TERC Information Collection (TIC) Index

The Dow Chemical Company

Title
GLYPHOSATE TGAI: MICRONUCLEUS TEST OF GLYPHOSATE TGAI IN MICE
Author(s)
[redacted], JAI RESEARCH FOUNDATION
Reviewer(s)
[redacted]

Patent Status	*Date Issued*	*Page Count*	*File Number*
No Action Required	13 September 2012	55	DR-0112-6927-003
Geographic Location	***Department***	***Archive Number***	***PTR***
EMEA	TERC	20121002131627	10001701-27-1

Abstract

This study was performed to assess the micronucleus induction potential of Glyphosate TGAI (supplied by Dow AgroSciences) in mice. The methods followed were as per the guidelines of OECD 474, OPPTS 870.5395, EEC B.12 and JMAFF 2-1-19-3.

Dose Range Finding Study

A dose range finding study was conducted to determine the doses (Maximum Tolerated Dose, MTD) to be employed in the main study. MTD is defined as the dose that produces clear signs of toxicity without having a major effect on thermal regulation or the survival of the animals. For changes in thermal regulation, a body temperature increase of at least 1 °C or a decrease of at least 3 °C for five or more hours was declared as having exceeded MTD.

Three animals per sex were treated at the dose levels of 2000 mg of Glyphosate TGAI/kg body weight by oral gavages for two consecutive days. All the animals were normal in the control and treatment groups, post treatment and pre-sacrifice. Mortality was not observed in any of the animals from the control and the treatment groups.

Based on the results of the dose range finding study, 2000 mg of Glyphosate TGAI/kg body weight was selected as the limit dose for the main study.

Main Study

Eighteen healthy Swiss albino mice (18 males) were divided into 3 groups, each group comprising of 6 male animals. The test item, Glyphosate TGAI, was suspended in vegetable oil and administered orally at

limit dose of 2000 mg/kg body weight (Group II) for two consecutive days. The mice from the vehicle control group (Group I) received only vegetable oil by oral gavage. Mice from the positive control group (Group III) received a single intraperitoneal injection of mitomycin-C at the dose level of 1.0 mg/kg body weight on day 2 of the treatment.

All the animals were normal in the vehicle control group (Group I), treatment group (Group II) and positive control group (Group III), both post-treatment and pre-sacrifice. Mortality was not observed in any of the animals from the controls and treatment groups. Body weights were comparable among the groups during the experimental period.

All the animals were sacrificed approximately 24 hours following the last treatment. The ratio of polychromatic erythrocytes (PCE) to total erythrocytes (P/E ratio) in the Glyphosate TGAI treated group was comparable to the vehicle control group. Hence, there was no evidence of cell proliferation or cytotoxicity to the target cells. No statistically significant increase in the number and percentage of micronucleated polychromatic erythrocyte (% MNPCE) was observed in animals treated with the dose level of 2000 mg/kg body weight when compared with vehicle control group. The positive control group treated with mitomycin-C yielded a statistically significant increase in the number of percent micronucleated polychromatic erythrocytes (% MNPCE) in comparison with vehicle control group.

From the results of the present study, it is concluded that Glyphosate TGAI does not have micronucleus induction potential up to the guidelines limit dose of 2000 mg/kg body weight, following oral administration for two consecutive days.

Summary

(In accordance with 40 CFR Part 152, this summary is available for public release after registration)

Study Title

Micronucleus Test of Glyphosate TGAI in Mice

Test Guidelines

GUIDELINES: OECD 474 (1997), OPPTS 870.5395 (1998), EEC B.12 (2008) and JMAFF 2-1-19-3 (2000)

Author(s)

STUDY DIRECTOR/REPORT AUTHOR: [redacted]

Study Completion Date

SEPTEMBER 13, 2012

Contact Phone

N/A

Sponsor

DOW AGROSCIENCES LLC
9330 ZIONSVILLE ROAD
INDIANAPOLIS, IN 46268
U.S.A.

Test Facility

JAI RESEARCH FOUNDATION
DEPARTMENT OF TOXICOLOGY
VALVADA - 396 108, DIST. VALSAD
GUJARAT
INDIA

SUMMARY

Eighteen healthy Swiss albino mice (18 males) were divided into 3 groups, each group comprising of 6 male animals. The test item, Glyphosate TGAI, was suspended in vegetable oil and administered orally at limit dose of 2000 mg/kg body weight (Group II) for two consecutive days.

All the animals were normal in the vehicle control group (Group I), treatment group (Group II) and positive control group (Group III), both post-treatment and pre-sacrifice. Mortality was not observed in any of the animals from the controls and treatment groups.

An increase in the number and percentage of micronucleated polychromatic erythrocyte (% MNPCE) was not observed in animals treated with the dose level of 2000 mg/kg body weight when compared to vehicle control group. The positive control group treated with mitomycin-C yielded a statistically significant increase in the number of micronucleated polychromatic erythrocytes (MNPCE) in comparison to the vehicle control group.

All criteria for a valid study were met as described in the protocol. Based on the results, it is concluded that Glyphosate TGAI does not have micronucleus induction potential in the animals treated up to the guidelines limit dose of 2000 mg/kg body weight following oral administration for two consecutive days.

STUDY TITLE

MICRONUCLEUS TEST OF GLYPHOSATE TGAI IN MICE

DATA REQUIREMENT
GUIDELINES: OECD 474, OPPTS 870.5395, EEC B.12 and JMAFF 2-1-19-3

STUDY DIRECTOR/REPORT AUTHOR: [redacted]

STUDY COMPLETION: SEPTEMBER 13, 2012

SPONSOR

DOW AGROSCIENCES LLC
9330 ZIONSVILLE ROAD
INDIANAPOLIS, IN 46268
U.S.A.

TEST FACILITY

JAI RESEARCH FOUNDATION
DEPARTMENT OF TOXICOLOGY
VALVADA - 396 108, DIST. VALSAD
GUJARAT
INDIA

LABORATORY PROJECT STUDY NUMBER 485-1-06-4696
DAS STUDY NUMBER 120709

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound : Glyphosate TGAI

No claim of confidentiality, on any basis whatsoever, is made for any information contained in this document. I acknowledge that information not designated as within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) and which pertains to a registered or previously registered pesticide is not entitled to confidential treatment and may be released to the public, subject to the provisions regarding disclosure to multinational entities under FIFRA sec. 10(g).

Company: Dow AgroSciences LLC

Company Agent: [redacted] 28. Sep. - 2012
(Date)

Regulatory Manager

THESE DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES

STATEMENT OF GOOD LABORATORY PRACTICE COMPLIANCE

Compound : Glyphosate TGAI

Study Title : Micronucleus Test of Glyphosate TGAI in Mice

Except as noted below, the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

Organisation for Economic Co-operation and Development (OECD)
ENV/MC/CHEM(98)17

Environmental Protection Agency (EPA-FIFRA)
Title 40 of the US Code of Federal Regulations Part 160
16 October 1989

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF)
12-Nousan- N° 8628, 6 December 2000

Exception: Test item characterization (identity, strength, purity and composition), stability, method of synthesis and location of documents for the synthesis is the responsibility of the Sponsor.

The dose solutions were used within 4 hours of formulating, but were not subject to analytical verification.

[redacted] M.Sc. [redacted] Date
Study Director

[redacted] September 13, 2012
Date
Test Facility Management

[redacted]
Regulatory Manager
Dow AgroSciences LLC

STATEMENT OF QUALITY ASSURANCE

Compound : **Glyphosate TGAI**

Study Title : **Micronucleus Test of Glyphosate TGAI in Mice**

This study was audited and the final report examined with respect to the protocol, standard operating procedures and raw data for conformance with OECD Principles of Good Laboratory Practice. The report was determined to be a full and accurate reflection of the procedures adopted and the raw data generated during the study.

The audits were carried out according to the standard operating procedures of the Quality Assurance Unit of Jai Research Foundation (JRF) and in compliance with OECD monograph N° 4, ENV/JM/MONO(99)20 (1999).

Findings resulting from the audits were reported to the Study Director and the Management on the dates specified below. These reports are kept in the GLP Archives at JRF.

Inspection/Audit			Reporting Dates to	
N°	Details	Date	Study Director	Facility Management
45304	Protocol	May 15, 2012	May 15, 2012	May 16, 2012
46755	Body weight, dose formulation preparation and dosing (Day 2)	July 17, 2012	July 17, 2012	July 18, 2012
46784	Sacrifice and bone marrow harvesting	July 18, 2012	July 18, 2012	July 19, 2012
47583	Raw data and report	August 23, 2012	August 23, 2012	August 24, 2012
48031	Final report	September 12, 2012	September 12, 2012	September 13, 2012

[redacted], M.Pharm.
QUALITY ASSURANCE OFFICER, JRF
DATE: September 13, 2012

CONTENTS

PAGE N°

PERSONNEL INVOLVED IN THE STUDY

Study Director

Deputy Study Director

Study Personnel

Statistical Analyst

REPORT APPROVAL

This study report is approved by:

September 13, 2012

, Ph.D.
TEST FACILITY MANAGEMENT

SUMMARY

This study was performed to assess the micronucleus induction potential of Glyphosate TGAI (supplied by Dow AgroSciences) in mice. The methods followed were as per the guidelines of OECD 474, OPPTS 870.5395, EEC B.12 and JMAFF 2-1-19-3.

Dose Range Finding Study

A dose range finding study was conducted to determine the doses (Maximum Tolerated Dose, MTD) to be employed in the main study. MTD is defined as the dose that produces clear signs of toxicity without having a major effect on thermal regulation or the survival of the animals. For changes in thermal regulation, a body temperature increase of at least 1 °C or a decrease of at least 3 °C for five or more hours was declared as having exceeded MTD.

