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Glyphosate Technical

Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse

Final Report

DATA REQUIREMENTS: OECD 474 (1997)
2000/32/EC (2000)
EPA OPPTS 870.5395 (1998)

AUTHOR(S): [redacted]

STUDY COMPLETION DATE: June 09, 2008

PERFORMING LABORATORY: RCC
Cytotest Cell Research GmbH (RCC-CCR)
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LABORATORY PROJECT ID: Report Number: 1158500
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SPONSOR: Syngenta Ltd
Jealott's Hill International Research Centre
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STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Number:	1158500
Test Item:	Glyphosate Technical
Study Director:	[redacted]
Title:	Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of the Mouse

This study performed in the test facility of RCC Cytotest Cell Research GmbH, 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: June 27, 2002.

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

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

Study Director [redacted]

Date: June 09, 2008

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 1158500

Test Item: Glyphosate Technical

Study Director: [redacted]

Title: Glyphosate Technical - Micronucleus Assay in Bone Marrow Cells of theMouse

The general facilities and activities of RCC Cytotest Cell Research GmbH are inspected periodically and the results are reported to the responsible person and the management.

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

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	February 11, 2008	February 11, 2008
1st Amendment to Study Plan:	March 07, 2008	March 07, 2008
Process Inspection Evaluation:	March 12, 2008	March 12, 2008
Draft Report:	May 30, 2008	May 30, 2008

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

Head of Quality Assurance Unit [redacted]

[redacted]

Date: June 09, 2008

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director
	Deputy Study Director
	Management
	Head of Quality Assurance Unit
	Study Monitor

Study dates

Experimental Start Date: 25 February, 2008
Experimental Completion Date: 13 March, 2008

Deviations from the guidelines

[None]

Retention

RCC Cytotest Cell Research will archive the following data for 15 years:
Raw data, study plan, a sample of the test item, and the final report.
Microscopic slides will be archived for at least 12 years.
No data will be discarded without the sponsors consent.

Performing laboratory test substance reference number

S 846011

Others

General

Contracting Institute:	RCC Ltd 4452 Itingen Switzerland
Reference Number:	B82800

Deviations to study plan

In the main experiment the animals treated with the high dose were not observed for clinical signs of toxicity at the planned 6 h post treatment interval.

The age of the animals was 7 weeks (beginning of acclimatization).

These deviations, however, does not affect the validity of the study.

Distribution of the report

Sponsor:	1 × pdf-file, 1 × Word-file, 1 Regulatory Summary
Study Director:	1 × original

Project staff signatures

Study Director

[redacted]

Date: June 09, 2008

Management

[redacted]

Date: June 09, 2008

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1.0 EXECUTIVE SUMMARY

1.1 Study design

This study was performed to investigate the potential of Glyphosate Technical to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

The test item was formulated in 0.5% CMC, which was also used as vehicle control. The volume administered orally was 20 mL/kg bodyweight (b.w.). At 24 h and 48 h after a single administration of the test item, the bone marrow cells were collected for micronuclei analysis.

Five males per test group were evaluated for the occurrence of micronuclei. At least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei.

To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

24 h preparation interval: 500, 1000, and 2000 mg/kg b.w.
48 h preparation interval: 2000 mg/kg b.w.

1.2 Results

The highest dose (2000 mg/kg; maximum guideline-recommended dose) was estimated by a pre-experiment to be suitable.

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control, thus indicating that Glyphosate Technical did not exert any cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle controls there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used.

A dose of 40 mg/kg b.w. cyclophosphamide administered orally was used as positive control, which showed a substantial increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg b.w.

1.3 Conclusion

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse.

Therefore, Glyphosate Technical is considered to be non-clastogenic in this bon marrow micronucleus assay.

2.0 INTRODUCTION

2.1 Purpose

This *in vivo* experiment was performed to assess the mutagenic properties of the test item by means of the micronucleus test in bone marrow cells of the mouse.

The occurrence of micronuclei in interphase cells provides an indirect but easy and rapid measure of chromosomal damage. Micronuclei arise from acentric chromosomal fragments or whole chromosomes induced by clastogens or agents affecting the spindle apparatus (1,2,3,4,5).

