REPORT

Glyphosate:
Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*

Study Director: [redacted]

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Harlan Study Number: **41401854**

Study Completion Date: 02 September 2014

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STUDY DIRECTOR STATEMENT OF GLP COMPLIANCE

Harlan Laboratories Ltd., Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK

Harlan Study Number: 41401854
Study Title: Glyphosate: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*

With the exception noted below this study was performed in compliance with UK GLP standards (Schedule 1, Good Laboratory Practice Regulations 1999 (SI 1999/3106 as amended by SI 2004/0994)). These Regulations are in accordance with GLP standards published as OECD Principles on Good Laboratory Practice (revised 1997, ENV/MC/CHEM(98)17); and are in accordance with, and implement, the requirements of Directives 2004/9/EC and 2004/10/EC.

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI).

No analysis was carried out to determine the homogeneity, concentration or stability of the test item formulation. The test item was formulated within four hours of it being applied to the test system; it is assumed that the formulation was stable for this duration. This exception is considered not to affect the purpose or integrity of the study.

This report fully and accurately reflects the procedures used and data generated. There were no circumstances considered to have affected the integrity of the study or the validity of the data.

Study Director: [redacted]

Date: 02 SEP 2014

QUALITY ASSURANCE STATEMENT

Harlan Study Number: 41401854
Study Title: Glyphosate: Reverse Mutation Assay 'Ames Test' using *Salmonella typhimurium* and *Escherichia coli*

The general facilities and activities are inspected at least once a year and the results are reported to the relevant responsible person and management.

Study-related procedures conducted at the test facility were audited and inspected. The details of these audits and inspections are given below.

Dates and Types of QA Inspections			**Reported to the relevant Study Director and Test Facility Management**
Date of Inspection	Type of Inspection	Phase Inspected	Report Date
18 July 2014	Study Plan Verification	N/A	18 July 2014
07 July 2014	Process – based	Test Item Preparation	07 July 2014
16 July 2014 22 July 2014	Process – based	Test System Preparation and Application	16 July 2014 22 July 2014
15 July 2014	Process – based	Assessment of Response	15 July 2014
20 August 2014	Report Audit	N/A	20 August 2014

This statement confirms that this report reflects the raw data and the procedures followed.

Quality Assurance: [redacted]

..... [redacted] .....
Date:

02 SEP 2014

SUMMARY

Introduction

The test method was designed to be compatible with the guidelines for bacterial mutagenicity testing published by the major Japanese Regulatory Authorities including METI, MHLW and MAFF, the OECD Guidelines for Testing of Chemicals No. 471 "Bacterial Reverse Mutation Test", Method B13/14 of Commission Regulation (EC) number 440/2008 of 30 May 2008 and the USA, EPA OCSPP harmonized guideline - Bacterial Reverse Mutation Test.

Methods

Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strain WP2*uvrA* were treated with suspensions of the test item using both the Ames plate incorporation and pre-incubation methods at up to eight dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolizing system (10% liver S9 in standard co-factors). The dose range for Experiment 1 was predetermined and was nominally 1.5 to 5000 µg/plate. The experiment was repeated on a separate day (pre-incubation method) using fresh cultures of the bacterial strains and fresh test item formulations. The dose range was amended following the results of Experiment 1 and was nominally 5 to 5000 µg/plate.

Seven test item dose levels were selected in Experiment 2 in order to achieve both four non-toxic dose levels and the potential toxic limit of the test item following the change in test methodology.

Results

The vehicle (dimethyl sulphoxide) control plates gave counts of revertant colonies within the normal range. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with or without metabolic activation. Thus, the sensitivity of the assay and the efficacy of the S9-mix were validated.

The maximum dose level of the test item in the first experiment was selected as the maximum recommended dose level of 5000 µg/plate. The test item induced a visible reduction in the growth of the bacterial background lawns of all of the tester strains dosed in the absence of S9-mix at 5000 µg/plate. Although there was no toxicity in the bacterial background lawns noted to any of the tester strains dosed in the presence of S9-mix, several strains exhibited lower frequencies of revertant colonies at the maximum dose level. These results were not indicative of toxicity sufficiently severe enough to prevent the test item being tested up to the maximum recommended dose level of 5000 µg/plate in the second mutation test. Second experiment results were identical to the first mutation test with weakened lawns noted at 5000 µg/plate to all of the strains dosed in the absence of S9-mix with several strains observed to have decreased numbers of colonies present in the presence of S9-mix at the same dose level. No test item

precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

There were no significant increases in the frequency of revertant colonies recorded for any of the bacterial strains, with any dose of the test item, either with or without metabolic activation in Experiment 1 (plate incorporation method). Similarly, no significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test item, either with or without metabolic activation in Experiment 2 (pre-incubation method).

Conclusion

Glyphosate was considered to be non-mutagenic under the conditions of this test.

GENERAL INFORMATION

Schedule

Experimental Starting Date: 16 July 2014
Experimental Completion Date: 31 August 2014

Deviations from Study Plan

There were no deviations (unplanned changes) from the study plan.

