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The Dow Chemical Company

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AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE
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Reviewer(s)
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Abstract

Aryloxyalkanoate dioxygenase (AAD-1) was submitted by Dow AgroSciences LLC for evaluation of acute oral toxicity. Five Crl:CD1(ICR) mice/sex were dosed with 5000 mg of test material (containing 2000 mg/kg of the active ingredient AAD-1) per kilogram (kg) body weight. Parameters evaluated during the study included, detailed clinical observations, clinical observations, and body weights. All animals were examined for gross pathological changes.

All animals survived the two-week observation period, and no clinical signs were observed during the study. Eight out of the ten animals gained or maintained weight by test day 2. All animals gained weight by study termination on test day 15. There were no treatment-related gross pathological observations.

Under the conditions of this study, the acute oral LD50 of AAD-1 in male and female mice is greater than 2000 mg/kg (5000 mg/kg of test substance at 40% purity).

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Report Title

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

Author(s) and ID's	Department
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	Report / File Number(s)
	DECO HET DR-0393-0378-002

Materials

X04; ; ; GENERAL OTHERS + UNSPECIFIED; + ; ; VAL

Keywords

ARYLOXYALKANOATE DIOXYGENASE; LD50; SINGLE EXPOSURE

Compound Number(s)	Batch/Lot Number(s)	Bayer Codes
	480-15 (BATCH 2); TSN105930	

Lab Notebooks	Language(s)	Performing Laboratory Name
	ENGLISH	DOW CHEMICAL COMPANY, MIDLAND, MI, USA

Data Requirement(s)

OPPTS; 870.1100; OECD; 423

Geo Area and Country (s) of Study		Protocol Number(s)	Study Number(s)	Published?	Date Study Completed
NA	US	071128	071128	NO	28-AUG-2007
				Vertebrate?	GLP?
				Vertebrate	YES

Method Number	Method Division	Method Status	Validated?

Analyte	Method Type	SMC Code(s)	Enforcement Method?

Method Technique(s)

Reviewer(s)

K. E. STEBBINS (U096209);

Summary

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

Study Title

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

Test Guidelines

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

Author(s)

C. M. Wiescinski, M.S.
R. M. Golden, B.S., LAT

Study Completion Date

28 August 2007

Sponsor

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Indianapolis, Indiana 46268

Performing Laboratory

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

SUMMARY

Aryloxyalkanoate dioxygenase (AAD-1) was submitted by Dow AgroSciences LLC for evaluation of acute oral toxicity. Five Crl:CD1(ICR) mice/sex were dosed with 5000 mg of test material (containing 2000 mg/kg of the active ingredient AAD-1) per kilogram (kg) body weight. Parameters evaluated during the study included, detailed clinical observations, clinical observations, and body weights. All animals were examined for gross pathological changes.

All animals survived the two-week observation period, and no clinical signs were observed during the study. Eight out of the ten animals gained or maintained weight by test day 2. All animals gained weight by 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 AAD-1 in male and female mice is greater than 2000 mg/kg (5000 mg/kg of test substance at 40% purity).

STUDY TITLE

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

Test Guidelines

USEPA OPPTS 870.1100 (2002)
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Laboratory Project Study ID

071128

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: AAD-1

Title: AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C) †.

Company: Dow AgroSciences LLC

Company Agent: K Hilbert for PL Hunst 08/28/07

P. L. Hunst (Date)
Regulatory Manager

†In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

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

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Compound: AAD-1

Title: AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

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

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

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

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

European Community (EC) -- European Parliament and Council Directive 2004/10/EC (O.J. No. L 50/44, 20/02/2004)

GLP Exception: Stability, dose confirmation, and homogeneity verifications were not performed on the dose suspension.

R. M. Golden, B.S., LAT (Date) 9/4/07
Study Director

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Sponsored and Submitted By:

P. L. Hunst (Date) 08/28/07
Regulatory Manager
Dow AgroSciences LLC

QUALITY ASSURANCE STATEMENT

Compound: AAD-1

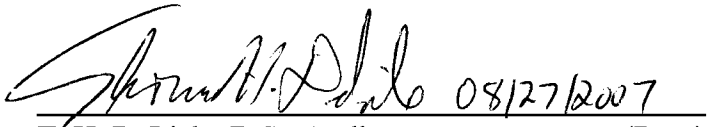
Title: AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

This study was examined for conformance with Good Laboratory Practices as published by the USEPA FIFRA, JMAFF, OECD, and EC. 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: 16 July 2007

TYPE OF AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Final protocol	17 July 2007	17 July 2007
Study conduct	31 July 2007	01 August 2007
Protocol, data, and draft report	23 August 2007	27 August 2007
Final report	The date of the signature below is the date of the final report audit.	

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



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SIGNATURE PAGE

Compound: AAD-1

Title: AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

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C. M. Wiescinski, M.S. (Date)
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R. M. Golden, B.S., LAT (Date)
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SUMMARY

Aryloxyalkanoate dioxygenase (AAD-1) was submitted by Dow AgroSciences LLC for evaluation of acute oral toxicity. Five Crl:CD1(ICR) mice/sex were dosed with 5000 mg of test material (containing 2000 mg/kg of the active ingredient AAD-1) per kilogram (kg) body weight. Parameters evaluated during the study included, detailed clinical observations, clinical observations, and body weights. All animals were examined for gross pathological changes.