Polychromatic erythrocytes (PCE) in the bone marrow of the mouse are the cell population of choice for mammalian cells *in vivo*. PCEs are newly formed red blood cells and are easily identifiable by their staining properties. These cells have the advantage that the micronuclei can be readily detected because the nucleus is extruded from the erythroblast after the last cell division.

The first appearance of micronuclei in PCEs is at least 10 - 12 hours after a clastogenic exposure. This lag is due to the time required for the affected erythroblast to differentiate into a PCE. This differentiation process includes:

1. The time required for the damaged erythroblast to proceed to mitosis.
2. The mitotic delay induced by the treatment.
3. The formation of micronuclei due to acentric fragments or chromosomes that are not included in the daughter nuclei.
4. The time required for the expulsion of the main nucleus after the last mitosis to become a micronucleated PCE.

This newly formed cell population persists for about 20 hours in the bone marrow of the mouse. During this time micronucleated PCEs can accumulate in the bone marrow in response to a clastogenic exposure, as the production of micronuclei extends over a considerable period of time.

The time at which the micronucleus frequency is at a maximum varies from agent to agent (6). Due to mitotic delay or metabolic and pharmacokinetic effects the appearance of micronucleated PCEs can be considerably delayed. Therefore, a single sampling time is not optimal. Results obtained with model mutagens showed that samples taken at 24 h and 48 h after treatment cover the intervals in which maximum frequencies of micronuclei occur.

For the initial assessment of clastogenic activity a single dose level at the maximum tolerated dose or that producing some indication of cytotoxicity (change in the ratio of polychromatic to normochromatic erythrocytes) and sampling at 24 h and 48 h after treatment is recommended. For verification two additional dose levels are tested at a sampling time of 24 h after treatment to establish a dose response effect.

To validate the test, a reference mutagen is 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:

9th Addendum to the OECD Guideline for Testing of Chemicals, Section 4, No. 474, adopted July 21, 1997, "Mammalian Erythrocyte Micronucleus Test".

Commission Directive 2000/32/EC, Annex 4C, dated May 19, 2000.

US EPA Health Effects Test Guidelines OPPTS 870.5395, Mammalian Erythrocyte Micronucleus Test, EPA 712-C-98-226, August (1998).

3.0 MATERIALS AND METHODS

3.1 Test substance

Internal RCC-CCR Test Item Number: S 846011

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

Identity:	Glyphosate Technical
Source:	Nantong Jiangshan
Batch No.:	20070545
CAS No.:	1071-83-6
Aggregate state at room temperature:	solid
Colour:	white
Purity:	99.1% w/w Glyphosate (estimated error ± 0.3%)
Storage:	2 - 8 °C
Reanalysis Date:	October, 2009

On the day of the experiment, the test item was formulated in 0.5% CMC. The vehicle was chosen based on its relative non-toxicity for the animals. All animals received a single standard volume of 20 mL/kg body weight orally.

3.2 Controls

3.2.1 Vehicle control

Name:	0.5% CMC (Carboxymethylcellulose)
Supplier:	FLUKA Chemie AG (CH-9471 Buchs, Switzerland)
Catalogue no.:	21902
Route and Frequency of Administration:	orally, once
Volume Administered:	20 mL/kg b.w.

3.2.2 Positive control

Name:	CPA; Cyclophosphamide
Supplier:	Sigma-Aldrich Vertriebs GmbH 82041 Deisenhofen
Catalogue no.:	C 0768 (purity: > 98 %)
Dissolved in:	deionised water
Dosing:	40 mg/kg b.w.
Route and frequency of administration:	orally, once
Volume administered:	10 mL/kg b.w.

Solution prepared on day of administration.

The stability of CPA at room temperature is sufficient. At 25 °C only 3.5 % of its potency is lost after 24 hours (7).

3.3 Experimental design

3.3.1 Animals

The mouse is an animal that has been used for many years as a suitable experimental animal in cytogenetic investigations. There are many data available from such investigations, which may be helpful in the interpretation of results from the micronucleus test. In addition, the mouse is an experimental animal in many physiological, pharmacological and toxicological studies. Data from such experiments also may be useful for the design and the performance of the micronucleus test (1,2,3,4,5,6).

