Glyphosate

Glyphosate Technical - Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay

Final Report

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SPONSOR(S): Syngenta Ltd
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STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of Harlan CCR, In den Leppsteinswiesen 19, 64380 Rossdorf, Germany 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.

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Dipl. Biol.
Study Director

.....
Date: 18 December 2009

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 1264500

Test Item: Glyphosate technical

Study Director: Dipl. Biol. [redacted]

Title: Glyphosate Technical - Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay

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:	May 05, 2009	May 05, 2009
Process Inspection Preparation for Application, Application:	October 14, 2009	October 14, 2009
Draft Report:	December 02, 2009	December 02, 2009

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

Head of Quality Assurance Unit [redacted]

Date: 18 December 2009

GENERAL INFORMATION

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Reference Number:	C45331

Study dates

Study initiation date:	15 September 2009
Experimental start date:	23 September 2009
Experimental termination date:	13 October 2009

Deviations from the guidelines

None

Retention of samples

Raw data and a sample of the test item.

Performing laboratory test item reference number

S 10083 22

Other

Harlan CCR will archive the following data for 15 years:

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

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

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]

Deviations to study plan

None

Distribution of the report

Sponsor	2 · electronic copy (1 · pdf-file, 1 · Word file)
Study Director	1 · (original)

Project staff signatures

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1.0 EXECUTIVE SUMMARY

1.1 Study design

This study was performed to investigate the potential of Glyphosate technical (via the Nantong Jiangshan (glycine-route)) 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 strains WP2 uvrA pKM 101 and WP2 pKM 101.

1.2 Results

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 six tester strains was observed following treatment with Glyphosate technical at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Positive control chemicals showed appropriate responses in the relevant strains.

1.3 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 is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

2.0 INTRODUCTION

2.1 Purpose

The experiments were performed to assess the potential of the test item Glyphosate technical 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 and experiment II was performed as a pre-incubation assay.

The most widely used assays for detecting gene mutations are those using bacteria (1). 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 assure reliable detection of mutagens that may be specific to one tester strain or locus. 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 S. 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, WP2 uvrA pKM 101, and WP2 pKM 101 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 six 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.

2.2 Regulatory 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

"United States Environmental Protection Agency, Health Effects Test Guideline OPPTS 870.5100 (1998). Bacterial Reverse Mutation Test."

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

3.0 MATERIALS AND METHODS

3.1 Test item

Internal Test Item Number: S10083 22

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

Identity:	Glyphosate technical
Batch No.:	569753
Purity:	96.3 % of Glyphosate Acid
Stability in Solvent:	Not indicated by the sponsor
Storage:	Room temperature
Reanalysis Date:	August 31, 2010
Source of Material:	Nantong Jiangshan (glycine-route)

On the day of the experiment, the test item Glyphosate technical was dissolved in deionised water. The solvent was chosen because of its solubility properties and its relative non-toxicity to the bacteria (2).

3.2 Controls

3.2.1 Negative controls

Concurrent untreated and solvent controls were performed.

3.2.2 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:	water deionised
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
Strains:	WP2 uvrA pKM 101, WP2 pKM 101
Name:	methyl methane sulfonate, MMS
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	water deionised
Concentration:	3 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA pKM 101, WP2 pKM 101
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 pKM 101, WP2 pKM 101))

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.

3.3 Experimental design

3.3.1 Characterisation of the Salmonella typhimurium and E. coli strains

The histidine dependent strains are derived from S. typhimurium strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (rfa$^-$) 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$^-$. In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker (3).

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 (excisable repair damage). Such a repair-deficient strain may be more readily mutated by agents. The E. coli strains WP2 uvrA pKM101 and WP2 pKM101 are constructed by introduction of the R-factor plasmid pKM101.

When summarised, the mutations of the TA and E. coli strains 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 pKM101	trp E 56 uvrA$^-$; R-factor	base-pair substitutions and others
WP2 pKM101	trp E 56; R-factor	" "

Regular checking of the properties of the Salmonella typhimurium and E. coli strains regarding the membrane permeability and ampicillin resistance as well as normal spontaneous mutation rates is performed by Harlan CCR according to B. Ames et al. (5) and D. Maron and B. Ames (3). In this way it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, WP2 uvrA pKM 101, and WP2 pKM 101 were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

3.3.2 Storage

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

3.3.3 Precultures

From the thawed ampoules of the strains 0.5 mL bacterial 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, TA 100, WP2 uvrA pKM 101, and WP2 pKM 101. 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. 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).

3.3.4 Selective agar

The plates with the selective agar were obtained from E. Merck, D-64293 Darmstadt.

3.3.5 Overlay agar

The overlay agar contains per 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-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.

3.4 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.

