Summary

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

Study Title

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

Test Guidelines

USEPA OPPTS 870.1100 (2002)
OECD Guideline 423 (2001)
JMAFF Acute Oral Toxicity Study (2002)
EC Number B.1 tris Acute Toxicity (2004)

Author(s)

YC. Jeong, Ph.D.
R. M. Golden, B.S., LAT

Study Completion Date

26 April 2011

Sponsor

Dow AgroSciences LLC
9330 Zionsville Road
Indianapolis, Indiana 46268

Performing Laboratory

Toxicology & Environmental Research and Consulting
The Dow Chemical Company
Midland, Michigan 48674

SUMMARY

2mEPSPS protein was submitted by Dow AgroSciences LLC for evaluation of acute oral toxicity. Five Crl:CD1(ICR) mice/sex were dosed with 7519 mg of the test material (containing 5000 mg/kg of the active ingredient 2mEPSPS) per kilogram (kg) body weight followed by a two week observation period. Parameters evaluated during the study included detailed clinical observations, clinical observations, and body weights. Necropsy was conducted on all animals at study termination to evaluate gross pathological changes.

All animals survived the two-week observation period, and no clinical signs were observed during the study. All animals gained weight by test day 8 and study termination on test day 15. There were no treatment-related gross pathological observations.

Under the conditions of this study, the acute oral LD_{50} of 2mEPSPS protein in male and female Crl:CD1(ICR) mice was greater than 5000 mg/kg (7519 mg/kg of test substance at 66.5% concentration in test material).

STUDY TITLE

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Midland, Michigan 48674

Laboratory Project Study ID

101168

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: 2mEPSPS

Title: 2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

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:  28 March 2011

M. Krieger (Date)
Regulatory Manager

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

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: 2mEPSPS

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All phases of this study were conducted in compliance with the following Good Laboratory Practice Standards:

The Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) -- Good Laboratory Practice Standards, 11 NohSan, Notification No. 6283 - 1 October 1999 revised by 12 NohSan, Notification No. 8628 - 6 December, 2000

US Environmental Protection Agency -- FIFRA GLPs Title 40 CFR, Part 160 - Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards, Final Rule

Organisation for Economic Co-Operation and Development (OECD) -- OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1. OECD Principles on Good Laboratory Practice (as revised in 1997) ENV/MC/CHEM(98)17

Exception: Concentration and homogeneity of the test material in the dose solution was not verified analytically.

R.M. Golden 4/26/11

R. M. Golden, B.S., LAT (Date)
Study Director

(on behalf of R.R. Albee) 3/29/11

R. R. Albee, M.S. (Date)
Manager
Toxicology & Environmental
Research and Consulting

Sponsored and Submitted By:

28 March 2011

M. Krieger (Date)
Regulatory Manager
Dow AgroSciences LLC

QUALITY ASSURANCE STATEMENT

Compound: 2mEPSPS

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This study was examined for conformance with Good Laboratory Practices as published by the USEPA FIFRA, JMAFF, and OECD. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Initiation Date: 06 December 2010

TYPE OF AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Final protocol	15 December 2010	15 December 2010
Study conduct	07-09 December 2010	15 December 2010
Protocol, data, and draft report	11-16 March 2011	23 March 2011
Final report audit.	The date of the signature below is the date of the final report	

The final report accurately reflects the raw data of the study.

3-25-2011

H. M. Mahan, B.S., M.T. (A.S.C.P.), Auditor (Date)
Quality Assurance
Toxicology & Environmental Research and Consulting
The Dow Chemical Company
1803 Building
Midland, Michigan 48674

SIGNATURE PAGE

Compound: 2mEPSPS

Title: 2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

04/26/2011

YC. Jeong, Ph.D. (Date)
Lead Scientist

4/26/11

R. M. Golden, B.S., LAT (Date)
Study Director

Reviewed by:

March 29, 2011

J. Thomas, M.V.Sc., Ph.D. (Date)
Diplomate, American College of Veterinary Pathologists

USEPA TSCA 8(e)/FIFRA 6(a)(2) ASSESSMENT
This test material has been reviewed for possible TSCA 8(e)/FIFRA 6(a)(2) submission YJ 04/26/11 RD 4/26/11
Initials/Date Initials/Date

TABLE OF CONTENTS

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SUMMARY

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All animals survived the two-week observation period, and no clinical signs were observed during the study. All animals gained weight by test day 8 and study termination on test day 15. There were no treatment-related gross pathological observations.