Strain:	NMRI
Source	Harlan Winkelmann GmbH D-33178 Borchen
Number of Animals:	42 males
Initial Age at Start of Acclimatisation:	7 - 8 weeks
Acclimatisation:	minimum 5 days
Initial Body Weight at Start of Treatment:	mean value 39.0 g (SD ± 2.6 g)

According to the suppliers assurance the animals were in healthy condition. The animals were under quarantine in the animal house of RCC - CCR for a minimum of five days after their arrival. During this period the animals did not show any signs of illness or altered behaviour.

The animals were distributed into the test groups at random and identified by cage number.

3.3.2 Husbandry

The animals were kept conventionally. The experiment was conducted under standard laboratory conditions.

Housing:	single
Cage Type:	Makrolon Type I, with wire mesh top (EHRET GmbH, D-79302 Emmendingen)
Bedding:	granulated soft wood bedding (Harlan Winkelmann GmbH, D-33178 Borchen)
Feed:	pelleted standard diet, ad libitum (Harlan Winkelmann GmbH, D-33178 Borchen)
Water:	tap water, ad libitum, (Gemeindewerke, D-64380 Roßdorf)
Environment:	temperature 22 ± 3 °C relative humidity 30 - 70 % artificial light 6.00 a.m. - 6.00 p.m.

3.3.3 Pre-experiment on toxicity

A preliminary study on acute toxicity was performed in both male and female mice (two animals per sex per dose level) under identical conditions as in the mutagenicity study concerning: animal strain, vehicle, route, frequency, and volume of administration.

The animals were treated orally with the test item and examined for acute toxic symptoms at intervals of around 1 h, 2-4 h, 6 h, 24 h, 30 h, and 48 h after administration of the test item.

3.3.4 Dose selection

It is generally recommended to use the maximum tolerated dose or the highest dose that can be formulated and administered reproducibly or 2000 mg/kg as the upper limit for non-toxic test items.

The maximum tolerated dose level is determined to be the dose that causes toxic reactions without having major effects on survival within 48 hours.

The volume to be administered should be compatible with physiological space available.

Three adequately spaced dose levels spaced by a factor of 2 were administered, and samples were collected at the central sampling interval of 24 h after treatment. For the highest dose level an additional sample was taken at 48 h after treatment.

3.3.5 Study procedure

3.3.5.1 Test groups

Six males were assigned to each test group. The animals were identified by their cage number as shown in Table 1.

3.3.5.2 Treatment

At the beginning of the treatment the animals (including the controls) were weighed and the individual volume to be administered was adjusted to the animals body weight. The animals received the test item, the vehicle or the positive control substance once. Six males were treated per dose group and sampling time. The animals of all dose groups were examined for acute toxic symptoms at intervals of around 1 h, 2 - 4 h, 6 h (high dose group animals, 2000 mg/kg were not observed at 6 hr), 24 h and 48 h after administration of the test item.

Sampling of the bone marrow was done 24 and 48 hours after treatment.

3.4 *Post mortem* investigations

3.4.1 Preparation of the animals

The animals were sacrificed using CO_2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with foetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 · g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald (Merck, D-64293 Darmstadt)/Giemsa (Merck, D-64293 Darmstadt). Cover slips were mounted with EUKITT (Kindler, D-79110 Freiburg). At least one slide was made from each bone marrow sample.

3.5 Data evaluation

3.5.1 Analysis of cells

Evaluation of the slides was performed using NIKON microscopes with 100x oil immersion objectives. At least 2000 polychromatic erythrocytes (PCE) were analysed per animal for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides.

Five males per test group were evaluated as described. The remaining 6^{th} animal in the respective test group is usually evaluated in case an animal dies in its test group spontaneously.

3.5.2 Data recording

The data generated are recorded in the laboratory records. The results are presented in tabular form, including experimental groups, negative, and positive control. The micronucleated cells per 2000 PCEs and the ratio of polychromatic erythrocytes to total erythrocytes are presented for each animal.

3.5.3 Acceptance criteria

The study was considered valid as the following criteria are met:
- the negative controls are in the range of our historical control data (Appendix 1).
- the positive controls are in the range of our historical control data (Appendix 1).
- at least 4 animals per group could be evaluated.
- PCE to erythrocyte ratio was not less than 20 % of the negative control value.