Archiving

Unless instructed otherwise by the Sponsor, the study plan, (general study plan and study specific supplement), all raw data (paper and electronic) and the final report will be retained in the Harlan Laboratories Ltd, Shardlow, UK archives for five years after which instructions will be sought as to further retention or disposal. Further retention or return of the data will be chargeable to the Sponsor.

No data will be discarded without contacting the Sponsor to obtain their written consent.

1 INTRODUCTION AND PURPOSE

The purpose of the study was to evaluate Glyphosate for the ability to induce reverse mutations, either directly or after metabolic activation, at the histidine or tryptophan locus in the genome of five strains of bacteria.

The study was based on the *in vitro* technique described by Ames *et al.,* (1975), Maron and Ames (1983) and Mortelmans and Zeiger (2000), in which mutagenic effects are determined by exposing mutant strains of *Salmonella typhimurium* to various concentrations of the test item. These strains have a deleted excision repair mechanism which makes them more sensitive to various mutagens and they will not grow on media which does not contain histidine. When large numbers of these organisms are exposed to a mutagen, reverse mutation to the original histidine independent form takes place. These are readily detectable due to their ability to grow on a histidine deficient medium. Using these strains of *Salmonella typhimurium* revertants may be produced after exposure to a chemical mutagen, which have arisen as a result of a base-pair substitution in the genetic material (miscoding) or as a frameshift mutation in which genetic material is either added or deleted. Additionally, a mutant strain of *Escherichia coli* (WP2*uvrA*) which requires tryptophan and can be reverse mutated by base substitution to tryptophan independence (Green and Muriel, 1976 and Mortelmans and Riccio, 2000) is used to complement the *Salmonella* strains.

Since many compounds do not exert a mutagenic effect until they have been metabolized by enzyme systems not available in the bacterial cell, the test item and the bacteria are also incubated in the presence of a liver microsomal preparation (S9-mix) prepared from rats pre-treated with a mixture known to induce an elevated level of these enzymes.

A copy of the Certificate of Compliance with GLP, issued by the UK Department of Health, is included as Appendix 3.

1.1 Guidelines / Regulations

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- OECD Guidelines for Testing of Chemicals No. 471 (1997) "Bacterial Reverse Mutation Test", Method B13/14 of Commission Regulation (EC) number 440/2008 of 30 May 2008.
- USA, EPA OCSPP harmonized guideline 870.5100 - Bacterial Reverse Mutation Test.
- Japanese Ministry of Economy, Trade and Industry, Japanese Ministry of Health, Labour and Welfare and Japanese Ministry of Agriculture, Forestry and Fisheries.

2 TEST ITEM

Information as provided by the Sponsor. A copy of the Certificate of Analysis supplied by the Sponsor is given in Appendix 1.

Identification:	Glyphosate
Batch:	04062014
Purity:	85.79% †
Physical state/Appearance:	White crystalline solid
Expiry Date:	04 June 2016
Storage Conditions:	Room temperature in the dark

Formulated concentrations were adjusted to allow for the stated water/impurity content (14.21%) of the test item.

† The purity has been amended since the completion of the experimental phase. The previous purity was stated as 87.8% w/w. Consequently, the test item has been slightly under tested by 2.01%. However, the test item caused toxicity at the maximum dose level tested in the majority of tester strains, thereby indicating that there had been adequate exposure of the strains to the test item. The test, therefore, is considered to be valid and the change in purity did not affect the integrity of the assay.

3 MATERIALS AND METHODS

3.1 Test System

3.1.1 Tester Strains

The five strains of bacteria used, and their mutations, are as follows:

Salmonella typhimurium

Strains	**Genotype**	**Type of mutations indicated**
TA1537	his C 3076; *rfa*$^-$; *uvrB*$^-$:	frame shift mutations
TA98	his D 3052; *rfa*$^-$; *uvrB*$^-$;R-factor	
TA1535	his G 46; *rfa*$^-$; *uvrB*$^-$:	base-pair substitutions
TA100	his G 46; *rfa*$^-$; *uvrB*$^-$;R-factor	

Escherichia coli

Strain	**Genotype**	**Type of mutations indicated**
WP2*uvrA*	trp$^-$; *uvrA*$^-$:	base-pair substitution

All of the *Salmonella* strains are histidine dependent by virtue of a mutation through the histidine operon and 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 coat to the bacterial cell surface thus increasing the cell permeability to larger molecules. A further mutation, through the deletion of the *uvrB*$^-$*bio* gene, causes an inactivation of the excision repair system and a dependence on exogenous biotin. In the strains TA98 and TA100, the R-factor plasmid pKM101 enhances chemical and UV-induced mutagenesis via an increase in the error-prone repair pathway. The plasmid also confers ampicillin resistance which acts as a convenient marker (Mortelmans and Zeiger, 2000). In addition to a mutation in the tryptophan operon, the *E. coli* tester strain contains a *uvrA*$^-$ DNA repair deficiency which enhances its sensitivity to some mutagenic compounds. This deficiency allows the strain to show enhanced mutability as the *uvrA* repair system would normally act to remove and repair the damaged section of the DNA molecule (Green and Muriel, 1976 and Mortelmans and Riccio, 2000).

