SafePharm Laboratories

Glyphosate Technical:

MICRONUCLEUS TEST IN THE MOUSE

SPL PROJECT NUMBER: 2060/014

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QUALITY ASSURANCE REPORT

This study type is classed as short-term. Inspection of the routine and repetitive procedures that constitute the study is carried out as a continuous process designed to encompass the major phases at or about the time this study was in progress.

This report has been audited by Safepharm Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed.

In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and schedule of this study were as follows:

§	09 June 2005	Protocol Compliance Audit
	06 July 2005	Test Material Preparation
	06 July 2005	Animal Preparation
	06 July 2005	Dosing
	08 July 2005	Assessment of Response
	08 July 2005	Cell Harvest
	01 July 2005	Staining/Slide Preparation
Φ	01 February 2005, 24 May 2005	Cytogenetic facility, reported to the management 03 February and 27 May 2005
§	18 August 2005	Draft Report Audit
§	Date of QA Signature	Final Report Audit

§ Evaluation specific to this study

Φ Inspection by Test Site QA

[redacted] ..... DATE: 13 FEB 2006

For Safepharm Quality Assurance Unit*

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GLP COMPLIANCE STATEMENT

The work described 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 international standards are acceptable to the Regulatory agencies of the following countries: Australia, Austria, Belgium, Canada, the Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Israel, Italy, Japan, Republic of Korea, Luxembourg, Mexico, The Netherlands, New Zealand, Norway, Poland, Portugal, Slovenia, South Africa, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and the United States of America.

This report fully and accurately reflects the procedures used and data generated.

[redacted] DATE: 08 FEB 2006

[redacted]

Study Director

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Glyphosate Technical:

MICRONUCLEUS TEST IN THE MOUSE

SUMMARY

Introduction. The study was performed to assess the potential of the test material to produce damage to chromosomes or aneuploidy when administered to mice. The method was designed to comply with the 1997 OECD Guidelines for Testing of Chemicals No.474 "Micronucleus Test", Method B12 of the EC Commission Directive 2000/32/EC, the USA EPA, TSCA and FIFRA guidelines and the Japanese METI/MHLW/MAFF (JMAFF 2-1-19-3, 12 Nohsan 8147 and 13 Seisan 3986) guidelines for testing of new chemical substances.

Methods. A range-finding test was performed to find suitable dose levels of the test material and to investigate to see if there was a marked difference in toxic response between the sexes. A review of existing toxicity data indicated little or no evidence of any absorption in animals dosed via the oral route and, therefore, only the intraperitoneal route was investigated. There was no marked difference in toxicity of the test material between the sexes; therefore the main test was performed using only male mice. The micronucleus test was conducted using the intraperitoneal route in groups of seven mice (males) at the maximum tolerated dose (MTD) 600 mg/kg and with 300 and 150 mg/kg as the two lower dose levels. Animals were killed 24 or 48 hours later, the bone marrow was extracted, and smear preparations made and stained. Polychromatic (PCE) and normochromatic (NCE) erythrocytes were scored for the presence of micronuclei.

Further groups of mice were given a single intraperitoneal dose of phosphate buffered saline (each of 7 mice) or dosed orally with cyclophosphamide (5 mice), to serve as vehicle and positive controls respectively. Vehicle control animals were killed 24 or 48 hours later, and positive control animals were killed after 24 hours.

Results. A statistically significant decrease in the %PCEs per 1000 erythrocytes was observed in the 24-hour 600 mg/kg test material dose group when compared to the concurrent control group. A similar decrease was also observed in the 48-hour 600 mg/kg test material dose group, but the larger standard deviation resulted in no statistical significance being applied. This accompanied by the presence of clinical signs was taken to indicate that systemic absorption had occurred and exposure to the bone marrow was confirmed.

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There was a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals dosed with the test material in the 24-hour 600 mg/kg dose group when compared to the concurrent vehicle control group. However, the response was very modest, within the historical range for vehicle control animals (Appendix 2) and did not include any individual animal values that would not be acceptable for vehicle control animals. The response seen is considered to be most likely due to a haematopoeitic effect induced by the cytotoxic effect of the test material on the bone marrow rather than any genotoxic mechanism. Therefore the response was considered to have no genotoxic significance.