3.5.4 Evaluation of results

A test item is classified as mutagenic if it induces either a dose-related increase or a clear increase in the number of micronucleated polychromatic erythrocytes in a single dose group. Statistical methods (nonparametric Mann-Whitney test (8)) were used as an aid in evaluating the results. However, the primary point of consideration is the biological relevance of the results.

A test item that fails to produce a biological relevant increase in the number of micronucleated polychromatic erythrocytes is considered non-clastogenic in this system.

4.0 RESULTS AND DISCUSSION

4.1.1 Pre-experiment for toxicity

In a pre-experiment 4 animals (2 males, 2 females) received orally a single dose of 2000 mg/kg b.w. Glyphosate Technical formulated in 0.5% CMC. The volume administered was 20 mL/kg b.w..

The animals treated with 2000 mg/kg b.w. did not express any toxic reactions.

On the basis of these data 2000 mg/kg b.w. was estimated to be suitable as the highest dose level. Since gender-specific differences in the sensitivity against the test item were not observed, the main experiment was performed using only males.

4.1.2 Toxic symptoms in the main experiment

In the main experiment for the highest dose group 12 males received orally a single dose of 2000 mg/kg b.w. Glyphosate Technical formulated in 0.5% CMC. For the mid and low doses 6 males per group received orally a single dose of 1000 or 500 mg/kg b.w. Glyphosate Technical formulated in 0.5% CMC . The volume administered was 20 mL/kg b.w.

Neither the test item treated animals nor those treated with the vehicle control (0.5% CMC) expressed any toxic reactions.

4.1.3 Micronucleus test results

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that Glyphosate Technical did not have any cytotoxic properties in the bone marrow (see Table 2).

In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item (see Tables 2 and 3).

4.2 Discussion

The test item Glyphosate Technical was assessed in the micronucleus assay for its potential to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse.

The test item was formulated in 0.5% CMC, which was also used as vehicle control. The volume administered orally was 20 mL/kg b.w.. At 24 h and 48 h after a single administration of the test item, the bone marrow cells were collected for micronuclei analysis.

Five males per test group were evaluated for the occurrence of micronuclei. At least 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei.

To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

24 h preparation interval: 500, 1000, and 2000 mg/kg b.w.

48 h preparation interval: 2000 mg/kg b.w.

As estimated by a pre-experiment in male and female mice, 2000 mg Glyphosate Technical per kg b.w. (the maximum guideline-recommended dose) was suitable as the highest dose. Since obvious gender-specific differences in the sensitivity against the test item were not observed, the main experiment was performed using male animals only.

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that Glyphosate Technical did not have any cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle controls there was no biologically relevant enhancement in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test item. The mean values of micronuclei observed after treatment with Glyphosate Technical were near to the value of the vehicle control group and within the historical vehicle control range.

A dose of 40 mg/kg b.w. cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg b.w.

5.0 CONCLUSIONS

In conclusion, it can be stated that during the study described and under the experimental conditions reported, the test item **did not** induce micronuclei as determined by the micronucleus test in the bone marrow cells of the mouse.

6.0 REFERENCES

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TABLES SECTION

TABLE 1 Identification of the Animals by their Cage Number

Test group	hours post-treatment 24	48
Negative control	1 - 6	31 - 36
Low dose	7 - 12	-
Medium dose	13 - 18	-
High dose	19 - 24	37 - 42
Positive control	25 - 30	

TABLE 2 Summary of Micronucleus Test Results

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythrocytes
Vehicle	0	24	0.070	0 - 3	1202
test item	500	24	0.080	1 - 2	1147
test item	1000	24	0.080	1 - 2	1162
test item	2000	24	0.070	0 - 2	1173
Positive control	40	24	3.150	44 - 92	1030
Vehicle	0	48	0.070	0 - 3	1153
test item	2000	48	0.080	0 - 3	1190

TABLE 3 Biometry

Statistical significance at the five per cent level ($p < 0.05$) for the incidence of micronuclei was evaluated by means of the non-parametric Mann-Whitney test.