The bacteria used in the test were obtained from the University of California, Berkeley, on culture discs, on 04 August 1995 and from the British Industrial Biological Research Association, on a nutrient agar plate, on 17 August 1987. All of the strains were stored at approximately -196 °C in a Statebourne liquid nitrogen freezer, model SXR 34.

In this assay, overnight sub-cultures of the appropriate coded stock cultures were prepared in nutrient broth (Oxoid Limited; lot number 1408880 10/18) and incubated at 37 °C for approximately 10 hours. Each culture was monitored spectrophotometrically for turbidity with titres determined by viable count analysis on nutrient agar plates.

3.2 Test and Control Items Preparation

3.2.1 Test Item

The test item was insoluble in sterile distilled water, dimethyl sulphoxide, dimethyl formamide and acetonitrile at 50 mg/mL, acetone at 100 mg/mL and tetrahydrofuran at 200 mg/mL in solubility checks performed in–house. The test item formed the best doseable suspension in dimethyl sulphoxide, therefore, this solvent was selected as the vehicle.

The test item was accurately weighed and approximate half-log dilutions prepared in dimethyl sulphoxide by mixing on a vortex mixer and sonication for 10 minutes at 40 °C on the day of each experiment. To aid the suspension of the 50 mg/mL preparation of test item in dimethyl sulphoxide, the formulation was maintained at approximately 40 °C throughout dosing. Formulated concentrations were adjusted to allow for the stated water/impurity content (12.2%) of the test item. The purity has been amended since the completion of the experimental phase. The previous purity was stated as 87.8% w/w. Consequently, the test item has been slightly under tested by 2.01%. However, the test item caused toxicity at the maximum dose level tested in the majority of tester strains, thereby indicating that there had been adequate exposure of the strains to the test item. The dose levels reported should be considered as nominal rather than actual values. The test, therefore, is considered to be valid and the change in purity did not affect the integrity of the assay.

All formulations were used within four hours of preparation and were assumed to be stable for this period. Analysis for concentration, homogeneity and stability of the test item formulations is not a requirement of the test guidelines and was, therefore, not determined. This is an exception with regard to GLP and has been reflected in the GLP compliance statement. Prior to use, the solvent was dried to remove water using molecular sieves i.e. 2 mm sodium alumino-silicate pellets with a nominal pore diameter of 4 x 10^{-4} microns.

3.2.2 Control Items

Vehicle, negative (untreated) and positive controls were used in parallel with the test item.

The vehicle control used was dimethyl sulphoxide.

The positive control items used in the series of plates without S9-mix were as follows:

N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	2 µg/plate for WP2*uvrA*
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	3 µg/plate for TA100
N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG):	5 µg/plate for TA1535
9-Aminoacridine (9AA):	80 µg/plate for TA1537
4-Nitroquinoline-1-oxide (4NQO):	0.2 µg/plate for TA98

In addition, 2-Aminoanthracene (2AA) and Benzo(a)pyrene (BP), which are non-mutagenic in the absence of metabolizing enzymes, were used in the series of plates with S9-mix at the following concentrations:

2-Aminoanthracene (2AA):	1 µg/plate for TA100
2-Aminoanthracene (2AA):	2 µg/plate for TA1535 and TA1537
2-Aminoanthracene (2AA):	10 µg/plate for WP*2uvrA*
Benzo(a)pyrene (BP):	5 µg/plate for TA98

3.3 Microsomal Enzyme Fraction

Lot No's PB/βNF S9 01 June 2014 (Experiment 1) and 02 March 2014 (Experiment 2) were used in this study. The S9 Microsomal fraction was prepared in-house from male rats induced with Phenobarbitone/β-Naphthoflavone at 80/100 mg/kg/day, orally, for 3 days prior to preparation on day 4. The S9 homogenate was produced by homogenizing the liver in a 0.15M KCl solution (1g liver to 3 mL KCl) followed by centrifugation at 9000 *g*. The protein content of the resultant supernatant was adjusted to 20 mg/mL. Aliquots of the supernatant were frozen and stored at approximately -196 °C. Prior to use, each batch of S9 was tested for its capability to activate known mutagens in the Ames test.

This procedure was designed and conducted to cause the minimum suffering or distress to the animals consistent with the scientific objectives and in accordance with the Harlan Laboratories Ltd, Shardlow, UK policy on animal welfare and the requirements of the United Kingdom's Animals (Scientific Procedure) Act 1986 Amendment Regulations 2012. The conduct of the procedure may be reviewed, as part of the Harlan Laboratories Ltd, Shardlow, UK Ethical Review Process.

3.4 S9-Mix and Agar

The S9-mix was prepared before use using sterilized co-factors and maintained on ice for the duration of the test.