3.4.1 S9 (Preparation by Harlan CCR)

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. The protein concentration in the S9 preparation is usually between 20 and 45 mg/mL. 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 31.6 mg/mL (lot no. R 130309) in both experiments.

3.4.2 S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor 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.(5).

3.5 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains. 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:

A minimum of five analysable dose levels should be present with at least four dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

The above criteria should be met for all valid experiments.

3.6 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 toxic effects were observed , six concentrations were tested and 5000 µg/plate was chosen as maximal concentration in experiment II.

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

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

3.7 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 solvent or 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 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 (6).

* Substitution buffer: 8.5 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 1.5 parts of KCl solution 0.15 M

3.8 Data evaluation

3.8.1 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).

3.8.2 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

3.8.3 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 the colony count of the corresponding solvent control is observed (1).

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration (6).

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.

3.8.4 Biometry

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

4.0 RESULTS AND DISCUSSION

4.1 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 toxic effects were observed , six concentrations were tested and 5000 µg/plate was chosen as maximal concentration in experiment II.

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

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

4.2 Discussion

The test item Glyphosate technical was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and the Escherichia coli strains WP2 uvrA pKM 101 and WP2 pKM 101.

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

Pre-Experiment /Experiment I:	3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate
Experiment II:	33; 100; 333; 1000; 2500; and 5000 µg/plate

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

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

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

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

The laboratory´s historical control range was exceeded in the untreated and solvent control of strain WP2 uvrA pKM101 with and without S9 mix in the pre-experiment/experiment I and in the untreated control (with S9 mix) and solvent (with and without S9 mix) of strain WP2 uvrA pKM101 in experiment II. In strain WP2 pKM101 the lower limit of the laboratorys historical control range was not quite reached in the untreated control with and without metabolic activation in experiment I. These elevated colony counts were considered to be the result of biologically irrelevant fluctuations in the number of colonies and had no detrimental impact on the outcome of the study.

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

5.0 CONCLUSION

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

6.0 REFERENCES

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TABLES SECTION

TABLE 1 Summary of Results Experiment I

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Deionised water		16 ± 5	15 ± 2	35 ± 5	135 ± 13	182 ± 12	480 ± 34
	Untreated		14 ± 1	14 ± 1	33 ± 9	138 ± 5	185 ± 6	476 ± 23
	Glyphosate technical	3 µg	14 ± 5	14 ± 1	28 ± 4	138 ± 18	193 ± 17	486 ± 27
		10 µg	18 ± 5	15 ± 2	33 ± 11	143 ± 10	202 ± 3	477 ± 34
		33 µg	13 ± 2	16 ± 2	29 ± 2	135 ± 2	188 ± 5	499 ± 38
		100 µg	14 ± 0	14 ± 2	29 ± 5	120 ± 10	189 ± 23	494 ± 27
		333 µg	15 ± 2	16 ± 2	31 ± 3	128 ± 7	207 ± 11	484 ± 20
		1000 µg	14 ± 3	12 ± 4	30 ± 6	138 ± 10	170 ± 15	451 ± 22
		2500 µg	11 ± 3	13 ± 3	24 ± 5	122 ± 8	167 ± 31	428 ± 9
		5000 µg	10 ± 2	14 ± 1	27 ± 4	89 ± 12	110 ± 16	427 ± 19
	NaN3	10 µg	1508 ± 52			1574 ± 118		
	4-NOPD	10 µg			302 ± 4			
	4-NOPD	50 µg		68 ± 4				
	MMS	3 µl					2705 ± 106	2997 ± 332
With Activation	Deionised water		18 ± 3	15 ± 2	38 ± 2	148 ± 18	193 ± 21	531 ± 37
	Untreated		17 ± 4	15 ± 5	39 ± 6	142 ± 24	212 ± 22	560 ± 10
	Glyphosate technical	3 µg	16 ± 4	16 ± 3	37 ± 6	157 ± 14	197 ± 26	536 ± 22
		10 µg	17 ± 3	16 ± 4	37 ± 6	145 ± 3	210 ± 22	493 ± 29
		33 µg	16 ± 3	16 ± 1	40 ± 3	157 ± 14	212 ± 42	545 ± 22
		100 µg	16 ± 2	16 ± 4	37 ± 7	149 ± 1	192 ± 12	489 ± 31
		333 µg	19 ± 3	18 ± 3	35 ± 6	159 ± 14	204 ± 25	496 ± 15
		1000 µg	17 ± 6	16 ± 1	33 ± 5	140 ± 12	179 ± 14	517 ± 26
		2500 µg	15 ± 3	14 ± 1	32 ± 3	132 ± 7	189 ± 29	456 ± 23
		5000 µg	20 ± 4	18 ± 3	30 ± 2	108 ± 7	136 ± 6	442 ± 4
	2-AA	2.5 µg	302 ± 50	378 ± 105	1188 ± 9	2215 ± 144		
	2-AA	10 µg					2333 ± 145	1930 ± 138