Three animals per sex were treated at the dose levels of 2000 mg of Glyphosate TGAI/kg body weight by oral gavages for two consecutive days. All the animals were normal in the control and treatment groups, post treatment and pre-sacrifice. Mortality was not observed in any of the animals from the control and the treatment groups.

Based on the results of the dose range finding study, 2000 mg of Glyphosate TGAI/kg body weight was selected as the limit dose for the main study

Main Study

Eighteen healthy Swiss albino mice (18 males) were divided into 3 groups, each group comprising of 6 male animals. The test item, Glyphosate TGAI, was suspended in vegetable oil and administered orally at limit dose of 2000 mg/kg body weight (Group II) for two consecutive days. The mice from the vehicle control group (Group I) received only vegetable oil by oral gavage. Mice from the positive control group (Group III) received a single intraperitoneal injection of mitomycin-C at the dose level of 1.0 mg/kg body weight on day 2 of the treatment.

All the animals were normal in the vehicle control group (Group I), treatment group (Group II) and positive control group (Group III), both post-treatment and pre-sacrifice. Mortality was not observed in any of the animals from the controls and treatment groups. Body weights were comparable among the groups during the experimental period.

All the animals were sacrificed approximately 24 hours following the last treatment. The ratio of polychromatic erythrocytes (PCE) to total erythrocytes (P/E ratio) in the Glyphosate TGAI treated group was comparable to the vehicle control group. Hence, there was no evidence of cell proliferation or cytotoxicity to the target cells. No statistically significant increase in the number and percentage of micronucleated polychromatic erythrocyte (% MNPCE) was observed in animals treated with the dose level of 2000 mg/kg body weight when compared with vehicle control group. The positive control group treated with mitomycin-C yielded a statistically significant increase in the number of percent micronucleated polychromatic erythrocytes (% MNPCE) in comparison with vehicle control group.

From the results of the present study, it is concluded that Glyphosate TGAI does not have micronucleus induction potential up to the guidelines limit dose of 2000 mg/kg body weight, following oral administration for two consecutive days.

1. INTRODUCTION

1.1 Study Objective

This study was performed to evaluate the micronucleus induction potential of Glyphosate TGAI in mice. The study was conducted in compliance with Principles of GLP (OECD 1998, EPA 1989, JMAFF 2000).

1.2 Study Guidelines

The present study was conducted according to:

The Organisation for Economic Co-operation and Development (OECD) Guidelines for Testing of Chemicals Volume II, N° 474 "Mammalian Erythrocyte Micronucleus Test" adopted by the Council on July 21, 1997;

The United States Environmental Protection Agency (EPA) Health Effects Test Guidelines OPPTS 870.5395, Mammalian Erythrocyte Micronucleus Test (August 1998);

Official Journal of the European Economic Community (L142): Part B: Methods for the Determination of Toxicity and other Health Effects, B.12 Mutagenicity: *In vivo* Mammalian Erythrocyte Micronucleus Test. EC 440/2008, adopted May 30, 2008;

and

Japanese Ministry of Agriculture, Forestry and Fisheries. Testing Guidelines for Toxicology Studies, 2000. Notification No. 12-Nousan-8147. 24 November 2000. Micronucleus Studies 2-1-19-3.

1.3 Justification for Selection of the Test System

The mouse was selected as the test system because it is a readily available rodent species. It has been historically shown to be a suitable model for assessing the micronucleus induction potential and is recommended by the OECD and other regulatory authorities. The results of the study are believed to be of value in predicting the micronucleus induction potential of the Glyphosate TGAI in humans.

1.4 Test Facility and Study Period

This study was performed at the Department of Toxicology, Jai Research Foundation, Valvada - 396 108, Dist. Valsad, Gujarat, India.

Study Initiation	:	May 17, 2012
Experiment Start	:	May 28, 2012
Experiment Completion	:	August 07, 2012
Study Completion	:	September 13, 2012

1.5 Archives

All the original raw data records, protocol, protocol amendment and final report will be transferred to the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, 1803 Building, Midland, MI 48674 U.S.A. Slides generated during the study will be transferred to the Dow Chemical Company, 1803 Building, Midland, MI 48674, U.S.A. A self attested photocopy of the raw data records, copy of protocol, copy of protocol amendment, draft report, copy of final report, electronically recorded data and the representative sample of test item will be retained in the GLP Archives of Jai Research Foundation for a period of ten years, after which time they will be disposed of by JRF.

2. EXPERIMENTAL PROCEDURE

2.1 Test Item

Details of the test item provided by the Sponsor (Ref. TIDS):

Test Item Name	:	Glyphosate TGAI
Test Item Number	:	TSN105914
CA Name	:	N/K
CAS Number	:	Glyphosate: 1071-83-6
Analysed Concentration	:	98.9 weight% (Refer Certificate of Analysis in APPENDIX 13)
Lot N°	:	20061109
Supplied by	:	Dow AgroSciences
Manufactured by	:	Good Harvest-Weien Agrochemical Co. Ltd., Jiangsu, China
Date of Manufacture	:	November 2006
Date of Expiry	:	April 20, 2014
Appearance/Colour/Odour	:	White to off-white crystals
Storage Condition (at JRF)	:	As per the instructions received from the Sponsor on the storage of the test item, the test item was stored in its original container, as supplied by the Sponsor at ambient condition in the Test Item Control Office (TICO). The stability of the test item in storage is the responsibility of the Sponsor.

2.2 Positive Control

Name	:	Mitomycin-C
Lot N°	:	010M0665
CAS Number	:	[50-07-7]
Route of Administration	:	Intraperitoneal
Dose	:	1 mg/kg body weight
Manufactured by	:	Sigma
Storage	:	2 - 8 °C
Expiry date	:	May 2014 (unopened vial) January 16, 2013 (working stock)

2.3 Solvent and Chemicals

Methanol	:	Qualigens, Mumbai
Foetal Bovine Serum	:	Himedia
Giemsa Powder	:	Merck
Potassium Dihydrogen orthophosphate	:	Qualigens, Mumbai
Sodium Hydroxide	:	Qualigens, Mumbai
Disinfectant	:	Dettol 2.5%; Reckitt Benckiser
NaH_2PO_4	:	Merck
Na_2HPO_4	:	Sigma
Glycerol	:	Qualigens, Mumbai
Vegetable oil	:	New Saffola Gold

2.4 Instruments and Equipment

Bench top Autoclave	:	Kumar, India
Electronic Balances	:	1. Adventurer ™, OHAUS (Capable of measuring 10 mg to 210 g) 2. Electronic Weighing Scale - SMART (Capable of measuring 5 g to 3000 g)
Metal Cannula	:	CW12 ILA, England, size: 18 G x 5 cm.
Syringe	:	1mL disposable syringe, BD, Singapore
Needles	:	1. 26G ½ (0.45 x 13 mm), BD Precision Glide, BD, Singapore 2. 24 G x 1" (0.55 x 25 mm), BD Precision Glide, BD, Inida
Vacuum Desicator	:	Tarsons (CO_2 Chamber)
Centrifuge	:	R8C (REMI)
Binocular Microscopes	:	1. Nikon Optiphot-2 2. Eclipse E600 (Nikon, Japan) 3. Eclipse E 80i (Nikon, Japan)
pH Meter	:	CyberScan 500^{pH}
Tattoo Machine	:	AIMS™ Tattoo Machine
Microprobe Thermometer	:	Physitemp Instruments Inc., USA (Capable of measuring -100 °C to +200 °C)
Refrigerators	:	LG Electronics Inc. and Godrej
Bench Top Autoclave	:	Kumar, India
Horizontal Cylindrical Steam Sterilizer	:	Yorco, India
Deep-Freezer (-20 °C)	:	Labtop, Mumbai, India
Micropipettes	:	Eppendorf AG

2.5 Principle

The mammalian micronucleus test is used to detect cytogenetic damage (which results in a chromosomal break, fragment or lagging whole chromosome) caused by the test item. The damaged chromosomal fragments remain in the otherwise anucleated cytoplasm of the erythrocyte and are visible, when stained, as a small round or oblong structure called micronuclei. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage.

2.6 Test Animals

For the main study, eighteen (18 males) Swiss albino mice (*Mus musculus*) were received from the Animal Breeding Facility, JRF. The animals were 8 to 9 weeks old on day 1 of dosing. The male mice weighed between 35 and 50 g on day 1 of dosing.

2.7 Acclimatisation

The animals were received into the experimental room and acclimatised for a period of six days (6 male animals/cage). The animals were randomised into 3 groups using validated in-house developed software. The method of randomisation used was censored randomisation method (Gad S.C. and Weil, C.S., 1994)

2.8 Identification

Before randomisation, animals were marked with non toxic marker pen. After randomisation, individual animal number was tattooed on the tail of mice using a tattoo machine and appropriate labels were attached to the cages indicating the study number, test item code, group number and sex, dose, type of study, cage number and animal number.

2.9 Environmental Conditions

Animal Room	:	DCR Facility Room No. 406, Department of Toxicology
Temperature Range	:	19 - 22 °C
Relative Humidity Range	:	64 - 65 %
Photoperiod	:	The photoperiod was 12 h artificial light and 12 h darkness, light hours being 06:00 h - 18:00 h
Air Changes	:	Minimum 15 air changes per hour.

2.10 Husbandry Practices

Caging	:	Polypropylene mouse cages (size: 29 x 37.5 x 14 cm) with stainless steel grid top. Autoclaved clean rice husk was used as the bedding material.
Water Bottle	:	Each cage was supplied with a polypropylene water bottle (capacity 300 mL) with a stainless steel nozzle.
Housing	:	6 male animals per cage.
Room Sanitation	:	Each day the floor and all work tops were mopped with a disinfectant solution (Dettol 2.5%)

2.11 Feed and Water

The quality of feed and water is regularly monitored at Jai Research Foundation. There were no known contaminants in the feed or water at levels that would have interfered with the experimental results obtained.