The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

Conclusion. The test material was considered to be non-genotoxic under the conditions of the test.

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Glyphosate Technical:

MICRONUCLEUS TEST IN THE MOUSE

1. INTRODUCTION

The micronucleus test is a mammalian *in vivo* test that detects damage to the chromosomes induced by chemicals. In addition, numerical changes due to chromosome loss during cell division can be detected in this test.

The study was performed according to a method that was designed to comply with the 1997 OECD Guidelines for Testing of Chemicals No.474 "Micronucleus Test", Method B12 of the EC Commission Directive 2000/32/EC, the USA EPA, TSCA and FIFRA guidelines and the Japanese METI/MHLW/MAFF (JMAFF 2-1-19-3, 12 Nohsan 8147 and 13 Seisan 3986) guidelines for testing of new chemical substances. The results of the test are believed to be of value in predicting the mutagenic potential of the test material to man. The test system was chosen because the mouse has been shown to be a suitable model for this type of study and is recommended in the test method.

The experimental phases of the study were performed between 07 June 2005 and 20 July 2005.

2. PRINCIPLES OF INVESTIGATION

In mitotic cells in which chromosome damage has been caused by the test material or its metabolites, fragments (centric or acentric) or whole chromosomes tend to lag behind in the anaphase stage of cell division. After telophase, a large proportion of the fragments are not included in the nuclei of the daughter cells and hence form a single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. These micronuclei are seen in a wide variety of cell types, but erythrocytes are chosen since micronuclei are easily detected in these cells.

A few hours after the last mitosis is completed, erythrocytes expel their nuclei. Immature erythrocytes, less than 24 hours old, stain blue with May-Grünwald/Giemsa due to the presence of minute fragments of nuclear material in the cytoplasm. This material is mainly ribonucleic acid (RNA), which gradually disappears so that more mature erythrocytes (normochromatic erythrocytes) stain pink with May-Grünwald/Giemsa. The immature blue staining cells are known as polychromatic erythrocytes and mauve stained micronuclei are easily detected in this cell type. If scoring is restricted to polychromatic erythrocytes, all the chromosomal damage

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detected will have been caused during the final cell cycle of the nucleated precursor cells. Thus by examining polychromatic cells at various periods after administration, the effect of the test material over the previous 30 hours can be monitored.

Any toxic effects of the test material on the immature nucleated cells may lead to a reduction in cell division and cell death. This in turn leads to a reduction in cell volume and to compensate for this, peripheral blood is shunted into the bone marrow. If the ratio of polychromatic to normochromatic erythrocytes is scored and found to be significantly lower than the control value, this is taken as being indicative of cytotoxicity.

3. TEST AND CONTROL MATERIALS AND EXPERIMENTAL PREPARATION

3.1 Test Material

Sponsor's identification	:	Glyphosate Technical
Description	:	White crystalline solid
Batch number	:	H05H016A
Date received	:	11 April 2005
Storage conditions	:	Room temperature, in the dark

The integrity of supplied data relating to the identity, purity and stability of the test material is the responsibility of the Sponsor. A copy of the Certificate of Analysis is presented in Appendix 1.

For the purpose of this study the test material was freshly prepared as required as a suspension at the appropriate concentration in phosphate buffered saline (PBS).

Determination by analysis of the concentration, homogeneity and stability of the test material preparations was not appropriate because it was not specified in the Protocol.

3.2 Positive Control Material

The positive control material was supplied by Sigma-Aldrich, as follows:

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Supplier's identification	:	Cyclophosphamide
Supplier's lot number	:	084K1328
Safepharm serial number	:	R-3423
Date received	:	03 March 2005
Storage conditions	:	4°C in the dark

For the purpose of this study the positive control material was freshly prepared as required as a solution at the appropriate concentration in distilled water (Laboratoire Aguettant batch no. F315501).