Key to Positive Controls

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

TABLE 2 Summary of Results Experiment II

Study Name: 1264500
Experiment: 1264500 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 07/10/2009
Date Counted: 13/10/2009

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	Deionised water		16 ± 4	12 ± 3	27 ± 2	140 ± 20	222 ± 8	474 ± 7
	Untreated		16 ± 4	12 ± 3	30 ± 4	137 ± 32	226 ± 10	433 ± 33
	Glyphosate technical	33 µg	16 ± 4	15 ± 2	26 ± 3	145 ± 9	256 ± 28	456 ± 31
		100 µg	17 ± 4	11 ± 3	29 ± 4	151 ± 7	239 ± 7	456 ± 6
		333 µg	16 ± 5	12 ± 2	28 ± 4	143 ± 15	241 ± 7	456 ± 26
		1000 µg	16 ± 4	13 ± 1	29 ± 1	130 ± 7	235 ± 8	437 ± 23
		2500 µg	12 ± 2	11 ± 3	25 ± 4	116 ± 18	231 ± 19	419 ± 38
		5000 µg	9 ± 1	10 ± 2	21 ± 2	102 ± 15	140 ± 10	358 ± 8
	NaN3	10 µg	1521 ± 275			1673 ± 255		
	4-NOPD	10 µg			366 ± 31			
	4-NOPD	50 µg		80 ± 3				
	MMS	3.0 µL					1657 ± 34	1777 ± 67
With Activation	Deionised water		17 ± 5	18 ± 1	37 ± 3	152 ± 16	266 ± 13	533 ± 33
	Untreated		18 ± 5	17 ± 2	34 ± 4	142 ± 13	290 ± 16	527 ± 20
	Glyphosate technical	33 µg	17 ± 3	19 ± 2	35 ± 1	144 ± 12	274 ± 31	555 ± 14
		100 µg	18 ± 2	18 ± 2	33 ± 4	152 ± 6	274 ± 21	603 ± 29
		333 µg	19 ± 4	18 ± 2	36 ± 2	147 ± 12	249 ± 33	577 ± 32
		1000 µg	12 ± 3	16 ± 5	35 ± 3	139 ± 10	270 ± 12	546 ± 37
		2500 µg	13 ± 1	17 ± 3	36 ± 1	142 ± 11	255 ± 16	483 ± 27
		5000 µg	10 ± 2	15 ± 3	22 ± 1	97 ± 20	228 ± 5	466 ± 43
	2-AA	2.5 µg	297 ± 16	237 ± 11	1651 ± 162	1840 ± 169		
	2-AA	10.0 µg					1259 ± 7	2095 ± 20

Key to Positive Controls

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

TABLE 3 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

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 technical**	3 µg	*14.0*	*5.3*	*0.9*	12, 20, 10
		10 µg	*17.7*	*5.0*	*1.1*	17, 23, 13
		33 µg	*13.3*	*1.5*	*0.8*	12, 15, 13
		100 µg	*14.0*	*0.0*	*0.9*	14, 14, 14
		333 µg	*15.0*	*1.7*	*0.9*	16, 16, 13
		1000 µg	*14.3*	*2.5*	*0.9*	17, 14, 12
		2500 µg	*11.0*	*2.6*	*0.7*	13, 12, 8
		5000 µg	*10.0*	*2.0*	*0.6*	12, 8, 10
	Deionised water		*16.3*	*4.9*		22, 13, 14
	Untreated Control		*14.0*	*1.0*		13, 14, 15
TA 1537	**Glyphosate technical**	3 µg	*14.3*	*0.6*	*0.9*	14, 15, 14
		10 µg	*14.7*	*2.3*	*1.0*	16, 16, 12
		33 µg	*15.7*	*1.5*	*1.0*	17, 14, 16
		100 µg	*14.0*	*2.0*	*0.9*	14, 16, 12
		333 µg	*15.7*	*1.5*	*1.0*	17, 14, 16
		1000 µg	*12.0*	*4.0*	*0.8*	12, 16, 8
		2500 µg	*12.7*	*3.1*	*0.8*	12, 10, 16
		5000 µg	*14.3*	*0.6*	*0.9*	15, 14, 14
	Deionised water		*15.3*	*1.5*		15, 17, 14
	Untreated Control		*14.3*	*0.6*		15, 14, 14
TA 98	**Glyphosate technical**	3 µg	*28.0*	*3.6*	*0.8*	31, 29, 24
		10 µg	*33.0*	*10.8*	*1.0*	36, 21, 42
		33 µg	*28.7*	*2.1*	*0.8*	31, 27, 28
		100 µg	*29.0*	*4.6*	*0.8*	33, 30, 24
		333 µg	*30.7*	*2.5*	*0.9*	33, 31, 28
		1000 µg	*30.3*	*5.5*	*0.9*	33, 34, 24
		2500 µg	*24.3*	*5.1*	*0.7*	23, 20, 30
		5000 µg	*27.0*	*3.6*	*0.8*	26, 31, 24
	Deionised water		*34.7*	*4.9*		29, 37, 38
	Untreated Control		*33.3*	*8.7*		26, 31, 43