Feed : Mice pellet feed (Teklad, Certified Global 16% Protein Rodent Diet Sterilizable, USA) was provided *ad libitum* (except fasting for 2 h before dosing and 1 h after dosing) (Refer APPENDIX 9).

Water : UV sterilized drinking water filtered through reverse osmosis water filtration system was provided *ad libitum* (Refer APPENDIX 10).

2.12 Selection of Vehicle

Glyphosate TGAI formed suspension in vegetable oil. Hence vegetable oil was selected as the vehicle for the study.

2.13 Dose Range Finding Study

A dose range finding study was conducted to determine the doses to be employed in the main study (Maximum Tolerated Dose, MTD). Three animals per sex were treated at the dose level of 2000 mg/kg body weight by oral gavages for two consecutive days. Mortality, clinical symptoms and change in body temperature were monitored up to 72 h after the initial dose. The rectal temperatures of all the animals were measured before dosing (Day-1) and then approximately 2, 5 and 24 hours after each dosing and before sacrifice (72 h after initial dose) using microprobe thermometer.

Based on the results of the dose range finding study, 2000 mg of Glyphosate TGAI/kg body weight was selected as the limit dose for the main study.

2.14 Main Study

Dose formulation of 200 mg/mL of Glyphosate TGAI was freshly prepared on each day of dosing by suspending 2000 mg of test item in vegetable oil and volume was made up to 10 mL with vegetable oil. The dose formulation of Glyphosate TGAI was administered orally to mice using a metal cannula attached to a 1 mL BD disposable syringe. Mice from the vehicle control group (Group I) received only vegetable oil orally on both the days.

The mice from the positive control group (Group III) received a single injection of Mitomycin-C intraperitonially at the dose level of 1.0 mg/kg body weight on day 2 of treatment. Each day the dose solutions were freshly prepared prior to dosing. For the treatment and the control groups the dose volume was 10 mL/kg body weight.

Body weight was recorded before dosing on each day and before sacrifice. The clinical signs of toxicity were recorded post-dosing and pre-sacrifice.

2.15 Slide Preparation

All the animals of vehicle, treatment and positive control groups were sacrificed by CO_2 asphyxiation approximately at 24 hours after the last treatment. Femur bones from the sacrificed animals were excised and the epicondyle tips were removed. The bone marrow content was expelled by flushing and aspirating approximately 3 mL foetal bovine serum by using a 1 mL syringe and 24 gauge needle into centrifuge tubes. The aspirated bone marrow content was mixed using the syringe to dissociate the cells in order to avoid cell clump formation.

The tubes were centrifuged at around 2000 rpm for 5 minutes and the supernatant was discarded leaving about 0.2 - 0.3 mL of the medium with cell pellet. The cell pellet was dissociated thoroughly using a pasteur pipette and a drop of suspension was placed on a clean slide. A smear was prepared and allowed to air dry.

The slides were marked with study number, animal number and slide number. Two slides were prepared per animal and the cells were fixed with absolute methanol and allowed to air dry for 15 - 20 minutes. Slides were stained using 5% Giemsa in phosphate buffer for 25 minutes. Subsequently the slides were rinsed in distilled water, air-dried and mounted. In order to prevent bias in the scoring, the slide numbers were masked with code numbers provided by the Department of Bio-statistics and Systems Information, JRF.

2.16 Scoring of Bone Marrow Micronucleus

One out of two slides from each animal was used for screening of micronucleated erythrocytes whereas the other slide was kept in reserve, to be used for scoring when required. The slides were examined for the presence of micronuclei in polychromatic and normochromatic erythrocytes under a microscope (Nikon Optiphot-2, Nikon Eclipse E600 and Nikon Eclipse 80i). A minimum of 2000 polychromatic erythrocytes were screened per animal to evaluate the incidence of micronuclei. A minimum of 200 normochromatic erythrocytes to its corresponding polychromatic erythrocytes were recorded to determine the P/E ratio. The masked labels were removed and all the slides were decoded after scoring.

2.17 Calculation

The P/E ratios were calculated from polychromatic to total (polychromatic + normochromatic) erythrocytes. The percentage of micronucleated polychromatic erythrocytes was also calculated.

2.18 Statistical Evaluation of Results

The data of percent micronucleated polychromatic erythrocytes (% MNPCE) and P/E ratio was statistically analysed using Bartlett's test and Analysis of Variance (ANOVA) followed by the Dunnett's t-test (Gad and Weil, 1994) to determine the level of significant differences between the vehicle control and the treatment group. Where data did not meet the homogeneity of variance, Student's t-test (Gad and Weil, 1994) was performed to determine the level of significant difference between the vehicle control, treatment group and the positive control group.

3. RESULTS

3.1 Dose Range Finding Study

No adverse effects were observed in animals of vehicle control and treatment groups, at post treatment and pre-sacrifice. Mortality was not observed in any of the animals from the vehicle control and the treatment groups. Significant decrease i.e., > 3 °C or increase in body temperature i.e., at least 1 °C was not observed after day 1 and day 2 of dosing in both male and female animals.

Based on the results of the dose range finding study, 2000 mg of Glyphosate TGAI/kg body weight was selected as the limit dose for the main study.

Individual clinical observations are provided in APPENDIX 1. The summary of mean body temperature and individual body temperature of mice is provided in TABLE 1 and APPENDIX 3, respectively.

3.2 Main Study

3.2.1 Clinical Observations and Body Weight

All the animals were normal in the vehicle control, treatment and positive control groups, both post-treatment and pre-sacrifice.

Mortality was not observed in any of the animals from the vehicle control, treatment and positive control groups. Body weights were comparable among the groups during the experimental period.

Individual clinical observations are provided in APPENDIX 2. The summary of mean body weight and individual body weight are provided in TABLE 2 and APPENDIX 4, respectively.

3.2.2 Micronucleated Polychromatic Erythrocytes

The ratio of polychromatic erythrocytes (PCE) to total erythrocytes (P/E ratio) in Glyphosate TGAI treated group was comparable to the vehicle control group. Statistically significant difference in P/E ratio was not observed in male animals treated at the dose level of 2000 mg/kg body weight, hence there was no evidence of cell proliferation or cytotoxicity to the target cells.

The mean P/E ratios observed in the male animals were 0.525 and 0.531 at the dose levels of 0.0 (vehicle control group) and 2000 mg of Glyphosate TGAI/kg body weight, respectively. The mean polychromatic to total erythrocytes ratio (P/E) observed in the animals treated with mitomycin-C (1.0 mg/kg body weight) was 0.687.

Although statistically significant increase in the polychromatic to total erythrocytes ratio (P/E) was observed in the male animals of positive control group, increase in the P/E ratio was within historical range for positive control and hence considered biologically non-significant.

The mean percent micronucleated polychromatic erythrocytes (% MNPCE) observed in male animals was 0.033 and 0.000 at the dose levels of 0.0 (vehicle control group) and 2000 mg of Glyphosate TGAI/kg body weight, respectively. The mean percent micronucleated polychromatic erythrocytes (% MNPCE) observed in animals treated with Mitomycin-C (1.0 mg/kg body weight) was 2.492.

Statistical analysis of the results did not reveal any significant difference in percent micronucleated polychromatic erythrocytes (% MNPCE) in animals belonging to treatment group treated at the dose level of 2000 mg/kg body weight when compared with the vehicle control group.

Statistically significant increase in % MNPCE observed in the male animals treated with mitomycin-C (1.0 mg/kg body weight) demonstrated the sensitivity of the test system, suitability of the procedures and efficiency of the test conditions employed in the test. (TABLE 3, APPENDIX 5 and APPENDIX 6).

Group-wise total polychromatic erythrocytes (PCE), micronucleated polychromatic erythrocytes (MNPCE), percent MNPCE and mean P/E ratio in bone marrow cells are given in TABLE 3 with individual data presented in APPENDIX 5 and APPENDIX 6.

4. CONCLUSION

All criteria for a valid study were met as described in the protocol. From the results of the present study, it is concluded that Glyphosate TGAI does not have micronucleus induction potential in the animals treated up to the guideline limit dose of 2000 mg/kg body weight following oral administration for two consecutive days.

5. REFERENCES

Asanami, S., and Shimono, K., 1997a: Hypothermia induces micronuclei in mouse bone marrow cells. *Mutation Research*, 393, 91-98.

Asanami, S., and Shimono, K., 1997b: High body temperature induces micronuclei in mouse bone marrow. *Mutation Research*, 390, 79-83.

Asanami, S., Shimono, K., and Kaneda, S., 1998: Transient hypothermia induces micronuclei in mice. *Mutation Research*, 413, 7-14.

Gad, S.C. and Cassidy, C.D., 2006: Non-clinical Vehicles Use in Studies by Multiple Routes in Multiple Species, *International Journal of Toxicology*, 25, pp. 499-521.

Gad, S.C. and Weil, C.S., 1994: "Statistics for Toxicologists". In: *Principles and Methods of Toxicology*, 3rd edition, Hayes A.W. (Ed), Raven press Ltd., New York, pp. 221-274.

Krishna, G. and Hayashi, M., 2000: In vivo Rodent Micronucleus Assay: Protocol Conduct and Data Interpretation, Mutation Research, 455, 155-166.

MacGregor J.T., Heddle J.A., Hite M, Margolin B.H., Ramel C, Salamone M.F, Tice R.R. and Wild D., 1987: Guidelines for the Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes, *Mutation Research*, 189, 103-112.