The concentration, homogeneity and stability of the positive control material and its preparation were not determined by analysis.

3.3 Vehicle Control

The vehicle, PBS, was supplied as a 10X solution by Gibco Invitrogen, as follows:

Supplier's identification	:	D-PBS (10X)
Supplier's lot number	:	3085270A
Expiry date	:	February 2006
Description	:	Clear colourless liquid
Storage conditions	:	Room temperature

The single strength PBS was prepared by diluting the 10X concentrate 1 in 10 with sterile distilled water (Laboratoire Aguettant batch no. F315501).

4. METHODS

4.1 Animals and Animal Husbandry

Sufficient albino Crl:CD-1™(ICR)BR strain mice were supplied by Charles River (UK) Limited, Margate, Kent. At the start of the main test the male mice weighed 21 to 29g and were approximately five to eight weeks old. After a minimum acclimatisation period of seven days the animals were selected at random and given a number unique within the study by tail marking, and a group number written on a colour coded cage card.

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The animals were housed in groups of up to seven in solid-floor polypropylene cages with woodflake bedding. Free access to mains drinking water and food (Certified Rat and Mouse Diet Code 5LF2, BCM, IPS Limited, London, UK) was allowed throughout the study.

The temperature and relative humidity were set to achieve limits of 19 to 25°C and 30 to 70% respectively. Any occasional deviations from these targets were considered not to have affected the purpose or integrity of the study. The rate of air exchange was approximately fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours light and twelve hours darkness.

4.2 Procedure

4.2.1 Range-finding Toxicity Test

A range-finding toxicity test was performed to determine a suitable dose level for the micronucleus test. The dose level selected should ideally be the maximum tolerated dose level or that which produces some evidence of toxicity up to a maximum recommended dose of 2000 mg/kg. The range-finding toxicity test was also used to determine if the main test was to be performed using both sexes or males only. Using existing toxicology data it was considered to be unnecessary to investigate the oral route of administration.

Groups of mice were dosed via the intraperitoneal route as follows:

Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Mice	
			Male	Female
1000	100	10	2	2
800	80	10	1	1
600	60	10	1	1

All animals were dosed once only at the appropriate dose level using a hypodermic needle attached to a graduated syringe. The volume administered to each animal was calculated according to its bodyweight at the time of dosing.

Animals were observed one hour after dosing and subsequently once daily for up to two days. Any deaths and evidence of overt toxicity were recorded at each observation. No necropsies were performed.

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4.2.2 Micronucleus Test

Groups, each of seven mice, were dosed once only via the intraperitoneal route with the test material at 600, 300 or 150 mg/kg. One group of mice from each dose level was killed by cervical dislocation 24 hours following treatment and another group dosed with test material at 600 mg/kg was killed after 48 hours. In addition, three further groups of mice were included in the study; two groups (each of seven mice) were dosed via the intraperitoneal route with the vehicle alone (PBS) and a third group (five mice) was dosed orally with cyclophosphamide. Cyclophosphamide is a positive control material known to produce micronuclei under the conditions of the test. The vehicle controls were killed 24 or 48 hours following dosing and positive control group animals were killed 24 hours following dosing. The experimental design is summarised as follows:

Dose Group	Dose Level (mg/kg)	Concentration (mg/ml)	Dose Volume (ml/kg)	Kill Time (Hours After Dosing)	Animal Numbers
1. Vehicle Control (PBS)	0	0	10	48	1 - 7
2. Vehicle Control (PBS)	0	0	10	24	8 -14
3. Positive Control (Cyclophosphamide)	50	5	10	24	15 - 19
4. Glyphosate Technical	600	60	10	48	20 - 26
5. Glyphosate Technical	600	60	10	24	27 - 33
6. Glyphosate Technical	300	30	10	24	34 - 40
7. Glyphosate Technical	150	15	10	24	41 - 47

All animals were observed for signs of overt toxicity and death one hour after dosing and then once daily as applicable and immediately prior to termination.

