Glyphosate

Glyphosate Technical – Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

Final Report

DATA REQUIREMENT(S): OECD Test Guideline 425 (2008)
EPA OPPTS 870.1100 (2002)

AUTHOR(S): Judit Tavaszi, M.Sc.

STUDY COMPLETION DATE: 15 April 2011

PERFORMING LABORATORY: LAB Research Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

LABORATORY PROJECT ID: Report Number: 10/218-001P
Study Number: 10/218-001P
Task Number: TK0040339

SPONSOR(S): Syngenta Ltd.
Jealott's Hill International Research Centre
Bracknell, Berkshire, RG42 6EY, United Kingdom

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study has been performed in accordance with the Principles of Good Laboratory Practice (Hungarian GLP Regulations: 9/2001. (III. 30.) EüM-FVM joint decree of the Minister of Health and the Minister of Agriculture and Regional Development which corresponds to the OECD GLP, ENV/MC/CHEM (98) 17.).

This study was conducted in accordance with a written Study Plan and Amendment, authorized by the Sponsor and LAB Research Ltd. management, and followed applicable Standard Operating Procedures.

I, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the course of this study.

Signature: _____ Date: 15 April 2011

Judit Tavaszi, M.Sc.
Study Director

Performing Laboratory: LAB Research Ltd.
H-8200 Veszprém, Szabadságpuszta,
Hungary

FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Study Number: 10/218-001P

Study Title: Glyphosate Technical – Acute Oral Toxicity Study in the Rat (Up and Down Procedure)

Test Item: Glyphosate Technical

This study has been inspected, and this report audited by the Quality Assurance Unit in compliance with the Principles of Good Laboratory Practice. As far as it can be reasonably established the methods described and the results incorporated in this report accurately reflect the raw data produced during this study.

All inspections, data reviews and the report audit were reported in written form to the study director and to management. The dates of such inspections and of the report audit are given below:

Date of Inspection	Phase(s) Inspected/Audited	Date of report to	
		Management	Study Director
09 December 2010	Study Plan	09 December 2010	09 December 2010
14 December 2010	Clinical Observation	14 December 2010	14 December 2010
20 January 2011	Treatment	20 January 2011	20 January 2011
21 March 2011	Draft Report	21 March 2011	21 March 2011
15 April 2011	Final Report	15 April 2011	15 April 2011

Signature: Czuczay Diána Date: 15 April 2011

Diána Czuczay, M.Sc.
On behalf of QA

MANAGEMENT STATEMENT

According to the conditions of the research and development agreement between Syngenta Ltd. (as Sponsor) and LAB Research Ltd. (Test Facility) the study titled "Glyphosate Technical- Acute Oral Toxicity Study in the Rat (Up and Down Procedure)" has been performed in compliance with the Principles of Good Laboratory Practice.

Signature: _____ Date: 15 April 2011

Christopher Banks, DABT
Managing Director

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Function
Judit Tavaszi, M.Sc.	Study Director
Viktória Zelenák, M.Sc.	Assistant scientist
István Pásztor, DVM	Veterinary control
Peter Maslej, DVM, PhD	Head of Pathology Unit
Tamás Mészáros, PhD	Technical Team Leader of Central Dispensary
Ryan Doran, M.Sc.	Syngenta Study Manager

Study dates

Experimental Starting Date	20 January 2011
Experimental Completion Date	10 February 2011
Reception of Animals	30 December 2010
Acclimatization	At least 21 days
Treatment	20 January 2011 (female no. 3512) 25 January 2011 (female no. 3513) 27 January 2011 (female no. 3511)
Observation	20 January – 03 February 2011 (female no. 3512) 25 January – 08 February 2011 (female no. 3513) 27 January – 10 February 2011 (female no. 3511)

Deviations from the guidelines

There was no deviation from the guidelines.

Deviations from the study plan

No indication of hazard classification in accordance with EU guidelines was given in the final report.

The correct arrival date of the rats was 30 December 2010.

The Draft Report was sent later than indicated in the Study Plan.

These deviations have no impact on the outcome of the study.

Performing laboratory test substance reference number
10/197K/1 100817

Other
The study documents:
- study plan and amendment,
- all raw data,
- sample of the test item,
- study report and any amendments,
- correspondence

will be archived according to the Hungarian GLP and to applicable SOP's in the archives of LAB Research Ltd. 8200 Veszprém, Szabadságpuszta, Hungary.

After the retention time of 15 years has elapsed all the archived materials listed above will be returned to the Sponsor or retained for a further period if agreed by a contract. Otherwise the materials will be discarded.