Micronucleus Test of Glyphosate TGAI in Mice

TABLE 1: Summary of Mean Body Temperature - Dose Range Finding Study

Number of Animals = 3/Sex/Group Refer APPENDIX 3

Group and Dose of Glyphosate TGAI		Rectal Temperature (°C) After Dosing – Male							
		Day 1				Day 2			
		Before Dosing	2 h	5 h	24 h	2 h	5 h	24 h	48 h
G I Vehicle control	Mean	37.0	37.1	37.1	36.9	37.1	37.1	37.0	37.0
	SD	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1
G II 2000 mg/kg body weight	Mean	37.0	37.0	37.1	37.1	36.9	37.1	37.1	37.1
	SD	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Group and Dose of Glyphosate TGAI		Rectal Temperature (°C) After Dosing – Female							
		Day 1				Day 2			
		Before Dosing	2 h	5 h	24 h	2 h	5 h	24 h	48 h
G I Vehicle control	Mean	37.3	37.1	37.2	37.2	37.1	37.3	37.1	37.2
	SD	0.2	0.2	0.2	0.0	0.2	0.2	0.2	0.1
G II 2000 mg/kg body weight	Mean	37.2	37.1	37.2	36.9	37.1	37.1	37.1	37.2
	SD	0.2	0.1	0.1	0.1	0.2	0.2	0.3	0.1

Note: vegetable oil was used in vehicle control group.
Key: SD = Standard deviation.

Micronucleus Test of Glyphosate TGAI in Mice

TABLE 2: Summary of Mean Body Weight - Main Study

Number of Animals = 6 Males/Group **Refer APPENDIX 4**

Group and Dose of Glyphosate TGAI		Body Weight (g)		
		Male		
		Day 1	Day 2	Before Sacrifice
G I Vehicle control	Mean	41.67	41.17	41.00
	SD	5.05	5.08	4.90
G II 2000 mg/kg body weight	Mean	41.17	40.17	40.50
	SD	4.17	4.07	4.04
G III Positive control 1.0 mg/kg body weight	Mean	-	42.17	41.50
	SD	-	3.60	3.39

Note: 1. Vegetable oil was used in vehicle control group.
2. Mitomycin-C was used as the positive control.
3. Body weight of positive control animals was not taken on day one since positive control animals were not treated on day one.

Key: SD = Standard deviation, - = Not applicable.

Micronucleus Test of Glyphosate TGAI in Mice

TABLE 3: Summary of Micronucleated Polychromatic Erythrocytes in Bone Marrow Cells

Number of Animals = 6 Males/Group **Refer APPENDIX 5 and APPENDIX 6**

Group and Dose of Glyphosate TGAI	Male				
	Total PCE	MNPCE			Mean P/E Ratio
		Total	Mean	Mean % MNPCE	
G I Vehicle control	12014	4	0.667	0.033	0.525
G II 2000 mg/kg body weight	12019	0	0.000	0.000	0.531
G III Positive control (1.0 mg/kg body weight)	12030	300	50.000↑↑	2.492↑↑	0.687 ↑

Note: 1. Vegetable oil was used in vehicle control group.
2. Mitomycin-C was used as the positive control.

$$\% \text{ MNPCE} = \frac{\text{MNPCE} \times 100}{\text{Total PCE}}$$

Key: PCE = Polychromatic Erythrocytes
MNPCE = Micronucleated Polychromatic Erythrocytes
P/E = Polychromatic Erythrocytes corresponding to Normochromatic Erythrocytes/Total Erythrocyte
↑↑ = Significantly higher than the control at 1% level ($p \leq 0.01$)
↑ = Significantly higher than the control at 5% level ($p \leq 0.05$)

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 1: Individual Clinical Observations - Dose Range Finding Study

Group and Dose of Glyphosate TGAI	Sex	Animal N°	Clinical Signs Observed after Dosing on										48 h*		Before Sacrifice (72 h*)
			Day 1					Day 2							
			Before dosing	1h	2h	3h	4h	Before dosing	1h	2h	3h	4h	M	E	
G I Vehicle control	Male	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		2	1	1	1	1	1	1	1	1	1	1	1	1	1
		3	1	1	1	1	1	1	1	1	1	1	1	1	1
	Female	4	1	1	1	1	1	1	1	1	1	1	1	1	1
		5	1	1	1	1	1	1	1	1	1	1	1	1	1
		6	1	1	1	1	1	1	1	1	1	1	1	1	1
G II 2000 mg/kg body weight	Male	7	1	1	1	1	1	1	1	1	1	1	1	1	1
		8	1	1	1	1	1	1	1	1	1	1	1	1	1
		9	1	1	1	1	1	1	1	1	1	1	1	1	1
	Female	10	1	1	1	1	1	1	1	1	1	1	1	1	1
		11	1	1	1	1	1	1	1	1	1	1	1	1	1
		12	1	1	1	1	1	1	1	1	1	1	1	1	1

Key: 1= Normal, M = Morning and E = Evening, h = Hour and * = After initial Dosing

Note: Vegetable oil was used in vehicle control group.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 2: Individual Clinical Observations - Main Study

Group and Dose of Glyphosate TGAI	Sex	Animal N°	Clinical signs observed after dosing on										Before Sacrifice
			Day 1					Day 2					
			Before dosing	1h	2h	3h	4h	Before dosing	1h	2h	3h	4h	
G I Vehicle control	Male	1	1	1	1	1	1	1	1	1	1	1	1
		2	1	1	1	1	1	1	1	1	1	1	1
		3	1	1	1	1	1	1	1	1	1	1	1
		4	1	1	1	1	1	1	1	1	1	1	1
		5	1	1	1	1	1	1	1	1	1	1	1
		6	1	1	1	1	1	1	1	1	1	1	1
G II 2000 mg/kg body weight	Male	7	1	1	1	1	1	1	1	1	1	1	1
		8	1	1	1	1	1	1	1	1	1	1	1
		9	1	1	1	1	1	1	1	1	1	1	1
		10	1	1	1	1	1	1	1	1	1	1	1
		11	1	1	1	1	1	1	1	1	1	1	1
		12	1	1	1	1	1	1	1	1	1	1	1
G III Positive control (Mitomycin-C 1.0 mg/kg body weight)	Male	13	1	-	-	-	-	1	1	1	1	1	1
		14	1	-	-	-	-	1	1	1	1	1	1
		15	1	-	-	-	-	1	1	1	1	1	1
		16	1	-	-	-	-	1	1	1	1	1	1
		17	1	-	-	-	-	1	1	1	1	1	1
		18	1	-	-	-	-	1	1	1	1	1	1

Key: 1 = Normal, h = Hour and - = Not applicable.

Note: Vegetable oil was used in vehicle control group.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 3: Individual Rectal Temperature - Dose Range Finding Study

Temperature Data – Male

Group and Dose of Glyphosate TGAI	Animal N°	Before dosing (Day-1)	Day - 1 Hours after dosing 2 h	5 h	24 h	Day - 2 Hours after dosing 2 h	5 h	24 h	48 h
		°C	°C	°C	°C	°C	°C	°C	°C
G I Vehicle Control (Vegetable oil)	1	36.9	37.0	37.1	36.8	36.9	37.1	36.9	37.1
	2	37.1	37.1	37.3	36.9	37.1	37.2	37.0	36.9
	3	37.0	37.1	37.0	37.0	37.2	37.1	37.2	37.0
G II 2000 mg/kg body weight	7	36.8	36.9	37.1	37.0	36.9	37.2	37.1	37.0
	8	37.3	37.0	37.1	37.2	36.9	37.1	37.0	37.1
	9	36.9	37.0	37.2	37.1	37.0	37.0	37.1	37.2

Temperature Data – Female

Group and Dose of Glyphosate TGAI	Animal N°	Before dosing (Day-1)	Day - 1 Hours after dosing 2 h	5 h	24 h	Day - 2 Hours after dosing 2 h	5 h	24 h	48 h
		°C	°C	°C	°C	°C	°C	°C	°C
G I Vehicle Control (Vegetable oil)	4	37.4	37.3	37.3	37.2	37.3	37.4	37.0	37.3
	5	37.1	37.0	37.3	37.2	37.1	37.3	37.1	37.2
	6	37.3	37.1	37.0	37.2	36.9	37.1	37.3	37.2
G II 2000 mg/kg body weight	10	37.4	37.2	37.3	37.0	37.0	36.9	37.1	37.2
	11	37.2	37.0	37.1	37.0	37.1	37.2	37.4	37.1
	12	37.1	37.2	37.3	36.8	37.3	37.1	36.9	37.2

Note : 1. Range of microprobe thermometer is -100 °C to +200 °C.
2. Vegetable oil was used in vehicle control group.

Keys : °C = Degree Centigrade and h = Hour.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 4: Individual Body Weight (g) - Main Study

Group and Dose of Glyphosate TGAI	Sex	Animal N°	Body Weight (g)		
			Day 1	Day 2	Before Sacrifice
G I Vehicle control	Male	1	50	50	50
		2	43	43	42
		3	43	42	41
		4	41	38	38
		5	37	38	39
		6	36	36	36
G II 2000 mg/kg body weight	Male	7	46	45	46
		8	45	43	43
		9	42	42	42
		10	41	40	40
		11	38	37	37
		12	35	34	35
G III Positive control (Mitomycin-C 1.0 mg/kg body weight)	Male	13	-	46	45
		14	-	46	44
		15	-	44	44
		16	-	40	41
		17	-	39	37
		18	-	38	38

Note: Vegetable oil was used in the vehicle control group.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 5: Total Erythrocytes and P/E Ratio

Group and Dose of Glyphosate TGAI	Sex	Animal N°	Total PCE Scored	PCE Corr. to NCE	NCE Scored	Total Erythrocytes	P/E Ratio
G I Vehicle control	Male	1	2008	302	201	503	0.600
		2	2000	313	201	514	0.609
		3	2002	170	200	370	0.459
		4	2004	221	202	423	0.522
		5	2000	277	207	484	0.572
		6	2000	128	203	331	0.387
G II 2000 mg/kg body weight	Male	7	2007	298	206	504	0.591
		8	2001	158	207	365	0.433
		9	2000	278	201	479	0.580
		10	2002	171	206	377	0.454
		11	2008	254	206	460	0.552
		12	2001	281	205	486	0.578
G III Positive control (Mitomycin-C 1.0 mg/kg body weight)	Male	13	2000	239	200	439	0.544
		14	2005	413	204	617	0.669
		15	2002	679	202	881	0.771
		16	2000	404	203	607	0.666
		17	2011	339	205	544	0.623
		18	2012	1143	203	1346	0.849

Note: 1. Polychromatic erythrocytes corresponding to a minimum of 200 normochromatic erythrocytes were recorded for calculating the (P/E) ratio.