4.2.3 Slide Preparation

Immediately following termination (*i.e.* 24 or 48 hours following dosing), both femurs were dissected from each animal, aspirated with foetal calf serum and bone marrow smears prepared following centrifugation and re-suspension. The smears were air-dried, fixed in absolute methanol, stained in May-Grünwald/Giemsa, allowed to air-dry and coverslipped using mounting medium.

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4.2.4 Slide Evaluation

The analysis of the bone marrow smears for this study was sub-contracted to Microptic Cytogenetic Services, Swansea, UK (Principal Investigator: [redacted]).

Stained bone marrow smears were coded and examined blind using light microscopy at x1000 magnification. The incidence of micronucleated cells per 2000 polychromatic erythrocytes (PCE-blue stained immature cells) per animal was scored. Micronuclei are normally circular in shape, although occasionally they may be oval or half-moon shaped, and have a sharp contour with even staining. In addition, the number of normochromatic erythrocytes (NCE-pink stained mature cells) associated with 1000 erythrocytes was counted; these cells were also scored for incidence of micronuclei.

The percentage PCEs per 1000 erythrocytes was calculated together with appropriate group mean values and standard deviations.

4.2.5 Interpretation of Results

A comparison was made between the number of micronucleated polychromatic erythrocytes occurring in each of the test material groups and the number occurring in the corresponding vehicle control group.

A positive mutagenic response would be demonstrated when a statistically significant, dose-responsive, toxicologically relevant increase in the number of micronucleated polychromatic erythrocytes was observed for either the 24 or 48-hour kill times when compared to their corresponding control group.

If these criteria were not fulfilled, then the test material was considered to be non-genotoxic under the conditions of the test.

A positive response for bone marrow toxicity would be demonstrated when the dose group mean %PCEs per 1000 erythrocytes was shown to be statistically significantly lower than the concurrent vehicle control group.

All data were statistically analysed using appropriate statistical methods as recommended by the UKEMS Sub-committee on Guidelines for Mutagenicity Testing Report, Part III (1989). The data was analysed following a $\sqrt{(x+1)}$ transformation using Student's t-test (two tailed) and any significant results were confirmed using the one-way analysis of variance.

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5. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for five years, after which instructions will be sought as to further retention or disposal.

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6. RESULTS

6.1 Range-finding Toxicity Test

The mortality data are summarised as follows:

Dose Level (mg/kg)	Sex	Number of Animals Treated	Route	Deaths on Day			Total Deaths
				0	1	2	
1000	Male	2	ip	0	1[e]	0	2/4
	Female	2		0	1[e]	0	
800	Male	1	ip	0	1[e]		2/2
	Female	1		0	1[e]		
600	Male	1	ip	0	0	0	0/2
	Female	1		0	0	0	

In animals dosed with the test material via the intraperitoneal route animals were killed *in extremis* because of the severity of the clinical signs that were observed at and above 800 mg/kg, these included as follows: hunched posture, lethargy, ataxia, ptosis, pilo-erection, tip toe gait, distended abdomen and hypothermia. Moderate clinical signs were observed at 600 mg/kg as follows: hunched posture, ptosis, pilo-erection and ataxia.

The test material showed no marked difference in its toxicity to male or female mice, it was therefore considered to be acceptable to use males only for the main test. Clear evidence of test material toxicity was demonstrated via the intraperitoneal route of administration and, therefore, this was confirmed as the route for use in the main test. The maximum tolerated dose (MTD) of the test material, 600 mg/kg, was selected for use in the main test, with 300 and 150 mg/kg as the lower dose levels.

6.2 Micronucleus Test

6.2.1 Mortality Data and Clinical Observations

There were no premature deaths seen in any of the dose groups. Clinical signs were observed in animals dosed with the test material at and above 150 mg/kg in both the 24 and 48-hour groups where applicable, these included as follows: hunched posture, ptosis, ataxia and lethargy.

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e = killed *in extremis*
ip = Intraperitoneal

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6.2.2 Evaluation of Bone Marrow Slides

A summary of the results of the micronucleus test is given in Table 1. Individual data and group mean data are presented in Tables 2 to 8.