S9	5.0 mL
1.65 M KCl/0.4 M $MgCl_2$	1.0 mL
0.1 M Glucose-6-phosphate	2.5 mL
0.1 M NADP	2.0 mL
0.2 M Sodium phosphate buffer (pH 7.4)	25.0 mL
Sterile distilled water	14.5 mL

A 0.5 mL aliquot of S9-mix and 2 mL of molten, trace histidine or tryptophan supplemented, top agar were overlaid onto a sterile Vogel-Bonner Minimal agar plate in order to assess the sterility of the S9-mix. This procedure was repeated, in triplicate, on the day of each experiment.

Top agar was prepared using 0.6% Bacto agar (lot number 3218431 04/18) and 0.5% sodium chloride with 5 mL of 1.0 mM histidine and 1.0 mM biotin or 1.0 mM tryptophan solution added to each 100 mL of top agar. Vogel-Bonner Minimal agar plates were purchased from SGL Ltd (lot numbers 37143 07/14 and 37160 08/14).

3.5 Test Procedure

3.5.1 Test for Mutagenicity (Experiment 1) – Plate Incorporation Method

3.5.1.1 Dose selection

The test item was tested using the following method. The maximum concentration was 5000 µg/plate (the maximum recommended dose level). Eight concentrations of the test item (1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate) were assayed in triplicate against each tester strain, using the direct plate incorporation method.

3.5.1.2 Without Metabolic Activation

0.1 mL of the appropriate concentration of test item, vehicle or appropriate positive control was added to 2 mL of molten trace amino-acid supplemented media containing 0.1 mL of one of the bacterial strain cultures and 0.5 mL of phosphate buffer. These were then mixed and overlayed onto a Vogel-Bonner agar plate. Negative (untreated) controls were also performed on the same day as the mutation test. Each concentration of the test item, appropriate positive, vehicle and negative controls, and each bacterial strain, was assayed using triplicate plates.

3.5.1.3 With Metabolic Activation

The procedure was the same as described previously (see 3.5.1.2) except that following the addition of the test item formulation and bacterial culture, 0.5 mL of S9-mix was added to the molten trace amino-acid supplemented media instead of phosphate buffer.

3.5.1.4 Incubation and Scoring

All of the plates were incubated at 37 °C± 3 °C for approximately 48 hours and scored for the presence of revertant colonies using an automated colony counting system. The plates were viewed microscopically for evidence of thinning (toxicity).

3.5.2 Test for Mutagenicity (Experiment 2) – Pre-Incubation Method

As Experiment 1 was deemed negative, Experiment 2 was performed using the pre-incubation method in the presence and absence of metabolic activation.

3.5.2.1 Dose selection

The dose range used for Experiment 2 was determined by the results of Experiment 1 and was 5 to 5000 µg/plate.

Seven test item dose levels were selected in Experiment 2 in order to achieve both four non-toxic dose levels and the potential toxic limit of the test item following the change in test methodology.

3.5.2.2 Without Metabolic Activation

0.1 mL of the appropriate bacterial strain culture, 0.5 mL of phosphate buffer and 0.1 mL of the test item formulation, vehicle or 0.1 mL of appropriate positive control were incubated at 37 °C± 3 °C for 20 minutes (with shaking) prior to addition of 2 mL of molten amino-acid supplemented media and subsequent plating onto Vogel-Bonner plates. Negative (untreated) controls were also performed on the same day as the mutation test employing the plate incorporation method. All testing for this experiment was performed in triplicate.

3.5.2.3 With Metabolic Activation

The procedure was the same as described previously (see 3.5.2.2) except that following the addition of the test item formulation and bacterial strain culture, 0.5 mL of S9-mix was added to the tube instead of phosphate buffer, prior to incubation at 37 °C± 3 °C for 20 minutes (with shaking) and addition of molten amino-acid supplemented media. All testing for this experiment was performed in triplicate.

3.5.2.4 Incubation and Scoring

All of the plates were incubated at 37 °C± 3 °C for approximately 48 hours and scored for the presence of revertant colonies using an automated colony counting system. The plates were viewed microscopically for evidence of thinning (toxicity).

3.6 Acceptance Criteria

The reverse mutation assay may be considered valid if the following criteria are met:

All bacterial strains must have demonstrated the required characteristics as determined by their respective strain checks according to Ames *et al.,* (1975), Maron and Ames (1983) and Mortelmans and Zeiger (2000).

All tester strain cultures should exhibit a characteristic number of spontaneous revertants per plate in the vehicle and untreated controls (negative controls). Acceptable ranges are presented as follows:

TA1535	7 to 40
TA100	60 to 200
TA1537	2 to 30
TA98	8 to 60
WP2*uvrA*	10 to 60

Combined historical negative and solvent control ranges for 2012 and 2013 are presented in Appendix 2.

All tester strain cultures should be in the range of 0.9 to 9 x 10^9 bacteria per mL.

Diagnostic mutagens (positive control chemicals) must be included to demonstrate both the intrinsic sensitivity of the tester strains to mutagen exposure and the integrity of the S9-mix. All of the positive control chemicals used in the study should induce marked increases in the frequency of revertant colonies, both with or without metabolic activation. The historical ranges of the positive control reference items for 2012 and 2013 are presented in Appendix 2.

There should be a minimum of four non-toxic test item dose levels.

There should be no evidence of excessive contamination.

