Glyphosate

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

Final Report

DATA REQUIREMENTS: OECD 474 (1997)
EPA OPPTS 870 5395 (1998)
EC 440/2008 B. 12 (2008)

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PERFORMING LABORATORY: Harlan Cytotest Cell Research GmbH
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SPONSOR(S): Syngenta Ltd
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Bracknell, Berkshire RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study performed in the test facility of Harlan Cytotest Cell Research GmbH (Harlan CCR), In den Leppsteinswiesen 19, 64380 Rossdorf, Germany was conducted in compliance with Good Laboratory Practice Regulations:

"Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1), in its currently valid version.

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

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

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Date: 28 September 2012

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Harlan CCR Study: 1479200

Test Item: Glyphosate Technical

Study Director: [redacted]

Title: Glyphosate Technical – Micronucleus Assay in Bone Marrow Cells of the Mouse

The general facilities and activities of Harlan CCR are inspected periodically and the results are reported to the responsible person and the management.

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

Phases and Dates of QAU Inspections/ Audits		Dates of Reports to the Study Director and to Management
Study Plan:	04 April 2012	04 April 2012
1st Amendment to Study Plan:	05 April 2012	05 April 2012
Process Inspection Preparation for application & application:	24 April 2012	24 April 2012
Report:	30 May 2012	30 May 2012

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

Head of Quality Assurance Unit [redacted]

Date: 28 September 2012

PROJECT STAFF SIGNATURE

Study Director

.....

Date: 28 September 2012

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director
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Contracting Institute:	Harlan Laboratories Ltd. Zelgliweg 1 4452 Itingen Switzerland
Reference Number:	D52791

Study dates

Study initiation date:	04 April 2012
Experimental start date:	04 April 2012
Experimental termination date:	25 April 2012

Deviations from the study plan

In the main experiment the animals of the negative control group (48 h) and the high dose group (24 h) were not observed for clinical signs of toxicity at the 6 h post treatment interval, but at approximately at 5 h post treatment interval.

This deviation did not affect the validity of the study.

Retention of samples

Raw data, slides, and a sample of the test item.

Performing laboratory test substance reference number

S 1348611

Other

Harlan CCR will archive:

Raw data, study plan, original report, and specimens (if any) for at least 3 years at the test facility's archive. Thereafter, the material will be transferred to the GLP archive of Harlan Laboratories Ltd. in Füllinsdorf, Switzerland for archiving the remaining time up to a total archiving period of 15 years. No data will be discarded without the Sponsor's written consent.

A sample of the test item will be archived two years after the expiration date provided by the Sponsor. If no expiration date is given, the archiving period will be the required 15 years. Thereafter the samples will be discarded without further notice.

Distribution of the report

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

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

This study was performed in order 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 suspended in 1% CMC, which was also used as the vehicle control. The volume administered orally was 20 mL/kg body weight (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.

Seven males per test group (except the control groups with 5 males only) were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes (PCEs) 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 per slide and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

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

The tested dose (2000 mg/kg; maximum guideline-recommended dose) was estimated by a pre-experiment to be suitable. Since no toxic reactions were observed in the pre-experiment, and based on existing data for the test item, a limit dose test was performed using the highest dose only.

1.2 Results

The highest dose was estimated to be a suitable maximum tolerated dose based on a pre-experiment.

After treatment with the test item the number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the mean value of PCEs per 2000 erythrocytes of the vehicle control, thus indicating that glyphosate technical did not exert any significant 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 with any dose level used. The mean values of micronuclei observed after treatment with glyphosate technical were below 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 the 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-mutagenic in this bone 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.

2.2 Justification of Test System

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 were tested at a sampling time of 24 h after treatment to establish a dose response effect. Otherwise, for non toxic substances a single dose set at the maximum recommended level (2000 mg/kg b.w.) is sufficient.

To validate the test, a reference mutagen was tested in parallel to the test item.

2.3 Regulatory Guidelines

This study was conducted according to 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".

Environmental Protection Agency, Health Effects Test Guidelines OPPTS 870.5395 "Mammalian Erythrocyte Micronucleus Test", EPA 712-C-98-226, August 1998.

Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Part B: Methods for the determination of toxicity and other health effects: Mutagenicity – In vivo Mammalian Erythrocyte Micronucleus Test, No B.12; No L 142.