TABLE 3 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation (continued)

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Glyphosate**	3 µg	*137.7*	*18.4*	*1.0*	158, 133, 122
	technical	10 µg	*143.0*	*9.5*	*1.1*	154, 138, 137
		33 µg	*135.3*	*2.1*	*1.0*	133, 136, 137
		100 µg	*120.3*	*9.8*	*0.9*	109, 126, 126
		333 µg	*128.3*	*7.2*	*1.0*	132, 133, 120
		1000 µg	*137.7*	*10.0*	*1.0*	137, 148, 128
		2500 µg	*121.7*	*8.0*	*0.9*	114, 121, 130
		5000 µg	*89.0*	*12.1*	*0.7*	102, 78, 87
	Deionised water		*134.7*	*13.0*		134, 122, 148
	Untreated Control		*137.7*	*4.7*		136, 143, 134
WP2 pKM101	**Glyphosate**	3 µg	*193.3*	*17.1*	*1.1*	182, 213, 185
	technical	10 µg	*202.0*	*3.5*	*1.1*	198, 204, 204
		33 µg	*188.0*	*4.6*	*1.0*	192, 183, 189
		100 µg	*188.7*	*23.0*	*1.0*	183, 169, 214
		333 µg	*207.0*	*10.5*	*1.1*	206, 218, 197
		1000 µg	*169.7*	*14.6*	*0.9*	154, 183, 172
		2500 µg	*166.7*	*31.0*	*0.9*	198, 166, 136
		5000 µg	*110.0*	*16.1*	*0.6*	125, 93, 112
	Deionised water		*181.7*	*12.3*		168, 185, 192
	Untreated Control		*184.7*	*5.5*		185, 179, 190
WP2 uvrA pKM101	**Glyphosate**	3 µg	*485.7*	*27.2*	*1.0*	517, 468, 472
	technical	10 µg	*477.3*	*34.4*	*1.0*	460, 455, 517
		33 µg	*499.3*	*37.6*	*1.0*	456, 518, 524
		100 µg	*494.0*	*27.2*	*1.0*	483, 474, 525
		333 µg	*484.3*	*19.6*	*1.0*	482, 505, 466
		1000 µg	*450.7*	*22.3*	*0.9*	442, 434, 476
		2500 µg	*428.0*	*8.5*	*0.9*	429, 419, 436
		5000 µg	*427.0*	*19.1*	*0.9*	438, 438, 405
	Deionised water		*480.3*	*33.7*		516, 476, 449
	Untreated Control		*475.7*	*22.5*		492, 450, 485

TABLE 3 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation (continued)

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

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	*1508.3*	*52.5*	*92.3*	1520, 1554, 1451
TA 1537	**4-NOPD**	50 µg	*68.3*	*4.0*	*4.5*	69, 64, 72
TA 98	**4-NOPD**	10 µg	*301.7*	*4.0*	*8.7*	306, 301, 298
TA 100	**NaN3**	10 µg	*1573.7*	*117.6*	*11.7*	1568, 1459, 1694
WP2 pKM101	**MMS**	3 µl	*2704.7*	*106.2*	*14.9*	2686, 2609, 2819
WP2 uvrA pKM101	**MMS**	3 µl	*2997.0*	*332.3*	*6.2*	3361, 2710, 2920