2. Vegetable oil was used in vehicle control group.

Key: PCE = Polychromatic Erythrocytes, NCE = Normochromatic Erythrocytes,
P/E = Polychromatic Erythrocytes corresponding to Normochromatic Erythrocytes/Total Erythrocytes.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 6: Frequency of Micronucleated Polychromatic Erythrocytes

Group and Dose of Glyphosate TGAI	Sex	Animal N°	Total Number of PCE Scored	Number of MNPCE	Percent MNPCE
G I Vehicle control	Male	1	2008	1	0.05
		2	2000	0	0.00
		3	2002	0	0.00
		4	2004	1	0.05
		5	2000	1	0.05
		6	2000	1	0.05
G II 2000 mg/kg body weight	Male	7	2007	0	0.00
		8	2001	0	0.00
		9	2000	0	0.00
		10	2002	0	0.00
		11	2008	0	0.00
		12	2001	0	0.00
G III Positive control (Mitomycin-C 1.0 mg/kg body weight)	Male	13	2000	22	1.10
		14	2005	42	2.09
		15	2002	60	3.00
		16	2000	41	2.05
		17	2011	60	2.98
		18	2012	75	3.73

Key: PCE = Polychromatic Erythrocytes, MNPCE = Micronucleated Polychromatic Erythrocytes, Percent MNPCE = MNPCE x 100/Total PCE.

Note: Vegetable oil was used in vehicle control group.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 7: Signed Protocol and Protocol Amendment

PROTOCOL

MICRONUCLEUS TEST OF GLYPHOSATE TGAI IN MICE

GUIDELINES: OECD 474, OPPTS 870.5395, EEC B.12 and JMAFF 2-1-19-3

SPONSOR

DOW AGROSCIENCES LLC
9330 ZIONSVILLE ROAD
INDIANAPOLIS, IN 46268
U.S.A.

PERFORMING LABORATORY

JAI RESEARCH FOUNDATION
DEPARTMENT OF TOXICOLOGY
VALVADA - 396 108
DIST. VALSAD
GUJARAT
INDIA

MAY - 2012

APPENDIX 7 (Continued)

CONTENTS

APPENDIX 7 (Continued)

1. GENERAL INFORMATION

1.1 Study Director

[redacted]

Deputy Study Director

[redacted]

1.2 Facility Management

[redacted]

1.3 Agreed Study Period

Experiment Start*	:	Fourth week of May 2012
Experiment Completion*	:	Fourth week of June 2012
Draft Report Submission	:	First week of July 2012
Final Report Submission	:	Within two weeks from the date of receipt of comments on the final draft report from the Sponsor.

* Exact dates will be indicated in the study schedule.

1.4 Protocol and Amendment (if any) Distribution

a. Original copy in Archive; b. Electronic copy to Sponsor; c. Photocopy to Study Director and QAU.

2. INTRODUCTION

2.1 Objective

The objective of this study is to evaluate the micronucleus induction potential of Glyphosate TGAI in mice.

2.2 Regulatory Guidelines

This study is intended for regulatory submission and will be conducted and the final report formatted in accordance with the known requirement of international guidelines including the following:

The Organisation for Economic Co-operation and Development (OECD), Guidelines for Testing of Chemicals, Volume II, N° 474 (Adopted by the Council on 21st July 1997) "Mammalian Erythrocyte Micronucleus Test".

The United States Environmental Protection Agency (EPA) Health Effects Test Guidelines OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test (August 1998).

APPENDIX 7 (Continued)

Official Journal of the European Economic Community (L142): Part B: Methods for the Determination of Toxicity and other Health Effects, B.12 Mutagenicity: *In vivo* Mammalian Erythrocyte Micronucleus Test. Commission Regulation (EC) No. 440/2008 of 30 May 2008

Japanese Ministry of Agriculture, Forestry and Fisheries. Testing Guidelines for Toxicology Studies, 2000. Notification No. 12-Nousan-8147. 24 November 2000. Micronucleus Studies 2-1-19-3.

2.3 Principle of the Test Method

The mammalian micronucleus test is used to detect cytogenetic damage (which results in a chromosomal break, fragment or lagging whole chromosome) caused by the test item. The damaged chromosomal fragments remain in the otherwise anucleated cytoplasm of the erythro yte and are visible, when stained, as a small round or oblong structure called micronuclei. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage.

2.4 Test Item

The Test Item Data Sheet has been completed by the Sponsor. A representative sample of the test item will be retained for archiving. Any residual test item will be disposed of at JRF after the expiry date unless otherwise instructed by the Sponsor. The details relating to the test item (as provided by the study Sponsor) are as follows:

Test Item Name	:	Glyphosate TGAI
Test Item Number	:	TSN105914
CA Name	:	N/K
CAS Number	:	Glyphosate: 1071-83-6
Analysed Concentration	:	98.9 weight% (Information provided by Sponsor via CoA)
Lot N°	:	20061109
Supplied by	:	Dow AgroSciences
Manufactured by	:	Good Harvest-Weien Agrochemical Co. Ltd., Jiangsu, China
Date of Manufacture	:	November 2006
Date of Expiry	:	April 20, 2014
Appearance/Colour/Odour	:	White to off-white crystals
Storage Condition (at JRF)	:	As per the instruction received from the Sponsor on storage of the test item, the test item will be stored in its original container as supplied by the Sponsor at ambient condition in the Test Item Control Office (TICO). The stability of the test item in storage will be the responsibility of the Sponsor.
JRF Test Item Code	**:**	**GLP 268**

APPENDIX 7 (Continued)

3. GOOD LABORATORY PRACTICE (GLP)

3.1 GLP Compliance

This study will be conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98)17, N° 1, Environment Directorate, Organisation for Economic Co-operation and Development, Paris (1998) and also the following GLP Standards:

Environmental Protection Agency (EPA-FIFRA), Title 40 of the US Code of Federal Regulations Part 160, 16 October 1989.

Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF), 11-Nousan-No. 6283, 1 October, 1999. Revised by 12 Nousan, Notification No. 8628 - 6 December 2000.

3.2 Standard Operating Procedures (SOP)

Unless otherwise specified all procedures mentioned in the protocol are subject to the detailed Standard Operating Procedures of Jai Research Foundation.

3.3 Amendment to Protocol

This study protocol may be subject to amendment. Amendment to the protocol, whether initiated by the Sponsor or the Study Director, will be generated, authorised by the Study Director and sent to the Sponsor for approval.

In the event that circumstances dictate immediate action, the nature of these circumstances will be communicated to the Sponsor as soon as practicable (by telephone, facsimile transmission or e-mail) and will be confirmed as soon as possible by way of formal protocol amendment.

3.4 Deviation from the Protocol

Any deviation from the study protocol will be documented in the study file and reported in the study report.

3.5 Quality Assurance

The Quality Assurance Unit (QAU) of JRF will audit the critical phases of the study, the raw data, draft and final reports. The audit reports will be provided to the Study Director and Management. The dates of audits and reporting of findings to the Study Director and the Management will be incorporated in the study report.

4. ANIMAL WELFARE

The study will be undertaken in compliance with the 'Guidelines for Laboratory Animals Facility' issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. These guidelines promote the humane care of animals used in research by

providing specifications that will enhance animal well-being and experimental quality for the advancement of biological knowledge that is relevant to humans and animals.

JRF is committed to enhancing animal welfare and ensures that studies are designed and conducted to cause the minimum suffering or distress to the animals consistent with the scientific objectives and in accordance with JRF's policy on animal welfare.

Project proposal for the experimentation is subject to approval by Institutional Animal Ethics Committee (IAEC), JRF.

JRF is accredited with Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) that promotes the humane treatment of animals in science.

4.1 Humane Endpoint

Moribund animals or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed. Dependent on the time since dose administration, if moribund animals are sacrificed within two hours of the scheduled sacrifice, bone marrow may be collected and used as part of the interpretation of the results, at the discretion of the Study Director.

5. EXPERIMENTAL PROCEDURE

5.1 Initial Considerations

Test item, at doses that cause marked pain and distress due to corrosive or severely irritant actions, will not be administered and if required study will be terminated.

5.2 Animals

5.2.1 Justification

The mouse is selected as a test system because it is a readily available laboratory rodent species. It has been shown to be a suitable model for genotoxicity studies and is also recommended by the OECD and other regulatory authorities. The results of this study are believed to be of value in predicting the potential of the test item to cause mutations in humans.

5.2.2 Specification

Healthy, young, 6 - 9 weeks old Swiss albino mice will be obtained from the Animal Breeding Facility, at JRF. The female mice used will be nulliparous and non-pregnant. Body weight variation among the animals will not exceed ± 20% of the mean body weight for each sex at the time of initiation of dosing.

APPENDIX 7 (Continued)

5.3 Acclimatisation

After veterinary examination for good health, the mice will be acclimatised to the laboratory conditions for a minimum period of 5 days prior to commencement of treatment.

5.4 Housing and Animal Identification

The mice will be housed (6 per sex per cage) in polypropylene rat cages provided with rice husk as bedding material. Each day cages will be supplied with a polypropylene water bottle fitted with a stainless steel nozzle.