3.0 MATERIALS AND METHODS

3.1 Test Substance

Internal Test Item Number: S 1348611

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

Identity:	Glyphosate Technical
Product Code:	BX20070911
Batch No.:	569753
Purity:	Content of glyphosate technical: 96.3 % w/w (Dose calculation was not adjusted to purity.)
Stability in Solvent:	Not indicated by the sponsor
Storage:	At room temperature (< 30°C)
Reanalysis Date:	End of March 2015

On the day of the experiment, the test item was supended in 1% carboxymethylcellulose (CMC). Homogeneity of the test item in vehicle has been maintained during treatment using a magnetic stirrer. The vehicle was chosen by the Sponsor and due to its relative non-toxicity for the animals and ability to formulate a suitable dosing preparation. All animals received a single standard volume once orally. The oral route was used as this is of relevance to human risk assessment.

3.2 Controls

3.2.1 Negative control

The test item vehicle was used as negative control.

Name:	1% CMC
Source	FLUKA Chemie AG, 9471 Buchs, Switzerland
CAS No.:	9004-32-4
Catalogue no.:	21902
Batch no.:	1119535-12805109
Expiry Date:	January 2017
Route and Frequency of Administration:	Orally, once
Volume Administered:	20 mL/kg b.w.

3.2.2 Positive control

Name:	CPA; Cyclophosphamide
Supplier:	Fisher Scientific GmbH 61130 Nidderau, Germany
CAS No.:	6055-19-2
Catalogue no.:	203960010
Batch:	A0302605
Expiry Date:	July 2013
Dissolved in:	sterile 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 was sufficient. At 25 °C only 3.5 % of its potency was lost after 24 hours (7).

3.3 Test System

3.3.1 Reasons for the choice of the experimental animal species

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	Charles River Laboratories Research Models and Services Germany GmbH Sandhofer Weg 7, 97633 Sulzfeld, Germany
Number of Animals:	29 males
Initial Age at Start of Experiment:	8 - 9 weeks
Acclimation:	minimum 5 days
Initial Body Weight at Start of Treatment:	mean value 35.5 g (*SD ± 1.8 g); range 32.4 –38.8 g

According to the suppliers assurance the animals were in healthy condition. The animals were under acclimatisation in the animal house of Harlan 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.

*SD: Standard Deviation

3.3.2 Husbandry

The animals were kept conventionally. The experiment was conducted under standard laboratory conditions. The diet and water were routinely analysed to ensure the absence of any contaminant that could reasonably be expected to affect the purpose or integrity of the study. Certificates of analysis are retained at Harlan CCR.

Housing:	single
Cage type:	Makrolon Type II/III, with wire mesh top (Ehret, 79312 Emmendingen, Germany)
Bedding:	Granulated soft wood bedding (Rettenmaier & Söhne GmbH + Co. KG, 73494 Rosenberg, Germany)
Feed:	Pelleted standard diet, ad libitum (Harlan Laboratories B.V., Postbus 6174 5960 AD Horst / The Netherlands)
Water:	Tap water, ad libitum (Gemeindewerke Roßdorf, 64380 Roßdorf, Germany)
Environment:	Temperature 22 ± 2°C Relative humidity 45 - 65 % Artificial light 6.00 a.m. - 6.00 p.m.

3.4 Experimental Performance

3.4.1 Pre-Experiment

A preliminary study of 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. In a third preliminary experiment only female animals were used.

The animals were treated once 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.

The test dose levels were chosen using doses from the following scheme starting at 2000 mg/kg:

5 – 8 – 12.5 – 20 – 32 – 50 – 80 – 125 – 200 – 320 – 500 – 800 – 1250 – 2000 mg/kg b.w..

The pre-experiment demonstrated that the test item was non-toxic, therefore, a limit test was performed using the maximum dose of 2000 mg/kg b.w., omitting further lower doses.

3.4.2 Main-Experiment

It is generally recommended to use the maximum tolerated dose or the highest dose that can be dissolved 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 signs of toxicity without having major effects on survival within 48 hours.

The administered volume was 20 mL/kg b.w..

3.4.3 Study procedure

Seven males were assigned to each test group (except the negative and positive control groups with five animals each). The animals were identified by their cage number as shown in Table 1.

3.4.4 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 animal's body weight. The animals received the test item, the vehicle or the positive control substance once orally. Seven males were treated per dose group and sampling time. Five males each were treated for the vehicle and positive control group. The animals of all dose groups, except the positive control were

examined for acute toxic symptoms at intervals of around 1 h, 2 – 4 h, 6 h, 24 h, and 48 h after administration of the test item or the vehicle controls.