A statistically significant decrease in the %PCEs per 1000 erythrocytes was observed in the 24-hour 600 mg/kg test material dose group when compared to the concurrent control group. A similar decrease was also observed in the 48-hour 600 mg/kg test material dose group, but the larger standard deviation resulted in no statistical significance being applied. This accompanied by the presence of clinical signs was taken to indicate that systemic absorption had occurred and exposure to the bone marrow was confirmed.

There was a small but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in animals dosed with the test material in the 24-hour 600 mg/kg dose group when compared to the concurrent vehicle control group. However, the response was very modest, within the historical range for vehicle control animals (Appendix 2) and did not include any individual animal values that would not be acceptable for vehicle control animals. The response seen is considered to be most likely due to a haematopoeitic effect induced by the cytotoxic effect of the test material on the bone marrow rather than any genotoxic mechanism. The increased erythropoiesis caused by the test material toxicity might cause some cells to cycle more quickly than in the vehicle control animals and, therefore, there may also be less opportunity to repair spontaneously-occurring DNA damage before the final mitosis and enucleation, resulting in small increases in micronucleated cells (Kirkland, 1991). Therefore the response was considered to have no genotoxic significance.

The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test.

The test material was found not to produce any significant increases in the frequency of micronuclei in polychromatic erythrocytes of mice under the conditions of the test that were considered to due to any genotoxic activity.

7. CONCLUSION

The test material was considered to be non-genotoxic under the conditions of the test.

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8. REFERENCE

Kirkland, D.J. (1991) Selection of mutagenicity and cell transformation tests– interpretation of results. In *Preclinical Evaluation of Peptides and Recombinant Proteins*. Association of the Swedish Pharmaceutical Industry and the Swedish National Board of Health and Welfare, pp 49 – 56.

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Table 1 Micronucleus Test - Summary of Group Mean Data

Treatment Group	Number of PCE with Micronuclei per 2000 PCE		%PCE with Micronuclei		% PCE Per 1000 Erythrocytes	
	Group Mean	SD	Group Mean	SD	Group Mean	SD
1. Vehicle Control 10 ml/kg 48-hour Sampling Time	2.0	2.4	0.10	0.12	36.01	4.39
2. Vehicle Control 10 ml/kg 24-hour Sampling Time	1.3	1.1	0.06	0.06	38.46	4.58
3. Positive Control 50 mg/kg 24-hour Sampling Time	60.6***	9.7	3.03***	0.49	51.46	4.45
4. Glyphosate Technical 600 mg/kg 48-hour Sampling Time	1.9	2.1	0.09	0.11	28.16	14.23
5. Glyphosate Technical 600 mg/kg 24-hour Sampling Time	3.9*	1.5	0.19*	0.07	27.71**	4.95
6. Glyphosate Technical 300 mg/kg 24-hour Sampling Time	1.1	1.1	0.06	0.05	38.57	8.69
7. Glyphosate Technical 150 mg/kg 24-hour Sampling Time	1.4	0.8	0.07	0.04	45.23	6.12

PCE = Polychromatic erythrocytes
SD = Standard deviation
* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

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Table 2 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Vehicle Control (10 ml/kg) 48-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
1. VEHICLE CONTROL 10 ml/kg 48-hour sampling time	1	27	2000	7	0.35	630	4	37.00
	2	25	2000	1	0.05	591	1	40.90
	3	26	2000	2	0.10	600	1	40.00
	4	23	2000	3	0.15	698	1	30.20
	5	25	2000	0	0.00	613	1	38.70
	6	24	2000	0	0.00	650	0	35.00
	7	24	2000	1	0.05	697	1	30.30
	Group Mean	24.9	2000	2.0	0.10	640	1.3	36.01
	SD	1.3	0	2.4	0.12	44	1.3	4.39