3.7 Evaluation Criteria

There are several criteria for determining a positive result. Any, one, or all of the following can be used to determine the overall result of the study:

1. A dose-related increase in mutant frequency over the dose range tested (De Serres and Shelby, 1979).
2. A reproducible increase at one or more concentrations.
3. Biological relevance against in-house historical control ranges.
4. Statistical analysis of data as determined by UKEMS (Mahon *et al.,* 1989).
5. Fold increase greater than two times the concurrent solvent control for any tester strain (especially if accompanied by an out-of-historical range response (Cariello and Piegorsch, 1996)).

A test item will be considered non-mutagenic (negative) in the test system if the above criteria are not met.

Although most experiments will give clear positive or negative results, in some instances the data generated will prohibit making a definite judgment about test item activity. Results of this type will be reported as equivocal.

4 RESULTS

4.1 Mutation Test

Prior to use, the master strains were checked for characteristics, viability and spontaneous reversion rate (all were found to be satisfactory). The amino acid supplemented top agar and the S9-mix used in both experiments were shown to be sterile. The test item formulation was also shown to be sterile. These data are not given in the report.

Results for the negative controls (spontaneous mutation rates) are presented in Table 1 and were considered to be acceptable. Experiment 1, one value of the TA1535 was slightly outside of the historical profile minimum but still considered acceptable as the count was only two colonies out of range, all other counts were within range and the strain responded well to the appropriate positive control. These data are for concurrent untreated control plates performed on the same day as the Mutation Test.

The individual plate counts, the mean number of revertant colonies and the standard deviations, for the test item, positive and vehicle controls, both with and without metabolic activation, are presented in Table 2 and Table 3 for Experiment 1 and Table 4 and Table 5 for Experiment 2.

A history profile of vehicle, untreated and positive control values (reference items) is presented in Appendix 2.

The maximum dose level of the test item in the first experiment was selected as the maximum recommended dose level of 5000 µg/plate. The test item induced a visible reduction in the growth of the bacterial background lawns of all of the tester strains dosed in the absence of S9-mix at 5000 µg/plate. Although there was no toxicity in the bacterial background lawns noted to any of the tester strains dosed in the presence of S9-mix, several strains exhibited lower frequencies of revertant colonies at the maximum dose level. These results were not indicative of toxicity sufficiently severe enough to prevent the test item being tested up to the maximum recommended dose level of 5000 µg/plate in the second mutation test. Second experiment results were identical to the first mutation test with weakened lawns noted at 5000 µg/plate to all of the strains dosed in the absence of S9-mix with several strains observed to have decreased numbers of colonies present in the presence of S9-mix at the same dose level. No test item precipitate was observed on the plates at any of the doses tested in either the presence or absence of S9-mix.

There were no significant increases in the frequency of revertant colonies recorded for any of the bacterial strains, with any dose of the test item, either with or without metabolic activation in Experiment 1 (plate incorporation method). Similarly, no significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test item, either with or without metabolic activation in Experiment 2 (pre-incubation method).

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies thus confirming the activity of the S9-mix and the sensitivity of the bacterial strains.

5 CONCLUSION

Glyphosate was considered to be non-mutagenic under the conditions of this test.

6 REFERENCES

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TABLES

Table 1 Spontaneous Mutation Rates (Concurrent Negative Controls)

Experiment 1

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2*uvrA*		TA98		TA1537	
64		8		13		23		5	
63	(72)	19	(11)	16	(18)	20	(19)	8	(8)
88		5		25		15		12	

Experiment 2

Number of revertants (mean number of colonies per plate)									
Base-pair substitution type						Frameshift type			
TA100		TA1535		WP2*uvrA*		TA98		TA1537	
96		20		19		21		11	
112	(100)	17	(15)	15	(19)	19	(24)	15	(14)
92		8		24		32		15	