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

3.5 *Post mortem* Investigations

3.5.1 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 fetal 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, 64293 Darmstadt, Germany)/Giemsa (Merck, 64293 Darmstadt, Germany). Cover slips were mounted with EUKITT (Kindler, 79110 Freiburg, Germany). At least one slide was made from each bone marrow sample.

3.6 Data Evaluation

3.6.1 Slide analysis

Evaluation of the slides was performed using NIKON microscopes with 100× oil immersion objectives. Per animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined from the same slide and expressed in polychromatic erythrocytes per 2000 erythrocytes. The analysis was performed with coded slides. Immature and mature erythrocytes were identified by their pale and blue to green colour, respectively. Micronuclei are distinguished by being small nuclei separate from and additional to the main nuclei of the cells.

All animals per test group were evaluated as described.

3.6.2 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.

3.6.3 Acceptance criteria

The study was considered valid as the following criteria were met:

- at least 5 animals per group could be evaluated.
- PCE to erythrocyte ratio was not less than 20 % of the negative control.
- the positive control showed a statistically significant and biologically relevant increase of micronucleated PCEs compared to the negative control.

3.6.4 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 was the biological relevance of the results.

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

A test item failing to meet the criteria for a positive or negative response may be judged equivocal in this assay and may be considered for further investigation.

4.0 RESULTS AND DISCUSSION

4.1 Pre-experiment

In a pre-experiment 2 male and 2 female animals received a single oral dose of glyphosate technical (2000 mg/kg b.w.) suspended in 1% CMC (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 and based on pre-existing data for the test item, it was decided to perform the main experiment as limit test.

4.2 Signs of Toxicity in the Main Experiment

In the main experiment for the highest dose group 14 males received a single oral dose of 2000 mg/kg b.w. glyphosate technical formulated in 1 % 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 as well as the animals of the vehicle control (1 % CMC) group

4.2.1 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 significant cytotoxic properties in the bone marrow (Table 2).

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

4.3 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 suspended in 1% CMC, which was also used as vehicle (negative) 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.

Seven males per test group (except the control groups with five males only) were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes (PCEs) 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 and 48 h preparation interval: 2000 mg/kg b.w.

The tested dose (2000 mg/kg; maximum guideline-recommended dose) was estimated by a pre-experiment to be suitable. Since no toxic reactions were observed in the pre-experiment a limit dose test was performed using the highest does 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 significant cytotoxic properties in the bone marrow.

In comparison to the corresponding vehicle control values there was no biologically relevant enhancement and no statistically significant increase in the frequency of the detected micronuclei after administration of the test item. The mean value of micronuclei observed after treatment with glyphosate technical was below to the value of the respective vehicle control group and within the historical vehicle control range. Additionally no dose dependence was observed.

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. Therefore, glyphosate technical is considered to be non-mutagenic in this bone marrow micronucleus assay.

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 – 5	18 – 22
High dose	6 – 12	23 – 29
Positive control	13 – 17	

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	0.160	0 -8	1245
test item	2000	24	0.114	2 -3	1247
positive control	40	24	2.010	16 -67	1149
vehicle	0	48	0.070	0 -3	1197
test item	2000	48	0.057	0 -3	1092

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
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	n.t.	-

+ = significant;
- = not 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	1% CMC	0	6	1211
2	m			1	1364
3	m			0	1230
4	m			1	1154
5	m			8	1267
			sum	16	6226
			mean (± SD)	3.2 (± 3.6)	1245
		percent cells with micronuclei		**0.160**	

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
6	m	Glyphosate Technical	2000	2	1283
7	m			3	1296
8	m			2	1234
9	m			2	1232
10	m			2	1069
11	m			3	1252
12	m			2	1362
			sum	16	8728
			mean (± SD)	2.3 (± 0.5)	1247
		percent cells with micronuclei		**0.114**	

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

C. Positive control:

animal no.	sex	test group	dose mg/kg b.w.	micronucleated cells per 2000 PCEs per animal	PCE per 2000 erythrocytes
13	m	Cyclophosphamide	40	40	1151
14	m			67	1185
15	m			36	1132
16	m			42	1095
17	m			16	1181
			sum	201	5744
			mean (± SD)	40.2(± 18.2)	1149
			percent cells with micronuclei	**2.010**	

