TRADE SECRET

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STUDY TITLE: Acute Oral Toxicity Study in Rats - Up-and-Down Procedure

TEST GUIDELINES: U.S. EPA Health Effect Test Guidelines
OPPTS 870.1100 (2002)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 425) (2008)

AUTHOR:

ORIGINAL REPORT COMPLETED: November 18, 2010

REPORT REVISION 1 COMPLETED: November 23, 2010

PERFORMING LABORATORY:

LABORATORY PROJECT ID:

WORK REQUEST NUMBER:

SERVICE CODE NUMBER:

SPONSOR:

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices, except for the item documented below. The item listed does not impact the validity of the study.

1. The test substance was characterized by the sponsor prior to the initiation of this study. Although the characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

Study Director: 23-NOV-2010
Date

Sponsor: _____
Date

QUALITY ASSURANCE STATEMENT

Work Request Number:
Service Code Number:

Key inspections for the above referenced study were completed by the Quality Assurance Unit of and the findings were submitted on the following dates:

Audit Dates	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:		
August 20, 2010	August 20, 2010	August 23, 2010
Conduct:		
September 08, 2010	September 08, 2010	September 08, 2010
Report/Records:		
October 18-19, 2010	October 19, 2010	October 20, 2010
Report Revision 1:		
November 22, 2010	November 22, 2010	November 22, 2010

Reported by: 23 Nov. 2010
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Anatomic Pathology Evaluation Reported by: 23 Nov 2010
Date

Anatomic Pathology Evaluation Reviewed by: 23 Nov 2010
Date

Reviewed by: 23 Nov 2010
Date

Issued by Study Director: 23 Nov 2010
Date

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STUDY INFORMATION

Substance Tested:

Number:

Composition:

Purity: See composition, above

Physical Characteristics:

Study Initiated/Completed: August 20, 2010 / (see report cover page)

Experimental Start/Termination: August 25, 2010 / November 18, 2010

In-Life Initiated/Completed: August 25, 2010 / September 10, 2010

Notebook Number(s):

REASON FOR REVISION

Study Information page was revised at the request of the sponsor.

SUMMARY

A single dose of was administered by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg. One rat was initially dosed. The remaining 2 rats were simultaneously dosed 2 days later. The rats were observed for mortality, body weight effects, and clinical signs for up to 14 days after dosing. All rats were necropsied to detect grossly observable evidence of organ or tissue damage.

No deaths occurred. The rats exhibited no clinical signs or body weight losses during the study. No gross lesions were present in the rats at necropsy.

Under the conditions of this study, the oral LD_{50} for was greater than 5000 mg/kg for female rats.

In accordance with the provisions of Directive 67/548/EEC, classification is not required based on the results of this study.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of when administered by oral gavage to female rats. The starting dose level of 5000 mg/kg was chosen based on toxicity data for similar test substances.

ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR) and the Guidelines from the Guide for the Care and Use of Laboratory Animals (NRC 1996). All studies conducted by or for adhere to the following principles:

- The sponsor and/or the study director ensure that the study described in this report does not unnecessarily duplicate previous experiments, and is in compliance with the Policy on Animal Testing.
- Whenever possible, procedures used in this study have been designed to implement a reduction, replacement, and/or refinement in the use of animals in an effort to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study report or in written laboratory standard operating procedures.
- policy is that animals experiencing severe pain or distress that cannot be relieved are painlessly euthanized, as deemed appropriate by the veterinary staff and study director or appropriate designee.
- Methods of euthanasia used during this study were in conformance with the above referenced regulation and the recommendations of the American Veterinary Medical Association (AVMA), 2007 Guidelines on Euthanasia.
- Animals were provided with species-appropriate environmental enrichment.
- is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.1100: Acute Oral Toxicity, *Health Effects Test Guidelines* (2002)
- OECD, Section 4 (Part 425): Acute Oral Toxicity – Up-and-Down Procedure, *Guideline for the Testing of Chemicals* (2008)

B. Test Substance

The test substance, was supplied by the sponsor as an . The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

C. Test System

Female Crl:CD(SD) rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina.

The Crl:CD(SD) rat was selected based on consistently acceptable health status and on extensive experience with the strain at

D. Animal Husbandry

1. Housing

All animals were housed singly in polycarbonate pans that contained bedding with enrichment (i.e., Shepherd's™ Cob + PLUS™).

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26ºC (64-79ºF) and a relative humidity of 30-70%. Animal rooms were artificially illuminated (fluorescent light) on an approximate 12-hour light/dark cycle. Any excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

3. Feed and Water

PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and water were available *ad libitum* except as noted in section E. Dosing.