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Table 3 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Vehicle Control (10 ml/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
2. VEHICLE CONTROL 10 ml/kg 24-hour sampling time	8	28	2000	0	0.00	673	0	32.70
	9	25	2000	0	0.00	607	1	39.30
	10	26	2000	1	0.05	535	1	46.50
	11	27	2000	1	0.05	653	1	34.70
	12	24	2000	3	0.15	584	1	41.60
	13	28	2000	2	0.10	625	0	37.50
	14	23	2000	2	0.10	631	1	36.90
	Group Mean	25.9	2000	1.3	0.06	615	0.7	38.46
	SD	2.0	0	1.1	0.06	46	0.5	4.58

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Table 4 **Micronucleus Test – Individual Data and Group Means and Standard Deviations: Cyclophosphamide (50 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
3. CYCLOPHOSPHAMIDE 50 mg/kg 24-hour sampling time	15	25	2000	44	2.20	460	1	54.00
	16	26	2000	60	3.00	518	1	48.20
	17	23	2000	65	3.25	493	0	50.70
	18	27	2000	67	3.35	533	1	46.70
	19	21	2000	67	3.35	423	6	57.70
	Group Mean	24.4	2000	60.6	3.03	485	1.8	51.46
	SD	2.4	0	9.7	0.49	45	2.4	4.45

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Table 5 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Test Material (600 mg/kg) 48-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
4. Glyphosate Technical 600 mg/kg 48-hour sampling time	20	26	2000	1	0.05	529	0	47.10
	21	25	2000	3	0.15	827	1	17.30
	22	24	2000	0	0.00	575	0	42.50
	23	25	2000	6	0.30	863	2	13.70
	24	25	2000	1	0.05	616	3	38.40
	25	26	2000	0	0.00	758	2	24.20
	26	22	2000	2	0.10	861	2	13.90
	Group Mean	24.7	2000	1.9	0.09	718	1.4	28.16
	SD	1.4	0	2.1	0.11	142	1.1	14.23

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Table 6 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Test Material (600 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
5. Glyphosate Technical 600 mg/kg 24-hour sampling time	27	26	2000	1	0.05	705	1	29.50
	28	27	2000	5	0.25	741	1	25.90
	29	27	2000	5	0.25	684	0	31.60
	30	27	2000	4	0.20	672	0	32.80
	31	26	2000	5	0.25	746	1	25.40
	32	26	2000	3	0.15	816	1	18.40
	33	29	2000	4	0.20	696	0	30.40
	Group Mean	26.9	2000	3.9	0.19	723	0.6	27.71
	SD	1.1	0	1.5	0.07	49	0.5	4.95

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Table 7 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Test Material (300 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
6. Glyphosate Technical 300 mg/kg 24-hour sampling time	34	26	2000	0	0.00	665	1	33.50
	35	28	2000	1	0.05	620	2	38.00
	36	28	2000	0	0.00	665	0	33.50
	37	25	2000	1	0.05	464	1	53.60
	38	25	2000	2	0.10	653	1	34.70
	39	25	2000	1	0.05	527	2	47.30
	40	25	2000	3	0.15	706	0	29.40
	Group Mean	26.0	2000	1.1	0.06	614	1.0	38.57
	SD	1.4	0	1.1	0.05	87	0.8	8.69

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Table 8 **Micronucleus Test - Individual Data and Group Means and Standard Deviations: Test Material (150 mg/kg) 24-Hour Sampling Time**

TREATMENT GROUP	ANIMAL NUMBER	BODYWEIGHT (g) WHEN DOSED	POLYCHROMATIC ERYTHROCYTES (PCE)			NORMOCHROMATIC ERYTHROCYTES (NCE)		% PCE PER 1000 ERYTHRO-CYTES
			NUMBER SCORED	PCE + MN	%PCE + MN	NUMBER SCORED	NCE + MN	
7. Glyphosate Technical 150 mg/kg 24-hour sampling time	41	25	2000	2	0.10	594	0	40.60
	42	25	2000	2	0.10	574	0	42.60
	43	23	2000	2	0.10	604	3	39.60
	44	24	2000	0	0.00	531	0	46.90
	45	26	2000	1	0.05	598	0	40.20
	46	25	2000	2	0.10	465	1	53.50
	47	25	2000	1	0.05	657	0	34.30
	Group Mean	24.7	2000	1.4	0.07	575	0.6	42.53
	SD	1.0	0	0.8	0.04	61	1.1	6.12