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
18	m	1% CMC	0	1	1177
19	m			3	1280
20	m			2	1278
21	m			1	1097
22	m			0	1153
			sum	7	5985
			mean (± SD)	1.4 (± 1.1)	1197
		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
23	m	Glyphosate Technical	2000	0	1204
24	m			3	931
25	m			0	1168
26	m			1	1021
27	m			3	1141
28	m			0	1110
29	m			1	1067
			sum	8	7642
			mean	1.1 (± 1.3)	1092
		percent cells with micronuclei		**0.057**	

TABLE 6 Individual Animal Weights at the Start of the Experiment

Dose Group	Animal No.	Initial Weight (g)	Mean Weight (g)	Standard Deviation	Range (g)
Negative control Group; 1% CMC; 24h Interval	1	32.6			
	2	36.6			
	3	34.7			
	4	36.8			
	5	33.4	34.8	± 1.9	32.6 – 36.8
High Dose Group (2000 mg/kg b.w.); 24h Interval	6	36.6			
	7	36.3			
	8	35.9			
	9	37.9			
	10	34.0			
	11	33.4			
	12	37.6	36.0	± 1.7	33.4 .– 37.9
Positive Control (CPA, 40 mg/kg b.w.); 24h Interval	13	32.4			
	14	34.4			
	15	37.3			
	16	34.2			
	17	33.2	34.3	± 1.9	32.4 – 37.3
Negative Control Group;1% CMC; 48h Interval	18	37.7			
	19	34.8			
	20	33.6			
	21	34.9			
	22	35.1	35.2	± 1.5	33.6 – 37.7
High Dose Group (2000 mg/kg b.w.); 48h Interval	23	37.1			
	24	34.7			
	25	37.3			
	26	35.8			
	27	38.8			
	28	37.3			
	29	34.6	36.5	± 1.5	34.6 – 38.8
Summary			35.5	± 1.8	32.4 – 38.8

APPENDICES SECTION

APPENDIX 1 Historical Control Data

2006 – 2011

Micronucleated cells	Negative Controls Males	Positive Controls (CPA) Males
Mean ± SD (%)	0.108 ± 0.039	2.533 ± 0.632
Range of mean group value (%)	0.010 - 0.250	0.858 - 4.370
Range (individual animal data)	0 - 9	8 - 139
No. of Experiments	219	217

APPENDIX 2 Copy of GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Harlan Cytotest Cell Research GmbH
Harlan Cytotest Cell Research GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies
6 Prüfungen zur Bestimmung von Rückständen	**6** Residues
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical studies on biological materials

15.08. und 27. – 29.10.2008
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

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

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.

Im Auftrag

[redacted]

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

HESSISCHES MINISTERIUM FÜR UMWELT, LÄNDLICHEN RAUM UND VERBRAUCHERSCHUTZ 2

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

English name and address of the GLP Monitoring Authority: Hessian Ministry for Environment, Energy, Agriculture and Consumer Protection; Department II 10; P.O. Box 31 09; 65189 Wiesbaden
English translation of the GLP Monitoring Authority Stamp: Hessian Ministry for Environment, Area for Agricultural Use and Consumer Protection

APPENDIX 3 Copy of Certificate of Analysis

Syngenta Crop Protection, LLC
Technology & Engineering
Analytical & Product Chemistry
Greensboro, NC 27409

Certificate of Analysis

Glyphosate Technical Batch ID 569753 (BX20070911)	
Batch Identification	569753
Product Design Code	ASF71W
Other Product Information	Formerly known as BX20070911
Product by Common Name	Glyphosate Technical
Other Product Code(s)	BX20070911
Source	Nantong Jiangshan Agrochemicals & Chemicals Limited, Jiangsu, China
Chemical Analysis (Active Ingredient Content)	
Identity of the Active Ingredient*	Confirmed
Content of Glyphosate Technical*	96.3% (wt/wt)
Methodology Used for Characterization	HPLC
Physical Analysis	
Appearance*	white solid
Stability	
Storage Temperature	< 30°C
Recertification date	End of March 2015

If stored under the conditions given above, this test substance can be considered stable until the recertification date is reached.

The stability of this test substance has been determined through periodic reanalysis of this batch of test substance, held under GLP conditions at Syngenta Crop Protection, LLC, Greensboro, NC., and was found to be stable for at least 5 years at room temperature.

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

Authorization:

[redacted]

Sr. Analytical Chemist
Analytical & Product Chemistry Department

June 20, 2012
Date