Table 2 Test Results: Experiment 1 – Without Metabolic Activation

Test Period		From: 22 July 2014						To: 25 July 2014					
	Dose Level Per Plate †	Number of revertants (mean) +/- SD											
		Base-pair substitution strains								Frameshift strains			
		TA100			TA1535			WP2*uvrA*			TA98			TA1537	
S9-Mix (-)	Solvent Control (DMSO)	71 74 64	(70) 5.1#		9 13 25	(16) 8.3		11 23 20	(18) 6.2		24 19 13	(19) 5.5		13 7 15	(12) 4.2
	1.5 µg	64 68 80	(71) 8.3		9 7 21	(12) 7.6		21 16 27	(21) 5.5		17 11 15	(14) 3.1		17 3 7	(9) 7.2
	5 µg	67 63 63	(64) 2.3		21 8 8	(12) 7.5		11 13 27	(17) 8.7		16 12 20	(16) 4.0		19 15 9	(14) 5.0
	15 µg	74 61 71	(69) 6.8		23 13 21	(19) 5.3		15 24 23	(21) 4.9		25 20 13	(19) 6.0		17 19 8	(15) 5.9
	50 µg	67 74 76	(72) 4.7		8 11 16	(12) 4.0		24 19 20	(21) 2.6		15 13 8	(12) 3.6		15 15 12	(14) 1.7
	150 µg	80 63 79	(74) 9.5		11 24 12	(16) 7.2		20 17 24	(20) 3.5		9 19 20	(16) 6.1		5 9 9	(8) 2.3
	500 µg	74 68 68	(70) 3.5		16 9 9	(11) 4.0		9 20 21	(17) 6.7		15 20 24	(20) 4.5		13 13 13	(13) 0.0
	1500 µg	56 67 79	(67) 11.5		9 12 17	(13) 4.0		20 19 19	(19) 0.6		15 8 13	(12) 3.6		7 5 5	(6) 1.2
	5000 µg	24 S 40 S 33 S	(32) 8.0		8 S 4 S 8 S	(7) 2.3		9 S 15 S 8 S	(11) 3.8		8 S 3 S 17 S	(9) 7.1		5 S 5 S 3 S	(4) 1.2
Positive controls S9-Mix (-)	Name	ENNG			ENNG			ENNG			4NQO			9AA	
	Dose Level	3 µg			5 µg			2 µg			0.2 µg			80 µg	
	No. of Revertants	882 682 926	(830) 130.0		909 754 934	(866) 97.5		1050 911 954	(972) 71.2		335 258 210	(268) 63.1		470 417 334	(407) 68.5

† Dose levels should be considered nominal values due to the change in purity and the presence of toxicity.
ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
S Sparse bacterial background lawn
Standard deviation

Table 3 Test Results: Experiment 1 – With Metabolic Activation

Test Period		From: 22 July 2014						To: 25 July 2014					
	Dose Level Per Plate †	Number of revertants (mean) +/- SD											
		Base-pair substitution strains								Frameshift strains			
		TA100			TA1535			WP2*uvrA*			TA98			TA1537		
S9-Mix (+)	Solvent Control (DMSO)	79 74 92	(82)	9.3#	13 8 7	(9)	3.2	27 28 21	(25)	3.8	13 31 21	(22)	9.0	11 16 9	(12)	3.6
	1.5 µg	82 67 69	(73)	8.1	17 13 9	(13)	4.0	24 12 27	(21)	7.9	15 31 17	(21)	8.7	8 12 9	(10)	2.1
	5 µg	88 78 65	(77)	11.5	8 9 8	(8)	0.6	20 21 19	(20)	1.0	20 17 28	(22)	5.7	15 11 23	(16)	6.1
	15 µg	82 95 94	(90)	7.2	13 9 13	(12)	2.3	12 15 16	(14)	2.1	11 27 21	(20)	8.1	9 15 9	(11)	3.5
	50 µg	64 72 79	(72)	7.5	19 9 13	(14)	5.0	20 24 17	(20)	3.5	24 12 16	(17)	6.1	17 7 12	(12)	5.0
	150 µg	60 80 78	(73)	11.0	12 12 7	(10)	2.9	19 20 28	(22)	4.9	21 23 15	(20)	4.2	8 8 15	(10)	4.0
	500 µg	90 79 92	(87)	7.0	8 19 7	(11)	6.7	13 20 32	(22)	9.6	15 15 23	(18)	4.6	9 16 8	(11)	4.4
	1500 µg	71 63 68	(67)	4.0	12 12 13	(12)	0.6	23 20 24	(22)	2.1	19 8 15	(14)	5.6	5 19 8	(11)	7.4
	5000 µg	36 44 64	(48)	14.4	5 8 9	(7)	2.1	24 21 29	(25)	4.0	17 15 15	(16)	1.2	8 13 11	(11)	2.5
Positive controls S9-Mix (+)	Name	2AA			2AA			2AA			BP			2AA		
	Dose Level	1 µg			2 µg			10 µg			5 µg			2 µg		
	No. of Revertants	1610 1490 1684	(1595)	97.9	203 331 293	(276)	65.7	369 269 410	(349)	72.5	223 167 180	(190)	29.3	305 425 401	(377)	63.5

† Dose levels should be considered nominal values due to the change in purity and the presence of toxicity.
BP Benzo(a)pyrene
2AA 2-Aminoanthracene
Standard deviation