Key to Positive Controls

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

TABLE 4 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

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 technical**	3 µg	*16.3*	*3.5*	*0.9*	20, 13, 16
		10 µg	*17.3*	*2.5*	*0.9*	15, 20, 17
		33 µg	*16.0*	*2.6*	*0.9*	14, 15, 19
		100 µg	*15.7*	*1.5*	*0.9*	16, 17, 14
		333 µg	*19.3*	*2.5*	*1.1*	17, 19, 22
		1000 µg	*16.7*	*5.8*	*0.9*	10, 20, 20
		2500 µg	*14.7*	*2.5*	*0.8*	15, 12, 17
		5000 µg	*19.7*	*3.5*	*1.1*	20, 23, 16
	Deionised water		*18.3*	*3.2*		17, 22, 16
	Untreated Control		*17.3*	*4.0*		15, 22, 15
TA 1537	**Glyphosate technical**	3 µg	*16.0*	*3.0*	*1.0*	16, 13, 19
		10 µg	*16.3*	*3.5*	*1.1*	13, 20, 16
		33 µg	*16.3*	*0.6*	*1.1*	17, 16, 16
		100 µg	*15.7*	*3.8*	*1.0*	20, 14, 13
		333 µg	*17.7*	*2.9*	*1.2*	16, 21, 16
		1000 µg	*15.7*	*0.6*	*1.0*	15, 16, 16
		2500 µg	*14.3*	*0.6*	*0.9*	14, 15, 14
		5000 µg	*17.7*	*3.2*	*1.2*	20, 19, 14
	Deionised water		*15.3*	*2.1*		17, 13, 16
	Untreated Control		*14.7*	*4.5*		15, 19, 10
TA 98	**Glyphosate technical**	3 µg	*37.0*	*6.1*	*1.0*	30, 41, 40
		10 µg	*37.0*	*6.1*	*1.0*	40, 41, 30
		33 µg	*40.0*	*2.6*	*1.0*	37, 41, 42
		100 µg	*37.3*	*6.5*	*1.0*	37, 44, 31
		333 µg	*35.3*	*5.5*	*0.9*	41, 35, 30
		1000 µg	*33.3*	*4.6*	*0.9*	36, 28, 36
		2500 µg	*32.0*	*3.5*	*0.8*	36, 30, 30
		5000 µg	*29.7*	*1.5*	*0.8*	28, 30, 31
	Deionised water		*38.3*	*1.5*		40, 38, 37
	Untreated Control		*38.7*	*5.7*		34, 37, 45

TABLE 4 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation (continued)

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 100	**Glyphosate technical**	3 µg	*157.0*	*13.5*	*1.1*	170, 158, 143
		10 µg	*145.3*	*3.2*	*1.0*	144, 143, 149
		33 µg	*157.3*	*14.0*	*1.1*	156, 172, 144
		100 µg	*149.0*	*1.0*	*1.0*	149, 148, 150
		333 µg	*159.0*	*14.0*	*1.1*	159, 145, 173
		1000 µg	*140.3*	*11.5*	*0.9*	140, 129, 152
		2500 µg	*131.7*	*7.2*	*0.9*	140, 127, 128
		5000 µg	*108.0*	*7.2*	*0.7*	106, 116, 102
	Deionised water		*148.3*	*17.8*		129, 164, 152
	Untreated Control		*141.7*	*24.2*		152, 114, 159
WP2 pKM101	**Glyphosate technical**	3 µg	*196.7*	*25.7*	*1.0*	226, 178, 186
		10 µg	*210.3*	*21.9*	*1.1*	185, 223, 223
		33 µg	*212.0*	*42.2*	*1.1*	249, 221, 166
		100 µg	*192.3*	*11.9*	*1.0*	184, 187, 206
		333 µg	*203.7*	*24.9*	*1.1*	232, 194, 185
		1000 µg	*179.0*	*14.0*	*0.9*	179, 193, 165
		2500 µg	*189.0*	*28.9*	*1.0*	222, 177, 168
		5000 µg	*135.7*	*6.0*	*0.7*	135, 142, 130
	Deionised water		*192.7*	*21.1*		187, 175, 216
	Untreated Control		*212.3*	*21.5*		233, 214, 190
WP2 uvrA pKM101	**Glyphosate technical**	3 µg	*535.7*	*22.5*	*1.0*	555, 541, 511
		10 µg	*493.3*	*29.1*	*0.9*	470, 484, 526
		33 µg	*545.3*	*22.3*	*1.0*	562, 554, 520
		100 µg	*488.7*	*30.6*	*0.9*	472, 524, 470
		333 µg	*496.3*	*15.1*	*0.9*	479, 503, 507
		1000 µg	*517.3*	*25.7*	*1.0*	502, 503, 547
		2500 µg	*455.7*	*23.1*	*0.9*	429, 469, 469
		5000 µg	*442.0*	*4.4*	*0.8*	447, 439, 440
	Deionised water		*531.0*	*37.3*		563, 490, 540
	Untreated Control		*560.3*	*9.8*		566, 566, 549

TABLE 4 Pre-Experiment and Experiment I: 1264500 VV Plate Incorporation (continued)

Study Name: 1264500
Experiment: 1264500 VV Plate
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 23/09/2009
Date Counted: 29/09/2009