Under the conditions of this study, the acute oral LD_{50} of 2mEPSPS protein in male and female Crl:CD1(ICR) mice was greater than 5000 mg/kg (7519 mg/kg of test substance at 66.5% concentration in test material).

INTRODUCTION

Purpose

The purpose of the acute oral toxicity study was to assess the short-term toxicity of 2mEPSPS protein, in Crl:CD1(ICR) mice following administration by a single oral gavage. This study was intended to provide information on potential health effects that may arise from a single exposure by the oral route.

Test Guidelines

USEPA	United States Environmental Protection Agency, *Health Effects Test Guidelines*, OPPTS 870.1100 (Acute Oral Toxicity), EPA712-C-02-190, December 2002.
OECD	Organisation for Economic Co-Operation and Development. *OECD Guideline for the Testing of Chemicals*, Guideline Number 423 (Acute Oral Toxicity – Acute Toxic Class Method), 17 December 2001.
JMAFF	Japan MAFF Acute Oral Toxicity Study, 2002
EC	EEC Methods Number B.1 tris Acute Oral Toxicity, 2004

Quality Assurance

The study conduct, data, protocol, protocol changes/revisions, and final report were inspected by the Quality Assurance Unit, Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Archiving

The data, protocol, protocol changes/revisions, and final report were archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

Safety

All personnel involved in the study were advised of the safety precautions to follow when handling the test material and treated animals prior to study initiation. Chemical safety information was made available.

TEST MATERIAL INFORMATION

Test Material Name

2mEPSPS

Chemical Name

Not applicable

Synonyms

None

Supplier, City, State (Lot, Reference Number)

Dow AgroSciences LLC, Indianapolis, Indiana (Lot# TSN033171-0001).

Purity/Concentration/Characterization (Method of Analysis and Reference)

The total protein concentration of the test material was determined to be 665 µg/mg by SDS-PAGE/densitometry. The test material was lyophilized protein prepared from a buffer solution containing 10 mM Tris-HCl, pH 7.5, 1 mM DTT, 0.1 M NaCl, and 1% Trehalose. The purity, expressed as the percentage of the 2mEPSPS protein relative to total protein, was greater than 99% (Schafer, 2010).

Characteristics

Molecular Formula

Not applicable

Molecular Weight

~ 47.3 kDa

Chemical Structure

Not applicable

CAS Number

None

Previous Toxicity Information

No previous toxicity information was available.

STUDY DESIGN

With minor variations, including simultaneous dosing and the addition of extra animals, the study design followed the USEPA, OECD, JMAFF, and EC acute oral toxicity guidelines. Because the test material was a digestible protein, no toxicity was anticipated, and therefore the limit test of 5000 mg/kg was employed. Additional animals were dosed to be consistent with previously conducted acute oral toxicity studies. Five mice per sex were simultaneously given 5000 mg/kg 2mEPSPS protein (7519 mg/kg of test material). The test material was given as an approximately 37.5% test material solution (25% protein concentration) in 0.5% aqueous methylcellulose.

Details of the dose preparation are documented in the study file. The total volume administered was 20 ml/kg given as a single oral gavage dose. Animals were observed for signs of toxicity daily for 14 days after dosing.

TEST SPECIES

Species and Sex

Mice (male and female). Both males and females were tested because there was uncertainty regarding sex dependant acute oral toxicity from the test material. Females were nulliparous and non pregnant.

Strain and Justification

The Crl:CD1(ICR) mouse was the preferred strain because of its general acceptance and suitability for acute oral toxicity testing, the availability of historical data, and the reliability of the commercial supplier.

Supplier and Location

Charles River Laboratories Inc. (Portage, Michigan)

Age at Study Start

Approximately 8 weeks

Physical and Acclimation

During the acclimation period each animal was evaluated by a laboratory veterinarian, or a trained animal/toxicology technician under the direct supervision of a laboratory veterinarian, to determine the general health status and acceptability for study purposes (fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International - AAALAC International). The animals were housed one per cage in stainless steel cages, in rooms designed to maintain adequate conditions (temperature, humidity, and photocycle), and acclimated to the laboratory for at least one week prior to the start of the study.

Housing

After assignment, animals were housed one per cage in stainless steel cages. Cages had wire mesh floors and were suspended above absorbent paper. Non-woven gauze was placed in the cages to provide a cushion from the flooring for the rodents' feet. The gauze also provided environmental enrichment. Cages contained a hanging

feeder and a pressure activated lixit valve-type watering system. The following environmental conditions were maintained in the animal room.