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Glyphosate Technical: MICRONUCLEUS TEST IN THE MOUSE

Appendix 1 Copy of Certificate of Analysis

Huntingdon Life Sciences
Working for a better future

CERTIFICATE OF ANALYSIS

SPONSOR	:	Nufarm Asia Sdn Bhd
PRODUCT NAME	:	Glyphosate technical
CHEMICAL NAME	:	N-(phosphonomethyl)glycine
PHYSICAL FORM	:	White powder
LOT NUMBER	:	H05H016A
DATE OF ANALYSIS	:	20 December 2005
PURITY	:	95.7% w/w
ANALYTICAL METHOD	:	High performance liquid chromatography Huntingdon Life Sciences Report No: NUF0136/054020
EXPIRATION DATE	:	25 March 2008
STORAGE CONDITIONS	:	Ambient
TESTING FACILITY & ARCHIVE	:	Huntingdon Life Sciences Huntingdon, Cambridgeshire, PE28 4HS, England

The analysis of the product was conducted following the principles of Good Laboratory Practices.

Signed: [redacted] Date: 22 December 2005

(Study Director, Product Chemistry, Eye)

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Glyphosate Technical: MICRONUCLEUS TEST IN THE MOUSE

Appendix 2 Historical Vehicle Control Data from 60 Studies (120 Groups)

Table 1: Relative Group Frequency of Mean Micronuclei Per 1000 PCEs

Frequency of Micronuclei per 1000 PCEs	Number of Study Groups Per MN/PCE Frequency		
	24-Hour Groups	48-Hour Groups	Combined
0.0	3	3	6
0.1	1	1	2
0.2	2	6	8
0.3	0	1	1
0.4	9	10	19
0.5	0	0	0
0.6	13	10	23
0.7	2	3	5
0.8	7	5	12
0.9	3	0	3
1.0	5	2	7
1.1	2	4	6
1.2	4	6	10
1.3	2	1	3
1.4	1	1	2
1.5	0	0	0
1.6	2	6	8
1.7	0	0	0
1.8	0	0	0
1.9	0	1	1
2.0	1	0	1
2.1	0	0	0
2.2	1	0	1
2.3	1	0	1
2.4	1	0	1
Total	60	60	120

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Glyphosate Technical: MICRONUCLEUS TEST IN THE MOUSE

Appendix 2 (continued) Historical Vehicle Control Data from 60 Studies (120 Groups)

Table 2: Relative Group Frequency Categories of Micronuclei Per 1000 PCEs

48-Hour Control Group (60 Groups)

Frequency Categories	Groups	
0.0 - 0.4	21	(35%)
0.5 - 0.9	18	(30%)
1.0 - 1.4	14	(23%)
1.5 - 2.0	7	(12%)
2.1 - 2.5	0	(0%)

24-Hour Control Group (60 Groups)

Frequency Categories	Groups	
0.0 - 0.4	15	(25%)
0.5 - 0.9	25	(42%)
1.0 - 1.4	14	(23%)
1.5 - 2.0	3	(5%)
2.1 - 2.5	3	(5%)

Combined 24 and 48-Hour Groups (120 Groups)

Frequency Categories	Groups	
0.0 - 0.4	36	(30%)
0.5 - 0.9	43	(36%)
1.0 - 1.4	28	(23%)
1.5 - 2.0	10	(8%)
2.1 - 2.5	3	(3%)

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Appendix 3 Statement of GLP Compliance in Accordance with Directive 88/320/EEC

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THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320/EEC

LABORATORY
SafePharm Limited
Shardlow Business Park,
London Road,
Shardlow,
Derbyshire,
DE72 2GD

TEST TYPE
Analytical/Clinical
Chemistry
Environmental tox.
Environmental fate
Mutagenicity
Phys./Chem. tests
Toxicology

DATE OF INSPECTION

2nd December 2002

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

[redacted]

13/12/03

[redacted]

Head, UK GLP Monitoring Authority