Individual mice will be identified with a unique number tattooed on the tail using a tattoo machine. After acclimatisation the animals will be randomized into 5 groups using Censored Randomization Method (Gad and Weil, 1994) using validated in-house developed software. The cages will be labeled with details of the study number, test item code, group number, sex, dose, type of study, cage number and animal numbers. The labels used will be of different colours for different dose groups.

5.5 Animal Room Sanitation

Each day, the floor of the experimental room will be swept and all worktops and the floor will be mopped with disinfectant solution.

5.6 Feed and Water

The mice will be provided with laboratory mice pellet feed (Teklad, USA, certified Global 16% Protein Rodent Diet Sterilizable) and pure charcoal filtered, UV sterilized drinking water, filtered through Aquaguard water filtration system, *ad libitum*.

5.7 Environmental Conditions

The temperature of the experimental room will be maintained at 22 ± 3 °C and the relative humidity between 30 and 70 per cent. The photoperiod will be 12 hours light and 12 hours dark, light hours being 06:00 - 18:00 hours approximately and air changes will be a minimum 15/hour.

5.8 Selection of Vehicle

The test item will be mixed with distilled water or other suitable vehicle such as corn oil, 0.5% carboxy methyl cellulose (CMC). Fresh dose formulations will be prepared daily and administered within 2 hours of preparation. The concentration of the Glyphosate TGAI will be adjusted so as to permit constant volume dosing. All animals will receive a single standard volume of 10 mL/kg body weight by oral, gavage, administration. Vehicle control will receive the vehicle alone. Vehicle selected will be recorded in the raw data.

APPENDIX 7 (Continued)

5.9 Dose Range Finding Study

Dose range finding study will be conducted to determine the doses to be employed in the main study. Three males and three females will be used in at least two dose groups. Mortality, severity of clinical symptoms and change in the body temperature will be monitored for up to 72 h after the initial dose.

The dose levels for the micronucleus test (MNT) will be selected based upon the range-finding test. The maximum dose for the micronucleus study will be the estimated maximum tolerated dose (MTD). MTD is defined as the dose that produces clear signs of toxicity without having a major effect on thermal regulation or the survival of the animals. For changes in thermal regulation, a body temperature increase of at least 1°C or a decrease of at least 3°C for five or more hours will be declared as having exceeded an MTD. Body temperature changes outside this range have been previously reported to cause an increase in micronucleus formation in absence of chemical treatment (Asanami and Shimono, 1997; Asanami *et al.,* 1998). With some test items, death of the treated animals may be the only sign of toxicity at doses above the MTD and no other clinical signs of toxicity may be apparent at the MTD. In such cases, the MTD will be a dose in between the doses that induced some mortalities and none. These are only general guidelines in determining the MTD and the Study Director will take into consideration such other factors as the dose-mortality curve, any other available toxicological data, etc.

The rectal temperature of the treated animals will be monitored during the range-finding test and provisionally during the main study using digital laboratory thermometer. The temperatures will generally be measured before dosing (Day-1), approximately 2, 5 and 24 hours after each dosing.

Relatively non-toxic compounds will be tested up to 2 g/kg body weight/day. The middle- and low-doses will be approximately 1/2 and 1/4 of the maximum dose, respectively. The test item will be administered on two consecutive days, approximately 24 hours (± 1 hour) apart.

5.10 Main Study

If the results of the dose range finding study indicate there are no significant differences between the sexes in toxicity, then only male mice will be used for the main study. Five groups (comprising 6 animals/sex/group) will be used for this study. Group I will serve as the vehicle control, Group II, III and IV will be low, mid and high dose groups, respectively. Group V will be the positive control and will receive mitomycin-C (1.0 mg/kg body weight on day 2 of treatment) in distilled water by the intraperitoneal route on a single occasion. The dose levels of the test item for the main study will be specified by protocol amendment. Any requirement for the recording of body temperature in the main study will also be documented by protocol amendment.

APPENDIX 7 (Continued)

5.11 Test Performance

The test item will be dissolved or suspended in a suitable vehicle (Gad and Cassidy, 2006). The test item will be prepared freshly on the day of dose administration. The animals will be dosed (10 mL/kg body weight) by oral intubation for 2 consecutive days. The body weight will be recorded prior to dosing on each day, and also on the day of sacrifice. Clinical signs will be recorded after dosing on each day and also before sacrifice. The animals will be sacrificed by CO_2 asphyxiation approximately 24 h following the last treatment of the test item (MacGregor *et al*., 1987). Animals in the positive control group will be sacrificed by CO_2 asphyxiation approximately 24 hours after of treatment (Krishna and Hayashi, 2000).

Bone marrow cells from both femur bones will be expelled and flushed with fetal bovine serum and centrifuged. The supernatant will be discarded, and smears will be prepared on clean slides, air dried and fixed in methanol. A minimum number of 2 slides per mouse will be prepared and stained with Giemsa (Heddle *et al*., 1984). Slides will be coded prior to scoring and decoded after completion of scoring.

5.12 Microscopic Observation

Slides will be observed under a light microscope. The proportion of immature erythrocytes among the total (immature + mature) will be determined for each animal by counting a minimum of 200 normochromatic erythrocytes to its corresponding number of polychromatic erythrocytes. A minimum of 2000 polychromatic (immature) erythrocytes per animal will be scored for the incidence of micronuclei.

5.13 Statistical Analysis

The data of percent micronucleated polychromatic erythrocytes (% MNPCE) and P/E ratio for both the sexes will be subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett's t-test. Where the data do not meet the homogeneity of variance, Student's t-test will be performed to determine the level of significant difference between the control and the treated groups.

5.14 Assay Acceptance and Evaluation Criteria

5.14.1 Acceptance Criteria

The study will be considered valid as the following criteria are met:

i. The prepared slides should have uniform staining properties and sufficient PCE cells present to allow accurate micronucleus determination.
ii. The vehicle (or negative) controls are in the range of historical control data.
iii. The positive controls are in the range of historical control data.
iv. At least 5 animals per group and sex can be evaluated
v. PCE to erythrocyte ratio should not be less than 20 % of the negative control.

APPENDIX 7 (Continued)

vi. Deviations from any of the above criteria will be discussed on a case by case basis and explanations for the anomalous responses presented whenever possible.

5.14.2 Evaluation Criteria

i. A positive result is defined as a dose-dependent, statistically significant increase in the incidence of micronuclei or increase in a single dose group.
ii. Biological relevance of the results will be considered first.
iii. Statistical methods will be used as an aid in evaluating the results.
iv. A test will be judged negative if no evident statistically significant increases in the numbersof MNPCE are observed, relative to the concurrent and established historical control frequencies for MN-PCE induction.

6. REPORT

A QA audited draft report will be issued for the Sponsor's review and comment. The report will include the following information:

Test item:

- Identification and CAS number, if known
- Physical nature and purity
- Phys-chem. properties relevant to the conduct of the study
- Stability of the test item, if known.

Vehicle

- Justification for choice of vehicle
- Solubility of the test item in vehicle.

Test Animals:

- Species and strain of animals used;
- Number, age and sex of animals;
- Source and housing conditions, diet, etc.
- Individual weight of the animals at the start of the experiment, including body weight range, mean and standard deviation for each group.

Test conditions:

- Positive and vehicle (or negative) control data
- Data from range-finding study, if conducted
- Rationale for dose level selection
- Details of test item preparation
- Details of the administration of the test item
- Rationale for route of administration
- Detailed description of treatment and sampling schedules

APPENDIX 7 (Continued)

- Methods of slide preparation
- Methods for measurement of toxicity
- Criteria for scoring micronucleated immature erythrocytes
- Number of cells analysed per animal
- Criteria for considering studies as positive, negative or equivocal

Results

- Signs of toxicity
- Proportion of immature erythrocytes among total erythrocytes;
- Number of micronucleated immature erythrocytes, given separately for each animal;
- Mean ± standard deviation of micronucleated immature erythrocytes per group;
- Dose-response relationship, where possible;
- Statistical analyses and method applied;
- Concurrent negative and positive control data;
- Historical control data ranges of P/E ratio and % MNPCE for male and female

Conclusion
Signed protocol and protocol amendment(s) (if any)
Deviation(s) from the study protocol (if any)

Unless otherwise instructed by the Sponsor, the report will follow EPA formatting for pages 1 – 4 (PR Notice 86-5). In addition, a PMRA summary will be compiled from the final report. The final report will be issued within approximately 2 weeks of receipt of the Sponsors comments.

JRF will provide the following on finalisation of the report:
One (1) original signed final report - unbound
One (1) electronic copy of the final report and appendices in Word processing format
One (1) electronic linked copy of the final report and appendices in PDF format that is suitable for editing (Links must be in blue underlined text – required links are: Table of contents, all mentions of tables, figures, and appendices in text should be linked to the appropriate page)
One (1) original signed PMRA summary – unbound
One (1) electronic copy of the PMRA summary in Word and PDF format on CD
One (1) original of the protocol and all raw data for the study – unbound
One (1) hard copy and one (1) electronic copy of the Freedom of Information Act (FOIA) Pages for each report (this can be included on the front of the report hard copy and e-copy if preferred, but should not be included in the page count for the final report).

The final report texts/data as listed above will be sent to The Dow Chemical Company, 1803 Building, Midland, MI 48674, U.S.A.

APPENDIX 7 (Continued)

7. ARCHIVES

On completion of the study, all the original raw data records, protocol, any protocol amendment/deviation and final report will be transferred to the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, 1803 Building, Midland, MI 48674 U.S.A. Slides generated during the study will be transferred to the Dow Chemical Company, 1803 Building, Midland, MI 48674 U.S.A.. A self attested photocopy of the raw data records, copy of protocol, draft report, copy of final report, electronically recorded data and the representative sample of test item will be retained in the GLP Archives of Jai Research Foundation for a period of ten years, after which time they will be disposed of by JRF.

8. REFERENCES

Asanami, S., and Shimono, K., 1997a: Hypothermia induces micronuclei in mouse bone marrow cells. *Mutation Research*. 393, 91–98.