Temperature:	22°C with a tolerance of ± 1°C (and a maximum permissible excursion of ± 3°C)
Humidity:	40-70%
Air Changes:	12-15 times/hour (average)
Photoperiod:	12-hour light/dark (on at 6:00 a.m. and off at 6:00 p.m.)

Randomization and Identification

Mice were randomly designated for treatment using a computer program. Mice were identified via a code number transmitted by a subcutaneously implanted transponder (BioMedic Data Systems, Seaford, Delaware).

Feed and Water

Animals were provided LabDiet Certified Rodent Diet #5002 (PMI Nutrition International, St. Louis, Missouri) in pelleted form. Feed and municipal water were provided *ad libitum*. Analyses of the feed were performed by PMI Nutrition International to confirm the diet provided adequate nutrition and to quantify the levels of selected contaminants. Drinking water obtained from the municipal water source was periodically analyzed for chemical parameters and biological contaminants by the municipal water department. In addition, specific analyses for chemical contaminants were conducted at periodic intervals by an independent testing facility. Copies of these analyses are maintained in the study file. There were no chemical or biological contaminants in the feed or water at levels that would have adversely impacted the study or interpretation of its results.

Animal Welfare

In accordance with the U.S. Department of Agriculture animal welfare regulations, 9 CFR, Subchapter A, Parts 1-4, the animal care and use activities required for conduct of this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC). The IACUC determined that the proposed Activities were in full accordance with these Final Rules. The IACUC-approved Animal Care and Use Activities used for this study were Acute Tox 01, DCO 01, Humane Endpoints 01, and Animal ID 01.

STUDY SPECIFIC PARAMETERS

Preparation of Animals

A group of experimentally naive mice were fasted by removing feed but not water approximately 3 hours prior to dosing. After fasting, the mice were weighed and given a detailed clinical observation. Only healthy mice were used.

Dose Calculations

Individual doses were calculated based on the initial (fasted) body weights.

Dosing

Each animal was dosed by oral intubation using a ball-tipped gavage needle attached to an appropriate syringe. Each animal was administered one single dose. The dosing volume was 20 ml/kg. Feed was returned immediately following dosing.

Daily Observations

A cage-side examination was conducted at least once a day, generally at the same time each day (usually in the morning). This examination was typically performed with the animals in their cages and was designed to detect significant clinical abnormalities that were clearly visible upon a limited examination, and to monitor the general health of the animals. The animals were not hand-held for these observations unless deemed necessary. Significant abnormalities that could be observed included, but were not limited to: decreased/increased activity, repetitive behavior, vocalization, incoordination/limping, injury, neuromuscular function (convulsion, fasciculation, tremors and twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency, and fecal quantity. In addition, all animals were observed for morbidity, mortality, and the availability of feed and water at least twice daily, with the exception of two occurrences in which the animals were only observed once daily.

Detailed Clinical Observations

A Detailed Clinical Observation (DCO) was conducted for all mice prior to test material administration for comparison with the observations recorded throughout the study. Animals were observed a minimum of two times on the day of treatment. A DCO was done each day (including weekends and holidays) during the study. Hand-held and open-field observations included a careful physical examination according to an established format (Appendix A). For scored DCOs, only observations other than typically expected were recorded. Observations were dictionary based, and the dictionary contained most of the common physical and neurologic abnormalities seen

in toxicity studies. Since not all potential observations were contained in the dictionary, free-field descriptions also were allowed. Details of the specific observations, definitions of the ranks used for the ranked observations and explanations of the categorical data can be found in Appendix B.

Body Weights

All animals were weighed pre-exposure and on test days 1, 2, 8, and 15.

Pathology

Animals submitted alive for necropsy were anesthetized by the inhalation of carbon dioxide, the tracheas were exposed and clamped and the animals were euthanized by decapitation. A complete necropsy of all animals was conducted by a veterinary pathologist assisted by a team of trained individuals. The necropsy included an examination of the external tissues and all orifices. The eyes were examined *in situ* by application of a moistened glass slide to each cornea. The cranial cavity was opened and the brain, pituitary gland and adjacent cervical tissues examined. The skin was reflected from the carcass, the thoracic and abdominal cavities opened and the viscera examined. All tissues and the carcasses were discarded following the necropsy.