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	*302.0*	*50.3*	*16.5*	276, 360, 270
TA 1537	**2-AA**	2.5 µg	*377.7*	*105.5*	*24.6*	497, 339, 297
TA 98	**2-AA**	2.5 µg	*1188.3*	*9.3*	*31.0*	1178, 1191, 1196
TA 100	**2-AA**	2.5 µg	*2214.7*	*143.9*	*14.9*	2094, 2374, 2176
WP2 pKM101	**2-AA**	10 µg	*2333.0*	*144.5*	*12.1*	2476, 2187, 2336
WP2 uvrA pKM101	**2-AA**	10 µg	*1930.0*	*137.9*	*3.6*	1818, 2084, 1888

Key to Positive Controls

2-AA 2-aminoanthracene

TABLE 5 Experiment II: 1264500 HV2 Pre-Incubation

Study Name: 1264500
Experiment: 1264500 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 07/10/2009
Date Counted: 13/10/2009

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 technical**	33 µg	*16.3*	*3.5*	*1.0*	16, 13, 20
		100 µg	*17.0*	*3.6*	*1.1*	14, 16, 21
		333 µg	*15.7*	*4.6*	*1.0*	13, 13, 21
		1000 µg	*15.7*	*3.8*	*1.0*	14, 20, 13
		2500 µg	*12.0*	*1.7*	*0.8*	10, 13, 13
		5000 µg	*8.7*	*0.6*	*0.6*	9, 9, 8
	Deionised water		*15.7*	*3.8*		13, 20, 14
	Untreated Control		*15.7*	*4.0*		15, 12, 20
TA 1537	**Glyphosate technical**	33 µg	*14.7*	*2.3*	*1.2*	16, 16, 12
		100 µg	*11.3*	*3.1*	*0.9*	8, 14, 12
		333 µg	*11.7*	*1.5*	*1.0*	13, 12, 10
		1000 µg	*13.3*	*1.2*	*1.1*	14, 14, 12
		2500 µg	*11.3*	*2.9*	*0.9*	8, 13, 13
		5000 µg	*10.0*	*1.7*	*0.8*	12, 9, 9
	Deionised water		*12.0*	*3.5*		10, 10, 16
	Untreated Control		*12.0*	*3.5*		10, 16, 10
TA 98	**Glyphosate technical**	33 µg	*26.3*	*2.5*	*1.0*	26, 29, 24
		100 µg	*29.3*	*4.2*	*1.1*	34, 28, 26
		333 µg	*27.7*	*4.0*	*1.0*	23, 30, 30
		1000 µg	*29.3*	*1.2*	*1.1*	30, 28, 30
		2500 µg	*25.0*	*4.4*	*0.9*	22, 30, 23
		5000 µg	*20.7*	*1.5*	*0.8*	22, 21, 19
	Deionised water		*27.0*	*1.7*		26, 29, 26
	Untreated Control		*29.7*	*3.8*		34, 27, 28
TA 100	**Glyphosate technical**	33 µg	*145.0*	*8.9*	*1.0*	148, 135, 152
		100 µg	*151.0*	*6.9*	*1.1*	155, 143, 155
		333 µg	*142.7*	*15.3*	*1.0*	151, 152, 125
		1000 µg	*129.7*	*6.5*	*0.9*	136, 130, 123
		2500 µg	*115.7*	*18.3*	*0.8*	122, 130, 95
		5000 µg	*102.3*	*14.7*	*0.7*	97, 91, 119
	Deionised water		*140.0*	*20.3*		144, 118, 158
	Untreated Control		*137.0*	*32.2*		147, 163, 101

TABLE 5 Experiment II: 1264500 HV2 Pre-Incubation (continued)

Study Name: 1264500
Experiment: 1264500 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 07/10/2009
Date Counted: 13/10/2009

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	**Glyphosate technical**	33 µg	*256.3*	*28.3*	*1.2*	289, 241, 239
		100 µg	*239.0*	*7.0*	*1.1*	232, 246, 239
		333 µg	*241.0*	*7.2*	*1.1*	243, 247, 233
		1000 µg	*235.3*	*8.1*	*1.1*	244, 228, 234
		2500 µg	*231.3*	*18.6*	*1.0*	212, 233, 249
		5000 µg	*140.0*	*9.5*	*0.6*	141, 149, 130
	Deionised water		*222.0*	*8.0*		222, 230, 214
	Untreated Control		*226.3*	*9.6*		235, 228, 216
WP2 uvrA pKM101	**Glyphosate technical**	33 µg	*456.3*	*30.6*	*1.0*	421, 474, 474
		100 µg	*456.0*	*6.1*	*1.0*	460, 449, 459
		333 µg	*456.3*	*25.5*	*1.0*	456, 482, 431
		1000 µg	*437.0*	*22.9*	*0.9*	463, 420, 428
		2500 µg	*418.7*	*38.1*	*0.9*	385, 411, 460
		5000 µg	*358.3*	*7.6*	*0.8*	355, 367, 353
	Deionised water		*473.7*	*6.7*		472, 481, 468
	Untreated Control		*433.3*	*32.5*		398, 462, 440
TA 1535	**NaN3**	10 µg	*1521.0*	*274.6*	*97.1*	1675, 1204, 1684
TA 1537	**4-NOPD**	50 µg	*80.3*	*3.1*	*6.7*	83, 77, 81
TA 98	**4-NOPD**	10 µg	*366.3*	*30.5*	*13.6*	368, 335, 396
TA 100	**NaN3**	10 µg	*1672.7*	*255.4*	*11.9*	1856, 1381, 1781
WP2 pKM101	**MMS**	3.0 µL	*1657.0*	*34.0*	*7.5*	1657, 1623, 1691
WP2 uvrA pKM101	**MMS**	3.0 µL	*1777.3*	*67.2*	*3.8*	1822, 1700, 1810