Vehicle control versus test group	Significance	p
500 mg Glyphosate Technical/kg b.w.; 24 h	-	0.5000
1000 mg Glyphosate Technical /kg b.w.; 24 h	-	0.5000
2000 mg Glyphosate Technical /kg b.w.; 24 h	n.t	
40 mg CPA/kg b.w.; 24 h	+	0.0040
2000 mg Glyphosate Technical /kg b.w.; 48 h	-	0.5000

- = not significant
+ = significant;
n.t = not tested, as the mean micronucleus frequency was not above the vehicle control value

TABLE 4 Micronuclei in Polychromatic Erythrocytes (PCE) and Relationship PCE/Total Erythrocytes Scoring 24 Hours after Treatment

A. Vehicle control:

animal no.	sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
1	m	0.5% CMC	0	2	1289
2	m	"	"	0	1048
3	m	"	"	2	1057
4	m	"	"	0	1246
5	m	"	"	3	1372
			Sum	7	6012
			Mean	1.4	1202
		percent cells with micronuclei		**0.070**	

TABLE 4 cont. Micronuclei in Polychromatic Erythrocytes (PCE) and Relationship PCE/Total Erythrocytes Scoring 24 Hours after Treatment (Continued)

B. 500 mg/kg b.w. test item:

animal no.	sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
7	m	Glyphosate Technical	500	1	1129
8	m	"	"	1	1082
9	m	"	"	2	1145
10	m	"	"	2	1112
11	m	"	"	2	1265
			sum	8	5733
			mean	1.6	1147
		percent cells with micronuclei		**0.080**	

C. 1000 mg/kg b.w. test item:

animal no.	Sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
13	m	Glyphosate Technical	1000	1	1257
14	m	"	"	2	879
15	m	"	"	2	1072
16	m	"	"	2	1270
17	m	"	"	1	1332
			sum	8	5810
			mean	1.6	1162
		percent cells with micronuclei		**0.080**	

TABLE 4 cont. Micronuclei in Polychromatic Erythrocytes (PCE) and Relationship PCE/Total Erythrocytes Scoring 24 Hours after Treatment (Continued)

D. 2000 mg/kg b.w. test item:

animal no.	Sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
19	m	Glyphosate Technical	2000	2	1181
20	m		„	1	1206
21	m	„	„	2	1145
22	m	„	„	2	1159
23	m	„	„	0	1175
			sum	7	5866
			mean	1.4	1173
		percent cells with micronuclei		**0.070**	

E. Positive control:

animal no.	Sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
25	m	CPA	40	92	960
26	m	„	„	57	956
27	m	„	„	44	1139
28	m	„	„	64	1051
29	m	„	„	58	1046
			sum	315	5152
			mean	63.0	1030
		percent cells with micronuclei		**3.150**	

* In order to confirm data a total of 6000 PCEs were evaluated. For better comparison the value is adjusted to 2000 PCEs.

TABLE 5 **Micronuclei in Polychromatic Erythrocytes (PCE) and Relationship PCE/Total Erythrocytes Scoring 48 Hours after Treatment**

A. Vehicle control:

animal no.	sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
31	m	0.5% CMC	0	2	1181
32	m	"	"	1	1148
33	m	"	"	0	1181
34	m	"	"	1	1169
35	m	"	"	3	1086
			sum	7	5765
			mean	1.4	1153
		percent cells with micronuclei		**0.070**	

B. 2000 mg/kg b.w. test item:

animal no.	sex	test group	dose mg/kg b.w.	Micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
37	m	Glyphosate Technical	2000	2	1243
38	m		"	2	1133
39	m		"	1	1101
40*	m		"	1	1173
41	m		"	0	1289
42	m		"	3	1182
			sum	8	5948
			mean	1.6	1190
		percent cells with micronuclei		**0.080**	

*: The value of this animal was not considered for the calculation of the mean values, since the weight of the animal (47.0 g) was not within the acceptance range of ±20% of the mean weight (the weight was 20.6% of the mean value). For this purpose the slide of the reserve animal was scored.