STATISTICS

Means and standard deviations of body weights were calculated. Body weight gains relative to day 1 were also calculated.

The LD_{50} was estimated as indicated below:

< 50% mortality LD_{50} were estimated as greater than the administered dose.

= 50% mortality LD_{50} were estimated as equal to the administered dose.

> 50% mortality LD_{50} were estimated as less than the administered dose.

RESULTS

All animals were dosed on December 7, 2010 (Day 1) and all rats were necropsied on December 21, 2010 (Day 15).

Mortality

Mortality results of male and female mice are presented in Table 1. All animals survived treatment and the two-week observation period.

Clinical Observations

Summary data for daily detailed clinical observations are presented in Table 2. Summary data for clinical observations are presented in Table 3. Individual animal detailed clinical observations are presented in Tables 4 and 5. Individual animal clinical observations are presented in Tables 6 and 7. All animals appeared normal throughout the study.

Body Weights

Mean and individual body weights of males and females are presented in Tables 8 and 9, respectively. All animals gained body weight by test day 8 and 15 (study termination).

Necropsy

Gross pathologic observations are presented in Table 10. There were no gross pathological observations noted at necropsy.

CONCLUSIONS

Under the conditions of this study, the acute oral LD_{50} of 2mEPSPS protein in male and female Crl:CD1(ICR) mice was greater than 5000 mg/kg (7519 mg/kg of test substance at 66.5% concentration in test material).

ACKNOWLEDGEMENTS

K. J. Gallagher	Animal Husbandry, Weights and Data Collection
D. P. Curell	Document Management
H. M. Mahan	Quality Assurance Unit
Pathology Staff	Necropsy

REFERENCES

Schafer, B. W. (2010). Certification of the purity, concentration, and identity of 2mEPSPS, TSN033171-0001, for use in a study. Study ID BIOT10-255698. Dow AgroSciences LLC, Indianapolis, Indiana.

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 1. Mortality - Male And Female Mice

Dose (mg/kg)	#/Sex/Dose	#Dead		Approximate Observed Time of Death (Day)	
		Males	Females	Males	Females
5000	5	0	0	---	---

---No deaths noted.

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 2. Detailed Clinical Observations – Summary

SEX			MALES	FEMALES
DOSE (MG/KG)			5000	5000
Number of Animals Examined				
	DAY	1	5	5
	DAY	2	5	5
	DAY	3	5	5
	DAY	4	5	5
	DAY	5	5	5
	DAY	6	5	5
	DAY	7	5	5
	DAY	8	5	5
	DAY	9	5	5
	DAY	10	5	5
	DAY	11	5	5
	DAY	12	5	5
	DAY	13	5	5
	DAY	14	5	5
	DAY	15	5	5
All Categories, Within Normal Limits				
	DAY	1	5	5
	DAY	2	5	5
	DAY	3	5	5
	DAY	4	5	5
	DAY	5	5	5
	DAY	6	5	5
	DAY	7	5	5
	DAY	8	5	5
	DAY	9	5	5
	DAY	10	5	5
	DAY	11	5	5
	DAY	12	5	5
	DAY	13	5	5
	DAY	14	5	5
	DAY	15	5	5

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 3. Clinical Observations – Summary

SEX		MALES	FEMALES
DOSE (MG/KG)		5000	5000
Number of Animals Examined			
DAY	1	5	5
DAY	2	5	5
DAY	3	5	5
DAY	4	5	5
DAY	5	5	5
DAY	6	5	5
DAY	7	5	5
DAY	8	5	5
DAY	9	5	5
DAY	10	5	5
DAY	11	5	5
DAY	12	5	5
DAY	13	5	5
DAY	14	5	5
DAY	15	5	5
All Categories, Within Normal Limits			
DAY	1	5	5
DAY	2	5	5
DAY	3	5	5
DAY	4	5	5
DAY	5	5	5
DAY	6	5	5
DAY	7	5	5
DAY	8	5	5
DAY	9	5	5
DAY	10	5	5
DAY	11	5	5
DAY	12	5	5
DAY	13	5	5
DAY	14	5	5
DAY	15	5	5

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 4. Individual Animal Detailed Clinical Observations – Males

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 MG/KG				
	3035	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3036	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3037	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3038	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3039	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 5. Individual Animal Detailed Clinical Observations – Females

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 MG/KG				
	3040	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3041	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3042	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3043	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3044	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 6. Individual Animal Clinical Observations – Males

