP-05068)	333 µg	*15.3*	*5.0*	*1.4*	20, 10, 16
		1000 µg	*15.0*	*5.6*	*1.3*	14, 10, 21
		2500 µg	*10.7*	*3.1*	*0.9*	8, 14, 10
		5000 µg	*10.7*	*4.0*	*0.9*	13, 6, 13
	Deionised water		*11.3*	*2.1*		12, 9, 13
	Untreated Control		*17.3*	*6.4*		22, 10, 20
TA 98	**Glyphosate**	33 µg	*31.0*	*6.2*	*1.1*	33, 36, 24
	Technical	100 µg	*29.3*	*1.5*	*1.0*	31, 28, 29
	(NUP-05068)	333 µg	*29.7*	*1.5*	*1.0*	28, 31, 30
		1000 µg	*29.0*	*6.0*	*1.0*	35, 29, 23
		2500 µg	*23.3*	*8.3*	*0.8*	26, 30, 14
		5000 µg	*23.7*	*2.1*	*0.8*	22, 23, 26
	Deionised water		*28.3*	*8.7*		26, 38, 21
	Untreated Control		*29.7*	*7.0*		23, 29, 37
TA 100	**Glyphosate**	33 µg	*179.7*	*9.3*	*1.0*	186, 184, 169
	Technical	100 µg	*168.7*	*37.1*	*0.9*	130, 172, 204
	(NUP-05068)	333 µg	*190.7*	*29.3*	*1.0*	223, 166, 183
		1000 µg	*192.0*	*16.6*	*1.0*	204, 199, 173
		2500 µg	*162.7*	*4.9*	*0.9*	157, 166, 165
		5000 µg	*126.3*	*3.8*	*0.7*	129, 128, 122
	Deionised water		*185.7*	*9.2*		191, 175, 191
	Untreated Control		*185.3*	*7.2*		190, 177, 189
WP2 uvrA	**Glyphosate**	33 µg	*81.0*	*9.8*	*1.1*	92, 78, 73
	Technical	100 µg	*66.0*	*2.6*	*0.9*	64, 69, 65
	(NUP-05068)	333 µg	*68.7*	*5.1*	*0.9*	63, 70, 73
		1000 µg	*67.3*	*9.1*	*0.9*	66, 59, 77
		2500 µg	*53.0*	*2.6*	*0.7*	50, 55, 54
		5000 µg	*55.0*	*12.1*	*0.8*	69, 48, 48
	Deionised water		*73.0*	*6.5*		79, 74, 66
	Untreated Control		*68.3*	*5.0*		73, 69, 63

Study Name: 1061401
Experiment: 1061401 HV2 Pre
Assay Conditions:

Study Code: RCC-CCR 1061401
Date Plated: 22/01/2007
Date Counted: 25/01/2007

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*365.0*	*17.1*	*19.6*	379, 370, 346
TA 1537	**2-AA**	2.5 µg	*178.7*	*17.9*	*15.8*	159, 183, 194
TA 98	**2-AA**	2.5 µg	*763.7*	*76.8*	*27.0*	738, 703, 850
TA 100	**2-AA**	2.5 µg	*2004.0*	*333.7*	*10.8*	1826, 1797, 2389
WP2 uvrA	**2-AA**	10.0 µg	*260.7*	*20.2*	*3.8*	248, 250, 284

Key to Positive Controls

2-AA 2-aminoanthracene

15 ANNEX II: COPY OF GLP CERTIFICATE OF ANALYSIS

Nufarm

V/Réf. :

N/Réf. :

CERTIFICAT D'ANALYSE
CERTIFICATE OF ANALYSIS

Nom du produit / *Product name* : Glyphosate Technical (NUP 05068)

Réf. lot / *Batch ref.* : 200809062

Réf. d'analyse / *Analytical ref.* : n° 00222 **Date d'analyse** / *Analytical date* : November 21, 2006

Date de fabrication / *Date of Manufacture* : September 14, 2006 **Date de péremption** / *Expiry date* : September 14, 2008

Caractéristiques *Characteristics*	Réf. Méthode *Test method ref.*	Unités *Units*	Valeur type *Typical value*	Spécifications *Certified limits* mini *Lower*	Maxi *Upper*	Résultats *Results*
Aspect *Appearance*	Visuel *Visual*	Poudre blanche *White powder*				conforme *conform*
Glyphosate	CIPAC MT 284/TC/(M)-	%	-	95	-	95.1

Ce produit a été analysé en conformité avec les principes des Bonnes Pratiques de Laboratoire. Les données brutes relatives à l'analyse de cet échantillon sont archivées chez Nufarm SAS, Laboratoire de Chimie Analytique, Gennevilliers, France.
This product was analysed in compliance with Good Laboratory Practice standards. The derivation and characterisation data for this material are located at Nufarm SAS, Analytical Chemistry Laboratory, Gennevilliers, France.

Date : 27 novembre 2006
Date : November 27, 2006

Date de la copie certifiée :
Date of certified copy: November 29, 2006

Approbateur du certificat :
Certificate approver : [redacted]

Opérateur :
Operator : [redacted]

Version n°3 du 01/02/02 DQBPL9.110

Nufarm S.A.S.
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GAILLON 27600
Notre-Dame-de-la-Garenne
Tél. : 02 32 64 74 00
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16 ANNEX III: COPY OF GLP CERTIFICATE

Hessisches Ministerium für Umwelt,
ländlichen Raum und Verbraucherschutz

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 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

RCC Cytotest Cell Research GmbH
RCC Cytotest Cell Research GmbH
In den Leppsteinwiesen 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)
8 Analytische Prüfungen an biologischen Materialien
9 Virussicherheitsprüfungen

2 Toxicity studies
3 Mutagenicity studies
8 Analytical studies on biological materials
9 Virus validation studies

03.06.; 19.07.-22.07.2004
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] Im Auftrag

[redacted], Referent, Wiesbaden, den **Wiesbaden, den 06. Januar 2005**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

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

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