Table 4 Test Results: Experiment 2 – Without Metabolic Activation

Test Period		From: 28 July 2014					To: 31 July 2014				
	Dose Level Per Plate †	Number of revertants (mean) +/- SD									
		Base-pair substitution strains						Frameshift strains			
		TA100		TA1535		WP2*uvrA*		TA98		TA1537	
S9-Mix (-)	Solvent Control (DMSO)	87 87 95	(90) 4.6#	16 19 12	(16) 3.5	20 20 17	(19) 1.7	17 25 21	(21) 4.0	21 15 16	(17) 3.2
	5 µg	88 88 96	(91) 4.6	8 11 8	(9) 1.7	20 17 15	(17) 2.5	33 19 13	(22) 10.3	17 15 8	(13) 4.7
	15 µg	96 82 98	(92) 8.7	7 20 13	(13) 6.5	17 23 21	(20) 3.1	27 28 21	(25) 3.8	13 15 9	(12) 3.1
	50 µg	107 94 96	(99) 7.0	11 8 21	(13) 6.8	25 19 23	(22) 3.1	23 24 7	(18) 9.5	16 17 8	(14) 4.9
	150 µg	86 75 88	(83) 7.0	11 7 11	(10) 2.3	21 12 33	(22) 10.5	29 20 16	(22) 6.7	8 16 13	(12) 4.0
	500 µg	60 64 61	(62) 2.1	7 11 15	(11) 4.0	16 19 17	(17) 1.5	13 24 27	(21) 7.4	12 12 12	(12) 0.0
	1500 µg	88 60 61	(70) 15.9	8 13 13	(11) 2.9	21 15 11	(16) 5.0	20 21 19	(20) 1.0	8 8 7	(8) 0.6
	5000 µg	75 S 45 S 75 S	(65) 17.3	5 S 7 S 5 S	(6) 1.2	12 S 12 S 12 S	(12) 0.0	20 S 15 S 13 S	(16) 3.6	5 S 8 S 5 S	(6) 1.7
Positive controls S9-Mix (-)	Name	ENNG		ENNG		ENNG		4NQO		9AA	
	Dose Level	3 µg		5 µg		2 µg		0.2 µg		80 µg	
	No. of Revertants	1491 1298 1046	(1278) 223.2	1890 2609 1741	(2080) 464.1	1208 1346 996	(1183) 176.3	390 444 388	(407) 31.8	901 861 1168	(977) 166.9

† Dose levels should be considered nominal values due to the change in purity and the presence of toxicity.
ENNG N-ethyl-N'-nitro-N-nitrosoguanidine
4NQO 4-Nitroquinoline-1-oxide
9AA 9-Aminoacridine
S Sparse bacterial background lawn
Standard deviation

Table 5 Test Results: Experiment 2 – With Metabolic Activation

Test Period		From: 28 July 2014			To: 31 July 2014	
		Number of revertants (mean) +/- SD				
	Dose Level Per Plate †	Base-pair substitution strains			Frameshift strains	
		TA100	TA1535	WP2*uvrA*	TA98	TA1537
S9-Mix (+)	Solvent Control (DMSO)	82 71 107 (87) 18.4#	15 11 12 (13) 2.1	27 17 20 (21) 5.1	25 27 17 (23) 5.3	7 11 5 (8) 3.1
	5 µg	76 61 91 (76) 15.0	8 23 16 (16) 7.5	24 16 23 (21) 4.4	19 19 16 (18) 1.7	7 8 11 (9) 2.1
	15 µg	98 86 80 (88) 9.2	16 15 8 (13) 4.4	21 24 21 (22) 1.7	29 20 32 (27) 6.2	9 8 8 (8) 0.6
	50 µg	86 64 65 (72) 12.4	7 9 9 (8) 1.2	20 25 15 (20) 5.0	16 33 16 (22) 9.8	20 12 7 (13) 6.6
	150 µg	88 103 82 (91) 10.8	11 12 12 (12) 0.6	21 19 13 (18) 4.2	20 32 25 (26) 6.0	13 15 15 (14) 1.2
	500 µg	75 78 84 (79) 4.6	13 16 13 (14) 1.7	23 21 19 (21) 2.0	19 16 17 (17) 1.5	9 23 8 (13) 8.4
	1500 µg	80 76 82 (79) 3.1	7 8 8 (8) 0.6	15 11 17 (14) 3.1	20 21 17 (19) 2.1	9 4 9 (7) 2.9
	5000 µg	84 95 102 (94) 9.1	8 9 9 (9) 0.6	16 8 8 (11) 4.6	20 12 27 (20) 7.5	5 12 11 (9) 3.8
Positive controls S9-Mix (+)	Name	2AA	2AA	2AA	BP	2AA
	Dose Level	1 µg	2 µg	10 µg	5 µg	2 µg
	No. of Revertants	1215 1368 1255 (1279) 79.3	214 253 216 (228) 22.0	287 277 329 (298) 27.6	168 158 163 (163) 5.0	519 521 596 (545) 43.9

† Dose levels should be considered nominal values due to the change in purity and the presence of toxicity.
BP Benzo(a)pyrene
2AA 2-Aminoanthracene
Standard deviation

APPENDICES

Appendix 1 A copy of the Certificate of Analysis

Certificate ALB45110

Spectral Service

Spectral Service AG
Emil Hoffmann Strasse 33
50996 Köln, Germany

Phone: [redacted]
Fax:
eMail:
http: //www.spectralservice.de

The sample named below was prepared in our laboratory in GLP study SSL01814 under study code ALB45110 from equal amounts of Glyphosate wetcake Albaugh batch # GWC070307, 070607, 070707, 070807 and 071307 and defined amounts of impurities as shown below.