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

1.1 Study Design

Two limit tests with different dose levels were performed. As there were no clinical signs or macroscopic findings observed at the dose level of 2000 mg/kg a limit test at a higher dose level (5000 mg/kg bw) was requested by the Sponsor, data from the animals treated at 2000 mg/kg bw will be archived in the raw data without any further reporting.

A single oral (gavage) administration was performed followed by a 14 day observation period. The animals were fasted overnight prior to treatment. Animals were weighed before dosing and food was returned 3 hours after dosing.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs.

The first animal was treated at a dose level of 5000 mg/kg body weight (bw). As no mortality or significant clinical signs were observed, 2 additional animals were sequentially dosed at 5000 mg/kg bw such that a total of 3 animals were tested. No mortalities were observed, therefore the study was terminated.

Animals were observed individually after dosing at 30 minutes, then 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter. Body weight was measured on Day -1, just before treatment and weekly thereafter. All animals were examined macroscopically at the end of the study.

1.2 Results

No deaths occurred during the study.

No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

There were no treatment related changes in the body weights. The body weights of the animals were within the range commonly recorded for this strain and age.

No test item-related macroscopic findings were observed.

1.3 Conclusion

Under the conditions of this study, the acute oral median lethal dose LD_{50} of the test item, Glyphosate Technical, was greater than 5000 mg/kg bw in female RjHan:WI rats.

2.0 INTRODUCTION

2.1 Purpose

The purpose of the study was to assess the oral toxicity of the test item Glyphosate Technical when administered as a single oral gavage dose to rats at one defined dose level. The results of the study allowed the test item to be ranked according to most classification systems currently in use.

2.2 Guidelines

The study was performed according to the following guidelines:

OECD guideline reference 425 (2008): Acute Oral Toxicity - Up-and-Down Procedure.

United States Environmental Protection Agency, Health Effects Test Guidelines, OPPTS 870.1100 Acute Oral Toxicity EPA 712-C-02-190, December 2002.

3.0 MATERIALS AND METHODS

3.1 Test Substance

Data as supplied by the Sponsor.

Name:	Glyphosate Technical
Batch number:	569753
Product code:	ASF71W
Source:	Nantong Jiangshan Agrochemicals & Chemicals Limited, Jiangsu, China (Glycine route of synthesis)
Appearance:	Dry white powder
Expiry date:	31 August 2011
Storage conditions:	< 30˚C
Safety Precautions:	Routine safety precautions (lab coat, gloves, goggles, face mask) for unknown materials were applied to assure personnel health and safety

The Certificate of Analysis is attached in Appendix 2.

3.1.1 Identification, Receipt

The test item of a suitable chemical purity together with all precautions required in the handling and disposal of the test item were supplied by the Sponsor. The identification of the test item was made in the Central Dispensary Unit of LAB Research Ltd. on the basis of the information provided by Sponsor.

3.1.2 Formulation

The test item was formulated for treatment doses at 5000 mg/kg bw (dose volume of 10 mL/kg) in 0.5% Carboxymethylcellulose (CMC).

Vehicle:	**0.5% Carboxymethylcellulose**	
	CMC	Distilled water
Batch number:	BCBC9753V	7530810
Expiry Date:	31 October 2011	August 2013
Produced by:	Sigma Aldrich	Teva

3.2 Experimental Animals

Species and strain:	RjHan:WI rats
Source:	Laboratoire Elevage Janvier, B.P. 4105, Route des Chênes Secs, 53940 Le Genest-St-Isle CEDEX FRANCE
Hygienic level at arrival:	SPF
Hygienic level during the study:	Standard housing conditions
Justification of strain:	Recognized by international guidelines as a recommended test system.
Number of animals:	3
Sex:	Female rats, nulliparous and non-pregnant.
Age when treated:	Young adult rats, 10-11 weeks old.
Body weight (at dosing):	Not more than 231 g
Randomization:	Selected by hand at time of delivery.
Acclimatization time:	At least 21 days

3.2.1 Husbandry

Animal health:	Only healthy animals were used for the test. The health status was certified by the veterinarian.
Room number:	522/1
Housing:	Individual caging
Cage type:	Type II. polypropylene/polycarbonate
Bedding:	Lignocel Bedding for Laboratory Animals was available to animals during the study.
Light:	12 hours daily, from 6.00 a.m. to 6.00 p.m.
Temperature:	22 ± 3 °C
Relative humidity:	30 – 70%
Ventilation:	15-20 air exchanges/hour
Enrichment:	Rodents were housed with deep wood sawdust bedding to allow digging and other normal rodent activities.

The temperature and relative humidity was recorded twice daily during the study and the acclimation period.