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 MG/KG				
	3035	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3036	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3037	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3038	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3039	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 7. Individual Animal Clinical Observations – Females

Dose	Animal Number	Day Observed First	Day Observed Last	Observation/Comment
5000 MG/KG				
	3040	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3041	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3042	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3043	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	3044	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 8. Body Weights (G) – Males

DOSE MG/KG	ANIMAL NUMBER	DAYS ON TEST 1	2	GAIN	8	GAIN	15	GAIN
5000	3035	27.8	28.1	0.3	30.2	2.4	30.6	2.8
	3036	31.0	30.8	-0.2	31.2	0.2	32.3	1.3
	3037	28.5	29.6	1.1	31.4	2.9	32.8	4.3
	3038	31.1	29.9	-1.2	32.7	1.6	34.9	3.8
	3039	27.1	27.4	0.3	29.3	2.2	30.6	3.5
	MEAN	29.1	29.2	0.1	31.0	1.9	32.2	3.1
	S.D.	1.8	1.4	0.8	1.3	1.0	1.8	1.2
	N=	5	5	5	5	5	5	5

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 9. Body Weights (G) – Females

DOSE MG/KG	ANIMAL NUMBER	DAYS ON TEST 1	2	GAIN	8	GAIN	15	GAIN
5000	3040	22.6	23.4	0.8	22.9	0.3	25.0	2.4
	3041	24.5	24.8	0.3	24.8	0.3	26.1	1.6
	3042	24.2	24.6	0.4	26.7	2.5	28.6	4.4
	3043	24.1	25.3	1.2	25.5	1.4	27.4	3.3
	3044	22.7	24.5	1.8	26.2	3.5	25.6	2.9
	MEAN	23.6	24.5	0.9	25.2	1.6	26.5	2.9
	S.D.	0.9	0.7	0.6	1.5	1.4	1.5	1.0
	N=	5	5	5	5	5	5	5

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 10. Individual Animal Pathology Reports

Group: 1 Dose: 5000 MG/KG Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3035	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3036	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3037	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3038	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3039	SCHEDULED NECROPSY	15	(3)	No Visible Lesions

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

TABLE 10. Individual Animal Pathology Reports (continued)

Group: 1 Dose: 5000 MG/KG Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3040	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3041	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3042	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3043	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
3044	SCHEDULED NECROPSY	15	(3)	No Visible Lesions

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

APPENDIX A. DCO Parameters and Mode of Recording

Cage-Side Observations	Recorded As
Abnormal movements or behaviors	Description
Resistance to removal from cage	Rank

Hand-Held Observations	Recorded As
Ranked Observations	
Eye observations	Rank
- Palpebral closure	Rank
- Pupil Size	Rank
- Lacrimation (non-colored periocular wetness)	Rank
Salivation (non-colored perioral wetness)	Rank
Muscle tone	Rank
Extensor-thrust response	Rank
Reactivity to stimuli	Rank
Categorical Observations	
Abnormal behavior	Description
Abnormalities of the eye	Description
Abnormal urine or feces	Description
Abnormalities of the gastrointestinal (GI) tract	Description
Injury	Description
Missing extremity	Description
Abnormal muscle movements	Description
Palpable mass/swellings	Description
Abnormal posture	Description
Abnormalities of the reproductive system	Description
Abnormal respiration	Description
Abnormal skin or hair-coat/mucous membranes	Description
Excessive soiling	Description
General abnormalities	Description

Open-Field Observations	Recorded As
Responsiveness to touch	Rank
Gait evaluation	Rank

2mEPSPS: ACUTE ORAL TOXICITY STUDY IN Crl:CD1(ICR) MICE

APPENDIX B. Explicitly Defined Scales for DCOs

A. Cage-side observations.

1. Abnormal movements or behaviors: Unusual body movements (*e.g.*, tremors, convulsions), abnormal behaviors (*e.g.*, circling, stereotypy) and changes in posture (*e.g.*, arched back, splayed stance).
2. Resistance to removal: The degree to which the animal attempts to escape capture is scored. The observer will slowly present a gloved hand into the cage and will grasp the animal over the shoulder area or by the tail.