Glyphosate wetcake spiked:

New composition corrected for a weight increase of 25.009 g to a total of 25.58 g:

Analyte	Mean [% m/m]	spike [% m/m]	New content corrected [% m/m]
Loss on drying	9.88	0.000	9.66
Insolubles	0.05	0.000	0.045
Formlaldehyde	0.014	0.180	0.19
A.I.	87.75	0.000	85.79
AMPA	0.46	0.250	0.70
IBMPA	0.43	0.219	0.63
MAMPA	0.38	0.209	0.57
NMG	0.10	0.180	0.28
Phosphate	0.18	0.221	0.39
IDA	0.18	0.120	0.29
Water (KF)	0.37	0.000	0.36
spiked material net		1.379	1.348
spiked material gross		2.282	2.231
Total	99.80		99.80
NNG [mg/kg]	<0.11	1.01	0.99

Date of preparation: 4 June 2014

Köln, 22 August 2014

[redacted]

(Study director, Chemist)

Company stamp:

Spectral Service AG
Emil-Hoffmann-Str. 33 · D-50996 Köln
☎ 0 22 36 / 9 69 47-0 · Fax 0 22 36 / 9 69 47-11
www.spectralservice.de

Appendix 2 History Profile of Vehicle and Positive Control Values

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2012

Strain S9-Mix	TA100		TA1535		TA102		WP2*uvrA*		TA98		TA1537		WP2*uvrA* pKM101		WP2pKM101	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	97	96	20	14	274	313	32	37	22	25	12	13	124	153	95	123
SD	16.9	17.9	5.1	3.3	26.8	38.2	8.0	7.8	5.8	6.7	2.7	2.8	27.0	37.4	15.9	18.0
Min	62	63	9	9	209	255	14	15	11	5	4	3	79	78	66	89
Max	162	169	39	33	321	355	58	59	48	42	24	25	216	242	161	168
Values †	780	560	561	347	20	8	668	479	898	369	880	347	64	43	56	34

POSITIVE CONTROL VALUES 2012

Strain S9-Mix	TA100		TA1535		TA102		WP2*uvrA*		TA98		TA1537		WP2*uvrA* pKM101		WP2pKM101	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Mean	546	1097	406	287	1033	840	734	330	167	204	778	245	764	1420	2027	633
SD	223.5	394.5	378.8	143.3	413.1	248.9	223.3	139.8	54.2	76.8	444.9	90.8	393.4	357.5	522.9	308.7
Min	266	268	85	108	541	418	256	120	81	85	146	98	158	714	1065	261
Max	3151	3241	4157	1335	1961	1284	1661	1409	420	677	3110	622	1910	2070	3071	1700
Values †	221	233	212	226	13	13	189	188	345	233	340	226	20	22	21	36

COMBINED VEHICLE AND UNTREATED CONTROL VALUES 2013

Strain S9-Mix	TA100		TA1535		TA102		WP2*uvrA*		TA98		TA1537		WP2*uvrA* pKM101		WP2pKM101	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Total Plates	843	855	1612	798	12	6	1542	765	1655	876	1646	807	42	36	42	36
Min	68	63	9	8	191	266	15	13	10	12	5	5	110	112	105	108
Max	147	153	37	29	292	292	47	54	42	43	26	23	162	181	154	162
Mean	103	101	20	15	255	279	28	33	22	26	11	13	138	152	124	137
SD	14.4	15.6	4.4	3.5	47.3	18.4	6.6	7.1	5.0	5.1	3.1	3.5	14.4	22.3	15.4	17.1

POSITIVE CONTROL VALUES 2013

Strain S9-Mix	TA100		TA1535		TA102		WP2*uvrA*		TA98		TA1537		WP2*uvrA* pKM101		WP2pKM101	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Total Plates	849	861	810	795	6	6	765	762	828	849	825	804	21	30	21	30
Min	240	349	91	103	668	673	129	101	102	84	113	86	501	840	302	385
Max	1429	3117	3750	1153	1016	694	1275	733	783	669	2161	1238	1124	2391	2889	1198
Mean	543	1211	644	250	842	684	611	320	207	226	813	286	698	1359	1288	665
SD	192.1	509.3	685.5	98.9	246.1	14.8	256.3	120.9	76.9	92.6	384.2	127.7	263.1	441.9	894.7	305.7

SD Standard deviation
Min Minimum value
Max Maximum value
† Number of mean values used to create dataset

Appendix 3 Monitoring Authority Statement of GLP Compliance

DH Department of Health

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE IN ACCORDANCE WITH DIRECTIVE 2004/9/EC

TEST FACILITY

Harlan Laboratories Limited
Shardlow Business Park
London Road
Shardlow
Derbyshire
DE72 2GD

TEST TYPE(S)

Analytical/Clinical Chemistry
Environmental Fate
Environmental Toxicity
Phys.Chem. Testing
Mutagenicity
Toxicology

DATE OF INSPECTION **12 to 14 March 2014**

An inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above test facility as part of the UK Good Laboratory Practice Compliance Monitoring Programme.

This statement confirms that, on the date of issue, the UK Good Laboratory Practice Monitoring Authority were satisfied that the above test facility was operating in compliance with the OECD Principles of Good Laboratory Practice.

This statement constitutes a Good Laboratory Practice Instrument (as defined in the UK Good Laboratory Practice Regulations 1999).

12/5/14

Head, UK GLP Monitoring Authority

MHRA