TABLE 6 Individual Animal Weights at the Start of the Experiment

Dose Group	Animal No.	Initial Weight (g)	% Deviation from Mean Weight	Mean (g)	Standard Deviation (g)
Negative control Group; 0.5% CMC; 24h Interval	1	35.3	9.4		
	2	35.1	10.0		
	3	38.9	0.2		
	4	37.3	4.3		
	5	40.4	3.6		
	6	37.4	4.0	37.4	2.0
Low Dose Group (500mg/kg b.w.)	7	38.5	1.2		
	8	41.7	7.0		
	9	38.5	1.2		
	10	40.4	3.6		
	11	37.5	3.8		
	12	38.6	1.0	39.2	1.5
Medium Dose Group (1000 mg/kg b.w.)	13	41.4	6.2		
	14	38.2	2.0		
	15	40.4	3.6		
	16	37.1	4.8		
	17	40.0	2.6		
	18	37.3	4.3	39.1	1.8
High Dose Group (2000 mg/kg b.w.); 24h Interval	19	37.4	4.0		
	20	36.2	7.1		
	21	33.5	14.1		
	22	39.9	2.4		
	23	38.6	1.0		
	24	37.2	4.6	37.1	2.2
Positive Control (CPA, 40 mg/kg b.w.)	25	41.8	7.2		
	26	37.2	4.6		
	27	40.9	4.9		
	28	40.9	4.9		
	29	34.9	10.5		
	30	36.4	6.6	38.7	2.9
Negative Control Group; 0.5% CMC; 48h Interval	31	40.0	2.6		
	32	40.8	4.7		
	33	41.2	5.7		
	34	40.2	3.1		
	35	41.4	6.2		
	36	44.9	15.2	41.4	1.8
High Dose Group (2000 mg/kg b.w.); 48h Interval	37	38.5	1.2		
	38	39.0	0.1		
	39	40.9	4.9		
	40	47.0	20.6		
	41	35.9	7.9		
	42	38.4	1.5	40.0	3.8
Summary		39.0			

APPENDICES SECTION

APPENDIX 1 Historical Control Data

2002 – 2007

	Vehicle Controls			Positive Controls (CPA)		
	Males	Females	Total	Males	Females	Total
Mean* ± SD	0.093 ± 0.040	0.074 ± 0.038	**0.084 ± 0.031**	2.202 ± 0.705	1.702 ± 0.647	**1.973 ± 0.630**
Range**	0.01 - 0.20	0.0 - 0.19	**0.01 - 0.18**	0.70 -4.52	0.56 -3.68	**0.77 - 3.69**
No. of Experiments	293	275	**294**	292	274	**293**

*: mean value (percent micronucleated cells)
**: range of the mean group values (percent micronucleated cells)

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 88/320/EG wurde durchgeführt in

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

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

RCC – Cytotest Cell Research GmbH
RCC – Cytotest Cell Research GmbH
In den 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
9 Virussicherheitsprüfungen

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

02.09.2006
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

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

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

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

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

, Referent, Wiesbaden, den **19. Januar 2007**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

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

APPENDIX 3 Certificate of Analysis

syngenta

Certificate of Analysis

Syngenta
Analytical Development & Product Chemistry
Jealott's Hill International Research Centre
Bracknell
Berkshire RG42 6EY
United Kingdom

Tel +44 (0) 1344 414212, Fax +44 (0) 1344 414858

Title GLYPHOSATE TECHNICAL MATERIAL | Reference [illegible] Bx 20070945

Purity: 99.1% w/w Glyphosate (estimated error ± 0.3%)

Structure

Chemical Nomenclature

IUPAC: *N*-(phosphonomethyl)glycine

CAS: *N*-(phosphonomethyl)glycine (9CI)

CAS Reg Number: [1071-83-6]

Methods of Characterisation	Study Number	Report Number
IC, NMR, Karl Fisher Titrimetry	[illegible]	[illegible]

Handling Procedure

Handle in a fumecupboard, wearing laboratory coat, safety spectacles and gloves. For further information see attached Safety Data information.

Storage and Expiration

Store at less than [illegible]. Expires end October [illegible]

Supplementary Information

[illegible] (ex [illegible])

Physical Appearance

White crystalline solid.

Supplied By

Certified by:

(Research Leader)

25 Jan 2008
Date

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