3.2.2 Food and water supply

Animals received ssniff® SM R/M-Z+H "Autoclavable complete feed for rats and rats – breeding and maintenance" produced by ssniff Spezialdiäten GmbH, D-59494 Soest Germany *ad libitum*, and tap water from municipal supply, as for human consumption from 500 mL bottle *ad libitum*. The food was considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study. Details of the diet are archived with the raw data at LAB Research Ltd.

Water quality control analysis is performed once every three months and microbiological assessment is performed monthly by Veszprém County Institute of State Public Health and Medical Officer Service (ÁNTSZ, H-8201 Veszprém, József A.u.36., Hungary). The quality control results are retained in the archive at LAB Research Ltd.

3.2.3 Identification

Animals were individually identified by numbers written on the tail with a permanent marker pen. The numbers were given on the basis of LAB Research Ltd.'s master file, for each animal allocated to the study. The boxes were identified by cards holding information on the study code, the sex of animals, the dose group, the cage number and the individual animal number.

3.3 Administration of the Test Item

3.3.1 Dosages

Justification of the doses:

A limit dose of 5000 mg/kg bw was selected as there is a special regulatory requirement to test this dose level. The test item was formulated in 0.5% Carboxy Methylcellulose. The applied dose volume was 10 mL/kg bw.

Rationale: Oral administration was considered to be an appropriate dose route as it is a possible route of human exposure.

Animal Number	Dosage [mg/kg body weight]
3512	5000
3513	5000
3511	5000

3.3.2 Procedure

A single oral (gavage) administration was followed by a 14 day observation period. The day before treatment the animals were fasted. The food, but not water, was withheld overnight. Animals were weighed before dosing and the food was returned 3 hours after the treatment.

Single animals were dosed sequentially following an interval of at least approximately 48 hours. The time intervals between dosing were determined by the onset, duration and severity of toxic signs. Treatment of an animal at the next dose was only performed when no significant clinical signs were noted in the previous animal.

3.4 Observations

3.4.1 Clinical observations

Animals were observed individually after dosing at 30 minutes, then 1, 2, 3, 4, and 6 hours after dosing and once each day for 14 days thereafter. Individual observations were performed on the skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

3.4.2 Body weight measurement

The body weights were recorded on Days -1, 0 (beginning of the experiment), 7 and 14.

3.5 Necropsy

All animals were subjected to macroscopic examination. All animals were exsanguinated under pentobarbital anaesthesia (Euthasol® 40%, Lot No.: 10C25 7, Expiry Date: February 2013, Produced by: AST Beheer B.V. Oudewater Netherlands (Produlab Pharma, Raamsdonksveer). After examination of the external appearance the cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. All gross pathological changes were recorded for each animal on the post mortem record sheets.

3.6 Data Evaluation

Type, severity and duration of clinical observations are described. Body weight and body weight changes are summarised in tabular form. Necropsy findings are described and summarised in tabular form.

4.0 RESULTS AND DISCUSSION

4.1 Results

Individual clinical observations and mortality results are presented in Table 1. Individual body weights and necropsy results are presented in Tables 2 and 3, respectively.

4.2 Mortality

There were no mortalities observed.

4.3 Body Weights

The body weight and the body weight gain did not show any test item related effect.

4.4 Clinical Signs

No clinical signs were observed in the 3 animals treated at 5000 mg/kg bw.

4.5 Macroscopic Findings

No test item-related macroscopic findings were observed at a dose level of 5000 mg/kg bw.

5.0 CONCLUSIONS

Under the conditions of this study, the acute oral median lethal dose LD_{50} of the test item, Glyphosate Technical, was greater than 5000 mg/kg bw in female RjHan:WI rats.

TABLES SECTION

TABLE 1 Individual Findings – Clinical Signs

DOSE LEVEL: 5000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal Number	Observations	Observation days							Frequency
			0						1-14	
			30'	1h	2h	3h	4h	6h		
1	3512	Symptom Free	+	+	+	+	+	+	+	20/20
2	3513	Symptom Free	+	+	+	+	+	+	+	20/20
3	3511	Symptom Free	+	+	+	+	+	+	+	20/20

Remarks: +: present

h=hour (s) Treatment day= Day 0

Frequency of observation = number of occurence of observation / total number of observations

TABLE 2 Body Weight and Body Weight Gain

DOSE LEVEL: 5000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal No.	Body weight (g) Days				Body Weight Gain (g)			
		-1	**0**	**7**	**14**	**-1-0**	**0-7**	**7- 14**	**-1 - 14**
1	3512	242	229	252	266	-13	23	14	24
2	3513	247	228	253	263	-19	25	10	16
3	3511	241	231	245	257	-10	14	12	16
Mean:		243.3	229.3	250.0	262.0	-14.0	20.7	12.0	18.7
Standard deviation:		3.2	1.5	4.4	4.6	4.6	5.9	2.0	4.6