Asanami, S., and Shimono, K., 1997b: High body temperature induces micronuclei in mouse bone marrow. *Mutation Research*. 390, 79–83.

Asanami, S., Shimono, K., and Kaneda, S., 1998: Transient hypothermia induces micronuclei in mice. *Mutation Research*. 413, 7–14.

Gad, S.C. and Cassidy, C.D., 2006: Non-clinical Vehicles Use in Studies by Multiple Routes in Multiple Species, *International Journal of Toxicology*, 25, pp.499 – 521.

Gad, S.C. and Weil, C.S., 1994: "Statistics for Toxicologists". In: *Principles and Methods of Toxicology*, 3rd edition, Hayes A.W. (Ed), Raven press Ltd., New York, p. 221-274.

Krishna, G. and Hayashi, M., 2000: *In vivo* Rodent Micronucleus Assay: Protocol Conduct and Data Interpretation, *Mutation Research*.455, 155-166.

Heddle J. A., Stuart, E. and Salamone, M.F., 1984: The Bone Marrow Micronucleus Test, In: *Hand Book of Mutagenicity Test Procedures*. Second edn. Kilbey B. J, M. Legator, W. Nichols, C. Ramel (Eds.) Elsevier, New York, pp. 441-457.

MacGregor J. T., Heddle J.A., Hite M, Margolin B. H., Ramel C, Salamone M. F,, Tice R. R. and Wild D., 1987: Guidelines for the Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes, *Mutation Research*, 189, 103-112.

APPENDIX 7 (Continued)

9. **PROTOCOL APPROVAL**

We, the undersigned have read the whole protocol for, "**Micronucleus Test of Glyphosate TGAI in Mice**" and confirm that the study will be performed as per this protocol.

Study Director :

Facility Management :

May 17, 2012
Signature and Date

For Study Sponsor : **DOW AGROSCIENCES LLC**
9330 ZIONSVILLE ROAD
INDIANAPOLIS, IN 46268
U.S.A.

Name of Sponsor's Representative :

Signature & Date :

APPENDIX 7 (Continued)

STUDY PLAN / PROTOCOL AMENDMENT RECORD

STUDY N°	485-1-06-4696	AMENDMENT N°	1	EFFECTIVE DATE	July 12, 2012
STUDY TITLE	Micronucleus Test of Glyphosate TGAI in Mice				

ORIGINAL DETAILS*	DETAILS AMENDED	REASON FOR AMENDMENT
Protocol Page 8 of 13 **5.10 Main Study** Five groups (comprising 6 animals/sex/group) will be used for the study. Group I....Group II, III and IV....... dose group respectively. Group V will be the positive control .... single occasion.	Three groups (comprising 6 male animals/group) will be used for the study. Group I will serve as the vehicle control and will receive vegetable oil. Group II will be treated with 2000 mg Glyphosate TGAI/kg body weight. Group III will serve as the positive control.	Based on the results of dose range finding study and sponsor's approval via e-mail dated July 09, 2012.

* Reference of page N°, paragraph number etc.

REVIEWED BY (QAU)	Name [redacted]	[redacted] July 12, 2012 Signature & Date

AUTHORISED BY	
For JRF	**For SPONSOR (S)**
Study Director: [redacted] July 12, 2012 Signature & Date: Name [redacted]	**DOW AGROSCIENCES LLC** **9330 ZIONSVILLE ROAD** **INDIANAPOLIS, IN 46268** **U.S.A.**
Facility Management: [redacted] July 12, 2012 Signature & Date: Name: [redacted]	Approved by sponsor [redacted] dated July 12, 2012 Signature & Date: [redacted]

The sponsor is requested to send one original, signed copy of the amendment to JRF.

Amendment Distribution: Archives (original) and photocopy to all the copy holders of study plan/protocol.

JRF/GEN/F 37/6

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 8: Record of Deviation from the Study Protocol

Serial N°	As in Study Protocol	Deviation from Study Protocol
1.	**Agreed Study Period** Experiment Completion : Fourth week of June 2012 Draft Report Submission : First week of July 2012	Experiment Completion : First week of August 2012 Draft Report Submission : Fourth week of August 2012

It is declared that, this deviation from the approved study protocol did not affect the outcome of the study or the interpretation of the results.

[redacted], M.Sc.
STUDY DIRECTOR, JRF
DATE: September 13, 2012

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 9: Feed Analysis Reports

JAI RESEARCH FOUNDATION

MICROBIOLOGICAL ANALYSIS CERTIFICATE OF ANIMAL FEED

Name of Sample : **Teklad Certified Global 16% Protein Rodent Diet**
Sample Received From : **Feed storage room**
Batch N° : **2016SC-013012MA**
Sample Analysed at : **Mutagenicity**

Parameters		Results Observed	Permissible Limits
Total viable organisms	Bacteria	Nil*	2 X 10^4 CFU/g
	Fungus	Nil*	200 CFU/g
Salmonella sp.		Absent	None/g
Coliform organisms		< 2/g	< 10/g
E.coli type I		Absent	None/g

*= Not detected in first dilution.

Conclusion: - The results of reanalysis indicate that the microbial load is within the permissible limit as recommended in JRF SOP N° 616.

[redacted]

[redacted]

APPENDIX 9 (Continued)

Laboratory Diet Certification Report

Teklad Certified Global 16% Protein Rodent Diet (Sterilizable)

2016SC

Lot Number 2016SC-013012MA
Date of Manufacture 01/30/12
Report Date 02/17/12

The following data is a consolidation of results obtained from one or more independent testing laboratories. The actual laboratory results are available upon request.

[redacted]
Quality Assurance Coordinator, Teklad Diets
Research Models and Services
Harlan Laboratories, Inc.

I have reviewed this document
2012.02.17
15:32:31 -06'00'

Proximate Analysis

Analysis	Result (%)
Protein	16.30
Fat	3.76
Fiber	3.47
Moisture	10.40
Ash	4.93
Calcium	0.69
Phosphorus	0.70

Feed Contaminant Screen

Analysis	Result	Units	Established Maximum Concentration
Heavy Metals			
Arsenic	< 0.10	ppm	1.00
Cadmium	< 0.10	ppm	0.50
Lead	< 0.20	ppm	1.50
Mercury	< 0.05	ppm	0.20
Selenium	0.44	ppm	0.50
Mycotoxin			
Aflatoxin B1, B2, G1, G2	< 5.00	ppb	5.00
Chlorinated Hydrocarbons			
Aldrin	< 0.01	ppm	0.03
Lindane	< 0.01	ppm	0.05
Chlordane	< 0.01	ppm	0.05
DDT & related substances	< 0.03	ppm	0.15
Dieldrin	< 0.02	ppm	0.03
Endrin	< 0.02	ppm	0.03
Heptachlor	< 0.01	ppm	0.03
Heptachlor Epoxide	< 0.01	ppm	0.03
Toxaphene	< 0.10	ppm	0.15
PCB's	< 0.10	ppm	0.15
a-BHC	< 0.01	ppm	0.05
b-BHC	< 0.01	ppm	0.05
d-BHC	< 0.01	ppm	0.05
Hexachlorobenzene	< 0.01	ppm	0.03
Mirex	< 0.01	ppm	0.02
Methoxychlor	< 0.05	ppm	0.50
Organophosphates			
Thimet	< 0.15	ppm	0.50
Diazinon	< 0.14	ppm	0.50
Disulfaton	< 0.15	ppm	0.50
Methyl Parathion	< 0.14	ppm	0.50
Malathion	< 0.14	ppm	0.50
Parathion	< 0.12	ppm	0.50
Thiodan	< 0.02	ppm	0.50
Ethion	< 0.14	ppm	0.50
Trithion	< 0.15	ppm	0.50

P.O. Box 44220 · Madison, WI 53744-4220 · 800-483-5523 · www.harlan.com

harlan™
Helping you do research better

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 10: Water Analysis Reports

JAI RESEARCH FOUNDATION

MICROBIOLOGICAL ANALYSIS CERTIFICATE OF WATER SAMPLE

Name of Sample : **R.O. Water Sample**
Sample Received From : **DCR 400**
Identification N° : **E & M 48**
Sample Analysed at : **Mutagenicity**

Parameters		Results Observed	Permissible Limits
Total viable organisms	Bacteria	Nil*	<20CFU/mL
	Fungus	Nil*	None/100mL
Salmonella sp.		Absent	None/100mL
Coliform organisms		< 2.2/100mL	< 10/100mL
E.coli type I		Absent	None/100mL

*= Not detected in first dilution.

Conclusion: - The results of analysis indicate that the microbial load is within the permissible limit as recommended in JRF/MIC/SOP/619.

Analysed by [redacted]

APPENDIX 10 (Continued)

JAI RESEARCH FOUNDATION

Research Number: RES-2-15-2622

CHEMICAL CONTAMINANT ANALYSIS REPORT

Type of Sample : Lab. Animal Drinking Water
Sample Source : Animal Experiment and Breeding Facility, Jai Research Foundation
Sample Received : October 29, 2011
Analysed on : November 29 to December 03, 2011

Drinking Water Samples	Test Performed	Water Samples Analysis	
		Acceptable Limit	Results
RO water DCR-100 ID No.(E & M-37)	(A) HEAVY METAL ANALYSIS		
RO water DCR-200 ID No.(E & M-38)	Chromium (Cr)	0.05 mg/L	<LOD
	Lead (Pb)	0.05 mg/L	<LOD
RO water DCR-300 ID No.(E & M-39)	(B) PESTICIDE RESIDUE		
RO water DCR-400 ID No.(E & M-40)	α-BHC	0.005 mg/L	<LOD
RO water Dog Facility ID No.(E & M-41)	gamma HCH	0.005 mg/L	<LOD
RO water Rabbit Facility ID No.(E & M-42)	Aldrin	0.005 mg/L	<LOD
	α-endosulfan	0.005 mg/L	<LOD
RO water Guinea pig Facility ID No. (E & M-43)	Dieldrin	0.005 mg/L	<LOD
RO water DCR-500 ID No. (E & M-44)	β-endosulfan	0.005 mg/L	<LOD
RO water DCR-500 ID No.(E & M-45)	Endrin	0.005 mg/L	<LOD
RO water Avian Facility ID No.(E & M-46)	Methoxychlor	0.005 mg/L	<LOD
	Dichlorvos	0.005 mg/L	<LOD
RO water BMRF ID No. (JRF/BMRF/40)	Phorate	0.005 mg/L	<LOD
RO water BMRF ID No.(JRF/BMRF/43)	Chlorpyrifos	0.005 mg/L	<LOD
	Quinalphos	0.005 mg/L	<LOD

Key: *Limit of detection = 0.001 mg/L for all organochlorine pesticides and 0.001 to 0.002 mg/L for all organophosphorus pesticides.
* Limit of detection of Cr and Pb were 0.001 and 0.001 mg/L.