4. Identification

Each rat was assigned an identification number which was recorded on a card affixed to the cage. The rats were tail-marked, using a water-insoluble marker, with the identification number.

5. Acclimation

The rats were weighed and observed for general health during the quarantine period (at least 6 days).

6. Animal Health and Environmental Monitoring Program

As specified in the animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.

- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cagewashers.

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

E. Dosing

A single oral dose of was administered neat by oral gavage to 3 fasted female rats at a dose of 5000 mg/kg. One rat was initially dosed. The remaining 2 rats were simultaneously dosed approximately 48.5 hours later.

The rats were approximately 10 or 11 weeks old on the day of dosing. The rats were fasted approximately 17.5 hours prior to dosing, with food being returned to the rats approximately 3-3.5 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing and the test substance density of 1.075 g/mL. The test substance was stirred prior to dosing.

F. Observations, Body Weights, and Anatomic Pathology

Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. The rats were observed for clinical signs at the beginning of fasting, just before dosing (test day 0), once during the first 30 minutes after dosing and 2 more times on the day of dosing, and once each day thereafter. The rats were weighed on test days –1, 0, 7, and 14. On test day 14, the rats were euthanized and necropsied to detect grossly observable evidence of organ or tissue damage. The rats were euthanized by exsanguination while under isoflurane anesthesia.

RESULTS AND DISCUSSION

In-life Toxicology

A. Dose Progression and Mortality

The dose progression and mortality are detailed below. No deaths occurred.

Test Sequence	Animal ID	Dose (mg/kg)	Result
1	3654	5000	O
2[a]	3655	5000	O
3[a]	3656	5000	O

a Sim ultaneously dosed
O Su rvived

The oral LD_{50} is greater than 5000 mg/kg.

B. Body Weights

(Appendices A-B)

No body weight loss occurred after dosing.

C. Clinical Signs

(Appendix C)

No clinical signs were observed.

Anatomic Pathology Evaluation

A. Gross Observations

(Appendix D)

No gross lesions were present in the rats at necropsy.

CONCLUSIONS

Under the conditions of this study, the oral LD_{50} for was greater than 5000 mg/kg for female rats.

In accordance with the provisions of Directive 67/548/EEC, classification is not required based on the results of this study.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, classification is not required.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at

APPENDICES

Appendix A
Individual Body Weights

Individual Body Weights (g)

Sex: Female Bodyweight (g)

5000 mg/kg	Day(s) Relative to Start Date			
	-1	0	7	14
3654	220.0	209.9	243.0	252.9

Sex: Female Bodyweight (g)

5000 mg/kg	Day(s) Relative to Start Date			
	-1	0	7	14
3655	219.0	217.7	266.8	285.9
3656	240.2	237.3	266.8	285.0

Appendix B
Individual Body Weight Gains

Individual Body Weight Gains (g)

Sex: Female Body Weight Gain

5000 mg/kg	Day(s) Relative to Start Date		
	0 → 7	7 → 14	0 → 14
3654	33.1	9.9	43.0

Sex: Female Body Weight Gain

5000 mg/kg	Day(s) Relative to Start Date		
	0 → 7	7 → 14	0 → 14
3655	49.1	19.1	68.2
3656	29.5	18.2	47.7

Appendix C
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

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   A  -  time slots for observations
 Ts1  -  postdose observation 1 (within 30 minutes of dosing)
 Ts2  -  postdose observation 2
 Ts3  -  postdose observation 3
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Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A	6 A	7 A	8 A	9 A
1	f	3654	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	10 A	11 A	12 A	13 A	14 A
1	f	3654	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X

Severity Codes: X = Present

Group 1 - 5000 mg/kg Group 2 - 5000 mg/kg

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A	6 A	7 A	8 A	9 A
2	f	3655	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.
		3656	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	10 A	11 A	12 A	13 A	14 A
2	f	3655	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X
		3656	No Abnormalities Detected		X	X	X	X	X
			Scheduled sacrifice		.	.	.	.	X

Severity Codes: X = Present

Group 1 - 5000 mg/kg Group 2 - 5000 mg/kg

Appendix D
Individual Animal Gross Observations

Individual Gross Observations in Female Rats

Group: 1 Dose: 5000 mg/kg Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3654	SCHEDULED SACRIFICE	14	(2)	No Visible Lesions

Group: 2 Dose: 5000 mg/kg Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3655	SCHEDULED SACRIFICE	14	(2)	No Visible Lesions
3656	SCHEDULED SACRIFICE	14	(2)	No Visible Lesions