Remark: Treatment day= Day 0

TABLE 3 Macroscopic Findings

DOSE LEVEL: 5000 mg/kg bw **SEX: FEMALE**

Cage No.	Animal ID	Necropsy Date	External Observations	Internal Observations	Organ/Tissue
1	3512	03 February 2011	No external observations recorded	No internal observations recorded	Not applicable
2	3513	08 February 2011	No external observations recorded	No internal observations recorded	Not applicable
3	3511	10 February 2011	No external observations recorded	No internal observations recorded	Not applicable

APPENDICES SECTION

APPENDIX 1 Pathology report

LAB Study code. 10/218-001P

PATHOLOGY REPORT

INTRODUCTION

The objective of the study was to assess the acute oral toxicity of Glyphosate Technical when administered in a single dose to female rats at one or more defined doses. The results of the study allows the calculation of the estimated oral LD_{50} of the test item and permits the test item to be ranked according to most classification systems currently in use.

RESULTS AND DISCUSSION

All rats survived until the scheduled termination of the study.

All animals were euthanized upon completion of the treatment period on Day 14. Rats were anesthetized with pentobarbital, followed by exsanguination. Gross pathology consisted of an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Histopathological examination was not performed.

TERMINAL (DAY 14)

Macroscopic Findings

There was no evidence of the macroscopic observations in animals at dose levels of 5000 mg/kg bw.

CONCLUSION

A single oral gavage of Glyphosate Technical to the RjHan:WI female rat at dose levels of 5000 mg/kg bw with a 14 day observation period, was not associated with any macroscopic findings.

15 April 2011

Peter Maslej, D.V.M., Ph.D. Date
Head, Pathology Department

APPENDIX 2 Certificate of Analysis

syngenta

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

Certificate of Analysis

Glyphosate Technical Technical
Batch ID 569753 (BX20070911)

Batch Identification	569753
Product Design Code	ASF71W
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

The Active Ingredient content is within the FAO limits.

Physical Analysis	
Appearance*	Dry white powder
Stability	
Storage Temperature	< 30°C
Recertification date	End of August 2011

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, Inc., Greensboro, NC., and was found to be stable for at least 1 year at the listed storage temperature (< 30°C).

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:

J Malek — Oct. 21, 2010

Jim Malek
Sr. Group Leader
Analytical & Product Chemistry Department

Date

APPENDIX 3 GLP Certificates

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

H-1051 Budapest, Zrínyi u. 3.
Mail: 1372 P.O. Box 450.
Phone: +36 1 8869-300
Fax: +36 1 8869-460
E-mail: ogyi@ogyi.hu

Budapest, 20th December 2008
No: 38625/48/2007
Our ref.: Szilvia Karsai
Subject: GLP Certificate

GOOD LABORATORY PRACTICE (GLP)
CERTIFICATE

Based on the Inspection report and the discussion of follow up activities it is hereby certified that the test facility

LAB Research Ltd.
H-8201 Veszprém, Szabadságpuszta, Hungary

is able to carry out **Physical-chemical testing, Toxicity studies, Mutagenicity studies, Environmental toxicity studies on aquatic and terrestrial organisms, Studies on behaviour in water, soil and air; bioaccumulation, Bioanalytical, Analytical and clinical chemistry testing** compliance with the Principles of GLP (Good Laboratory Practice).

Date of the inspection: **13-22 October 2008.**

This GLP Certificate is valid for 2 years.

Zsuzsanna Szepezdi, Ph. D.
Director-General

APPENDIX 3 GLP Certificates (Continued)

ORSZÁGOS GYÓGYSZERÉSZETI INTÉZET
National Institute of Pharmacy

FŐIGAZGATÓ
1051 Budapest, Zrínyi u. 3.
tel: (1) 8869-320
fax: (1) 8869-480
e-mail: szepezdi.zsuzsanna@ogyi.hu

Ref. no: OGYI/8242-11/2010
Admin.: Urbin Magdolna Zita
Date: 16 December, 2010

GOOD LABORATORY PRACTICE (GLP) CERTIFICATE

It is hereby certified that the test facility

LAB Research Kft.

(Base facility: H-8201 Veszprém, Szabadságpuszta, Hungary)

is able to carry out

physico-chemical testing, toxicity studies, mutagenicity studies, environmental toxicity studies on aquatic or terrestrial organisms, studies on behaviour in water, soil and air;bio-accumulation , safety pharmacology testing, reproduction toxicology, inhalation toxicology, analytical chemistry and contract archiving

in compliance with the Principles of GLP (Good Laboratory Practice) and also complies with the corresponding OECD/European Community requirements.

Date of the inspection: **4-8 October, 2010.**

Zsuzsanna Szepezdi, Ph. D.
Director-General