   1 = Decrease – clearly less resistance to capture than typical

   2 = Typical – minimally to actively avoids capture and may be mildly aggressive

   3 = Increase – clearly more resistance to capture than typical and is very aggressive (attempts to bite)

B. Hand-held observations recorded while handling an animal.

1. Ranked observations – the following use a defined scale to rank the degree of severity:
   a. Eye Observations: Eyes are bilaterally examined; however, if a unilateral observation is made, a concurrent observation is not made for the other eye if it is within typical limits.
      (1) Palpebral closure

      1 = Closed (50% to completely closed)

      2 = Open

      3 = Protruding eyes

      (2) Pupil size (aided by penlight): Under typical examination conditions (white light), the typical appearance of the pupils in albino animals is complete constriction. Therefore a decrease in pupil size cannot be observed.

      0 = Unable to evaluate

      1 = Decrease – clearly decreased pupil size compared to typical

      2 = Typical – completely constricted pupils

      3 = Increase – clearly increased pupil size compared to typical

      (3) Lacrimation (non-colored periocular wetness)

      1 = Decrease – extremely dry appearance of cornea

      2 = Typical – glistening cornea (moderate dryness or wetness)

      3 = Increase – extensive wetness around the eyes

   b. Degree of salivation:

1 = Decrease – oral dryness

2 = Typical – limited to moderate perioral wetness, but lips and chin are dry

3 = Increase – extensive wetness around the mouth and lips

c. Muscle tone: An assessment of muscle tone at the time of the hand-held observations.

1 = Decrease – clearly less muscle tone than typical

2 = Typical – animal is neither very relaxed nor very tense

3 = Increase – clearly more muscle tone than typical

d. Extensor-thrust response: Extent of reflex response to brisk pushes (by finger) on the plantar surface of the hindfeet.

1 = Decrease – clearly less response than typical

2 = Typical – clearly detectable extensor-thrust response

3 = Increase – clearly more response than typical

e. Reactivity to stimuli: The degree to which an animal struggles to get free from hand-held restraint is ranked.

1 = Decrease – very slight or no struggling

2 = Typical – mild to moderate struggling, animal may vocalize

3 = Increase – aggressive escape behavior, may try to bite observer and usually vocalizes

2. Categorical observations – The following use a description to record the severity. These observations can be made at any time during the examination.

a. Abnormal behavior: Description of unusual behaviors (*e.g.*, circling, stereotypy) and changes in posture (*e.g.*, arched back, splayed stance) not noted during the cageside portion of examination.

b. Abnormalities of the eye: Any additional descriptive observations concerning the eye, including, but not limited to, cloudiness, opaqueness, overall size, ruptures, etc.

c. Abnormal urine or feces: Description of animal excreta used to assess general health of animal, includes changes in color or quantity.

d. Abnormalities of the gastrointestinal (GI) tract: Description of atypical visual finding related to the gastrointestinal tract (*e.g.*, prolapsed rectum, decreased water or food intake, reflux of test material)

e. Injury: Recorded description of injury the animal has sustained.

f. Missing extremity: Description of missing body part, includes tail, ears, limbs, etc.

g. Abnormal muscle movements: Description of unusual movements (*e.g.*, tremors or convulsion)

h. Palpable mass/swellings: Description of unusual growths or swellings. Includes the location, onset, appearance and progression of any finding.

i. Abnormal posture: Description of unusual posture or stance.

j. Abnormalities of the reproductive system: Description of atypical visual findings in the reproductive organs, including but not limited to: prolapsed vagina, unretracted penis, scrotum bluish, enlarged testicles.

k. Abnormal respiration: Description of changes in respiration including shallow, slow, rapid or mouth breathing.

l. Abnormal skin or hair-coat/mucous membranes: Description of atypical skin or mucous membrane color, changes in hair coat, loss of fur, etc.

m. Excessive soiling: Description and location of increased body soiling.

n. General abnormalities: Description of any other atypical finding not fitting any of the previous observation categories.

C. Open-Field Observations – Ranked observations made by placing the animal on a level surface.

1. Responsiveness to touch: The ventral aspect of the tail is lightly stroked using a finger. Typically, the animal will lift its tail and wrap it around the finger when lightly touched.

   1 = Decrease – does not lift tail, but may briefly hold tail in the air when manually lifted; no response to touch

   2 = Typical – lifts tail when touched

   3 = Increase – lifts tail and acts startled, may turn towards finger in an attack response

2. Gait evaluation: Open-field observations are used for gait evaluation. If the animal remains motionless in the open-field, it may be forced to walk on its forelegs while the hindlegs are held off the floor.

   1 = Unable to walk

   2 = Clear knuckling, stumbling and poor coordination, may include falling and/or dragging of one or more limbs

   3 = Typical – smooth and coordinated gait