[redacted]

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 11: Bedding Material Analysis Reports

JAI RESEARCH FOUNDATION

MICROBIOLOGICAL ANALYSIS CERTIFICATE OF BEDDING MATERIAL

Name of Sample : **Sterilized Paddy Husk**
Sample Received From : **Conventional Facility**
Sample Analysed at : **Mutagenicity**

Result:

Parameter	Results Observed	Permissible Limit
1. Total Viable Count	**None/Plate**	**None/Plate**

Conclusion: The results of analysis indicate that the microbial load is within the permissible limit as recommended in IRFSOP NO 621.

Analysed by : [redacted]

[redacted]

APPENDIX 11 (Continued)

JAI RESEARCH FOUNDATION

Research Number: RES-2-13-3622

CHEMICAL CONTAMINANT ANALYSIS REPORT

Type of Sample : Bedding Material
Sample Source : Department of Toxicology, Jai Research Foundation
Sample Received : October 29, 2013
Analysed on : November 29 to December 03, 2013

Type of Bedding Material	Test Performed	Bedding Materials Analysis	
		Acceptable Limit	Results
Paddy Husk-PHBM (ID N° BM_)	(A) HEAVY METAL ANALYSIS		
	Chromium (Cr)	0.25 mg/L	<LOD
	Lead (Pb)	1.5 mg/L	<LOD
	(B) PESTICIDE RESIDUE		
Paddy Husk-DCRI (ID N° BM_)	a-HCH	0.02 mg/L	<LOD
	gamma HCH	2.25 mg/L	<LOD
	Aldrin	0.02 mg/L	<LOD
	a-endosulfan	2.00 mg/L	<LOD
	Dieldrin	0.02 mg/L	<LOD
	β-endosulfan	2.00 mg/L	<LOD
	Endrin	0.02 mg/L	<LOD
	Methoxychlor	0.05 mg/L	<LOD
	Dichlorvos	0.02 mg/L	<LOD
	Phorate	0.05 mg/L	<LOD
Corn Cobb (ID N° BM_)	Chlorpyrifos	0.02 mg/L	<LOD
	Quinalphos	0.05mg/L	<LOD

Key: *Limit of detection = 0.001 mg/L for all organ chlorine pesticides and 0.001 to 0.003 mg/L for all organ phosphorus pesticides.
*Limit of detection of Cr and Pb were 0.003 to 0.005 and 0.002 to 0.004 mg/L.

[redacted]

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 12: Historical Control Data

MOUSE (SWISS ALBINO) MICRONUCLEUS TEST - HISTORICAL CONTROL DATA SUMMARY (SEPTEMBER 2009 - FEBRUARY 2012)

Sex	Male		Female	
	P/E ratio	% MNPCE	P/E ratio	% MNPCE
Vehicle: 0.5% Carboxymethyl cellulose				
Mean	0.60	0.04	0.61	0.03
Standard Deviation	0.02	0.04	0.03	0.02
Maximum	0.61	0.06	0.63	0.04
Minimum	0.58	0.01	0.59	0.01
Vehicle: Distilled water				
Mean	0.59	0.03	0.61	0.05
Standard Deviation	0.04	0.02	0.03	0.03
Maximum	0.66	0.08	0.67	0.10
Minimum	0.48	0.00	0.53	0.00
Vehicle: Vegetable oil				
Mean	0.58	0.02	0.61	0.04
Standard Deviation	0.04	0.02	0.04	0.02
Maximum	0.65	0.07	0.71	0.08
Minimum	0.48	0.00	0.53	0.00
Positive control: Mitomycin-C @ 1 mg/kg body weight				
Mean	0.57	1.40	0.59	1.28
Standard Deviation	0.04	0.36	0.04	0.25
Maximum	0.69	2.52	0.68	1.93
Minimum	0.45	0.81	0.48	0.81

Note: Data of last 75 studies conducted during September 12, 2009 to February 03, 2012.

Key: % MNPCE = Percent Micronucleated Polychromatic Erythrocytes
P/E = Total Polychromatic Erythrocytes/Total Erythrocyte

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 13: Certificate of Analysis of Glyphosate TGAI

REPORT REPORT NUMBER FAPC12-000240

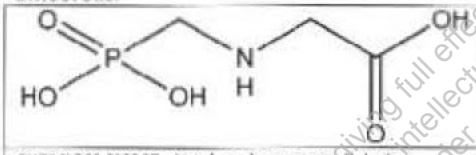
CERTIFICATE OF ANALYSIS FOR TEST/REFERENCE/CONTROL SUBSTANCES

TITLE OBJECTIVE: Determination of purity and/or identity of the following test/reference/control substance for use in a study.

TEST/REFERENCE/CONTROL SUBSTANCE:

TEST SUBSTANCE NUMBER:	TSN105914
LOT NO:	20061109
DESCRIPTION:	Glyphosate Technical Grade Active Ingredient - TGAI

STRUCTURE:



Molecular Formula $C_3H_8NO_5P$
Molecular Weight 169.07

CHEMICAL NAME: N-(phosphonomethyl)glycine

REFERENCE SUBSTANCE(S) USED: TSN003707-0003 (Glyphosate) **PURITY:** 99.8%

INITIATION DATE: April 19, 2012

METHODS USED:

PURITY:	IDENTIFICATION:
LC	IR, MS, ^{1}H NMR, ^{13}C NMR

RESULTS and CONCLUSIONS:

X **RECERTIFICATION: UNCHANGED:**
Current value of 99.3 weight% is within experimental variation of previously established purity of 98.9 weight%. Purity is unchanged and remains 98.9 weight%.

X **IDENTITY:**
Spectroscopic analysis is consistent with proposed structure.

N/A **OTHER:**
N/A

RE-CERTIFICATION DATE: April 20, 2014

CALCULATIONS:
Area Normalized: N/A Internal Standard: N/A External Standard: X
Other (explain): N/A

STUDY DIRECTOR SIGNATURE: [redacted] **STUDY COMPLETION DATE:** May 3, 2012

PEER REVIEWER SIGNATURE: [redacted] **DATE:** 30-April-2012

Sponsor and Testing Facility Address:
Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, IN 46268

All raw data and retainer samples associated with this study will be archived in the testing facility archive. Only descriptive statistics were used unless otherwise noted in the results. This study was conducted in accordance with the Good Laboratory Practice Standard, 40 CFR Part 160.135 (b) unless otherwise noted.

Micronucleus Test of Glyphosate TGAI in Mice

APPENDIX 14: GLP Endorsement of Compliance

NATIONAL GLP COMPLIANCE MONITORING AUTHORITY

GLP CERTIFICATE

GLP Inspection was carried out at **Jai Research Foundation, Near Daman Ganga Bridge, N.H. No. 8, Valvada-396 108, Dist. Valsad, Gujarat, India** in the following areas of expertise:

- **Physical-chemical testing**
- **Toxicity studies**
- **Mutagenicity studies**
- **Environmental Toxicity Studies on Aquatic and Terrestrial Organisms**
- **Studies on Behaviour in Water, Soil and Air, Bioaccumulation**
- **Residue Studies**
- **Analytical and clinical chemistry testing**
- **Toxicokinetics and bio analytical**

Based on the Inspection Report and the follow-up actions taken by the test facility, it is confirmed that the test facility is capable of conducting the above-mentioned tests in compliance with **OECD Principles of Good Laboratory Practice (GLP) and Norms,** as adopted by the National GLP Compliance Monitoring Authority.

This GLP Certificate is valid for a period of **three years** from August 05, 2010, subject to the condition that the test facility complies with the **Terms & Conditions of the National GLP Compliance Monitoring Authority's Document Number GLP-101**.

Certificate No.: GLP/C-0031

Issue Date: 03 -11-2010

Head
National GLP Compliance Monitoring Authority
Department of Science & Technology
Technology Bhavan New Delhi-110016

APPENDIX 14 (Continued)

ENDORSEMENT OF COMPLIANCE

WITH THE OECD PRINCIPLES OF GOOD LABORATORY PRACTICE

Pursuant to the Netherlands GLP Compliance Monitoring Programme and according to Directive 2004/9/EC the conformity with the OECD Principles of GLP was assessed on 7-11 March 2011 at:

Jai Research Foundation
Valvada – 396 108
Gujarat, India

It is herewith confirmed that the afore-mentioned test facility is currently operating in compliance with the OECD Principles of Good Laboratory Practice in the following areas of expertise: Physical-chemical, Toxicity, Mutagenicity, Ecotoxicity, Environmental fate and metabolism, Residue and Analytical and clinical chemistry studies.

[redacted]

Food and Consumer Product Safety Authority (VWA)
Catharijnesingel 59, 3511 GG Utrecht
PO Box 43006, 3540 AA Utrecht, The Netherlands