Key to Positive Controls

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

TABLE 6 Experiment II: 1264500 HV2 Pre-Incubation

Study Name: 1264500
Experiment: 1264500 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 07/10/2009
Date Counted: 13/10/2009

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 technical**	33 µg	*17.3*	*3.2*	*1.0*	21, 16, 15
		100 µg	*17.7*	*2.3*	*1.0*	19, 15, 19
		333 µg	*19.0*	*4.4*	*1.1*	16, 24, 17
		1000 µg	*12.0*	*3.5*	*0.7*	10, 10, 16
		2500 µg	*13.3*	*0.6*	*0.8*	13, 14, 13
		5000 µg	*9.7*	*2.1*	*0.6*	12, 9, 8
	Deionised water		*17.3*	*5.1*		16, 13, 23
	Untreated Control		*17.7*	*5.1*		12, 19, 22
TA 1537	**Glyphosate technical**	33 µg	*19.3*	*2.1*	*1.1*	21, 17, 20
		100 µg	*18.0*	*1.7*	*1.0*	20, 17, 17
		333 µg	*18.3*	*2.1*	*1.0*	19, 16, 20
		1000 µg	*16.0*	*4.6*	*0.9*	15, 12, 21
		2500 µg	*17.3*	*2.9*	*0.9*	19, 14, 19
		5000 µg	*15.0*	*2.6*	*0.8*	17, 12, 16
	Deionised water		*18.3*	*1.2*		19, 17, 19
	Untreated Control		*16.7*	*2.1*		19, 15, 16
TA 98	**Glyphosate technical**	33 µg	*34.7*	*1.2*	*0.9*	36, 34, 34
		100 µg	*33.3*	*3.8*	*0.9*	36, 35, 29
		333 µg	*36.0*	*1.7*	*1.0*	35, 35, 38
		1000 µg	*35.3*	*2.5*	*0.9*	38, 35, 33
		2500 µg	*35.7*	*1.2*	*1.0*	37, 35, 35
		5000 µg	*22.3*	*1.2*	*0.6*	23, 23, 21
	Deionised water		*37.3*	*2.5*		35, 40, 37
	Untreated Control		*33.7*	*4.2*		29, 35, 37
TA 100	**Glyphosate technical**	33 µg	*144.0*	*11.8*	*0.9*	141, 134, 157
		100 µg	*151.7*	*6.4*	*1.0*	159, 148, 148
		333 µg	*147.3*	*11.7*	*1.0*	156, 134, 152
		1000 µg	*139.0*	*10.4*	*0.9*	151, 132, 134
		2500 µg	*141.7*	*11.0*	*0.9*	154, 133, 138
		5000 µg	*97.0*	*20.0*	*0.6*	92, 119, 80
	Deionised water		*151.7*	*16.1*		145, 170, 140
	Untreated Control		*142.0*	*13.2*		127, 152, 147

TABLE 6 Experiment II: 1264500 HV2 Pre-Incubation (continued)

Study Name: 1264500
Experiment: 1264500 HV2 Pre
Assay Conditions:

Study Code: Harlan CCR 1264500
Date Plated: 07/10/2009
Date Counted: 13/10/2009

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 pKM101	**Glyphosate technical**	33 µg	*273.7*	*31.1*	*1.0*	283, 239, 299
		100 µg	*273.7*	*21.4*	*1.0*	265, 298, 258
		333 µg	*248.7*	*32.7*	*0.9*	286, 225, 235
		1000 µg	*270.3*	*12.0*	*1.0*	271, 282, 258
		2500 µg	*255.0*	*15.6*	*1.0*	264, 237, 264
		5000 µg	*228.0*	*4.6*	*0.9*	229, 223, 232
	Deionised water		*266.0*	*12.8*		263, 255, 280
	Untreated Control		*290.3*	*15.5*		308, 279, 284
WP2 uvrA pKM101	**Glyphosate technical**	33 µg	*554.7*	*13.5*	*1.0*	541, 568, 555
		100 µg	*603.0*	*28.6*	*1.1*	570, 619, 620
		333 µg	*577.3*	*31.9*	*1.1*	543, 606, 583
		1000 µg	*546.3*	*37.0*	*1.0*	589, 525, 525
		2500 µg	*482.7*	*27.3*	*0.9*	470, 464, 514
		5000 µg	*466.3*	*43.4*	*0.9*	516, 447, 436
	Deionised water		*532.7*	*33.0*		539, 497, 562
	Untreated Control		*527.3*	*20.4*		536, 504, 542
TA 1535	**2-AA**	2.5 µg	*297.0*	*15.7*	*17.1*	294, 314, 283
TA 1537	**2-AA**	2.5 µg	*237.0*	*11.4*	*12.9*	250, 232, 229
TA 98	**2-AA**	2.5 µg	*1651.3*	*161.6*	*44.2*	1826, 1621, 1507
TA 100	**2-AA**	2.5 µg	*1840.3*	*169.4*	*12.1*	1853, 2003, 1665
WP2 pKM101	**2-AA**	10.0 µg	*1259.3*	*6.7*	*4.7*	1255, 1256, 1267
WP2 uvrA pKM101	**2-AA**	10.0 µg	*2095.3*	*20.2*	*3.9*	2072, 2108, 2106

Key to Positive Controls

2-AA 2-aminoanthracene

APPENDICES SECTION

APPENDIX 1 Historical Control Data

These data represent the laboratory´s historical control data from January 2008 until October 2008 representing approx. 600 experiments (WP2 uvrA pKM101 the historical data from January 2008 until October 2008 are based on approx. 150 experiments; the control data for strain WP2 pKM101 are from January 2008 until October 2008 are based on approx. 80 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	17	5.17	9	39	21	5.82	8	41
	Negative control	17	5.33	9	38	20	6.23	10	46
	Positive control	2024	315.78	1041	3138	294	140.02	102	945
TA1537	Solvent control	13	3.12	6	25	17	3.90	9	35
	Negative control	13	3.38	5	26	18	4.05	8	31
	Positive control	116	30.52	68	407	204	69.54	72	454
TA 98	Solvent control	30	5.59	13	59	39	6.34	20	60
	Negative control	31	5.45	16	55	39	6.53	19	59
	Positive control	489	169.76	211	1694	1455	463.01	200	3553
TA 100	Solvent control	130	18.79	89	224	155	22.54	92	218
	Negative control	139	17.30	93	205	147	21.78	92	234
	Positive control	2160	342.67	588	3379	1839	621.27	404	3868
WP2uvrA pKM101	Solvent control	374	47.29	240	454	406	44.26	268	506
	Negative control	380	42.63	255	446	441	50.20	285	512
	Positive control	3058	168.37	1369	5367	1920	468.32	1163	3597
WP2 pKM 101	Solvent control	251	37.68	157	312	281	44.48	174	358
	Negative control	273	39.53	188	339	322	54.45	216	440
	Positive control	3020	830.78	1522	4451	1972	652.16	1043	3848

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

APPENDIX 2 Copy of GLP Certificate

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.

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

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

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]

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

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

APPENDIX 3 Certificate of Analysis

syngenta

Syngenta Crop Protection, Inc.
Technology & Projects
Analytical & Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

Glyphosate Technical
Batch ID 569753 (BX20070911)

Batch Identification	569753
Product Design Code	ASF71X
Product by Common Name	Glyphosate
Source	Nantong Jiangshan Agrochemicals & Chemicals Limited, Jiangsu, China
Other ID	BX20070911
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient*	Confirmed (LC/MS)
Glyphosate*	96.3% (wt/wt)
Methodology Used for Characterization	HPLC
Physical Analysis	
Appearance*	White powder (dry)
Stability[1]	
Storage Temperature	<10°C
Expiration date	AUG-2010

[1]Transport and handling for lab use at room temperature is acceptable. Refrigeration is recommended if storing beyond two weeks to minimize moisture uptake.

The stability of this test substance will be determined concurrently through reanalysis of material held in inventory under GLP conditions at Syngenta Crop Protection, Inc., Greensboro, NC.

This Certificate of Analysis is summarizing data (marked with an asterisk) from a study that has been performed in compliance with Good Laboratory Practices per 40 CFR Part 160. Raw data, documentation, protocols, any amendments to study protocols and reports pertaining to this study are maintained in the Syngenta Crop Protection Archives in Greensboro, NC.
Authorization:

[redacted] 9-11-09
Date

Senior Analytical Chemist
Analytical & Product Chemistry Department