All animals survived the two-week observation period, and no clinical signs were observed during the study. Eight out of the ten animals gained or maintained weight by test day 2. All animals gained weight by 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 AAD-1 in male and female mice is greater than 2000 mg/kg (5000 mg/kg of test substance at 40% purity).

INTRODUCTION

Purpose

The purpose of the acute oral toxicity study was to assess the short-term toxicity of AAD-1 in CD-1 mice when administered by 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 are 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

Aryloxyalkanoate dioxygenase (AAD-1)

Chemical Name

Aryloxyalkanoate dioxygenase

Synonyms

AAD-1

Supplier, City, State (Lot, Reference Number)

Dow AgroSciences LLC, Indianapolis, Indiana (lot # 480-15 (Batch 2), TSN105930)

Purity/Characterization (Method of Analysis and Reference)

The purity of the test material was determined to be 40% active ingredient (w/w). Purity expressed as percent protein is greater than 99% (Schafer, 2007).

Homogeneity and Concentration Verification

Homogeneity and concentration were verified by various biochemical tests using multiple replicates of the toxicological lot TSN105930 (Schafer, 2007).

Stability

Stability of the AAD-1 protein (in the lyophilized tox. lot) has been determined to be at least 2 months. This was the time period required to complete the AAD-1 Batch #2 GLP certification study (04-Jan-2007 to 01-Mar-2007 (Schafer 2007)).

Recertification Date

March 30, 2008

Characteristics

Appearance (physical state, color)

Solid

Molecular Formula

See Certificate of Analysis located in the study file for protein sequence.

Molecular Weight

Approximately 33 kDa

Chemical Structure

Not applicable

CAS Number

None

Previous Toxicity Information

In a previous study (Wilson *et. al*., 2004), 3 female CD-1 mice were dosed with 10 mg/kg body weight of AAD-1 protein via intraperitoneal injection. There were no treatment-related effects during the study.

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 is a 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 protein studies. Five mice per sex were simultaneously given 5000 mg/kg body weight of the test material (containing 2000 mg/kg AAD-1) as a 20% suspension in 0.5% aqueous methylcellulose. The total volume administered was 25 ml/kg given in two fractional oral gavage doses of 12.5 ml/kg, approximately 1 hour apart. Animals were observed for signs of toxicity daily for 14 days after dosing.

TEST SPECIES

Species and Sex

Mice (male and female).

Strain and Justification

Crl:CD1(ICR) mice were 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

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 upon arrival at the laboratory (fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International - AAALAC International). The animals were housed two-three 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 each cage to provide a cushion from the flooring for the rodents' feet and provided environmental enrichment. Cages contained a hanging feeder and a pressure activated lixit valve-type watering system.

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

Randomization and Identification

Mice were randomly assigned to dose groups using a computer program. Animals 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 provides 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. Copies of these analyses are maintained at Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. There were no contaminants found in either the feed or water that would adversely impact the results or interpretation of this study.

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 has determined that the proposed Activities were in full accordance with these Final Rules. The IACUC-approved Animal Care and Use Activities to be used for this study were Acute Tox 01, DCO 01, and Animal ID 01.

STUDY SPECIFIC PARAMETERS

Dose Calculations

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

Dosing

All animals were dosed on July 17, 2007. Each animal was fasted for approximately 3 hours prior to dosing. Animals were dosed by oral intubation using a stainless steel ball-tipped gavage needle attached to an appropriate syringe. Each animal was administered two equal fractional doses approximately 1 hour apart. The dosing volume was 12.5 ml/kg for each dose. Feed was returned to the animals immediately following the last dose.

Daily Observations

A cage-side examination was conducted at least once a day, preferably 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 are 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,

tremor, twitches), altered respiration, blue/pale skin and mucous membranes, severe eye injury (rupture), alterations in fecal consistency, and fecal/urinary quantity. In addition, all animals were observed for morbidity, mortality, and the availability of feed and water at least twice daily.

Detailed Clinical Observations

Detailed clinical observations (DCO) were conducted on all animals pre-exposure, a minimum of two times on the day of exposure, and daily thereafter. The DCO was conducted at approximately the same time each examination day, according to an established format. The examination included cage-side, hand-held and open-field observations, which were recorded categorically or using explicitly defined scales (ranks). Details of these observations can be found in Appendix Table 1 and Appendix A.

Body Weights

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

Pathology

Animals were necropsied on July 31, 2007. 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.

STATISTICS

Means and standard deviations of body weights were calculated. Statistical outliers were identified by a sequential test, but were not routinely excluded (Grubbs, 1969).

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

Mortality

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

Clinical Observations

Individual animal detailed clinical observations are presented in Tables 2 and 3. Individual animal clinical observations are presented in Tables 4 and 5. Summary data for daily detailed clinical observations are presented in Table 6. Summary data for clinical observations are presented in Table 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. Eight out of the ten animals gained or maintained weight by test day 2. All animals gained weight by study termination on test day 15.

Necropsy

Gross pathologic observations are presented in Table 10. There were no treatment-related gross pathological observations. Multifocal erosions/ulcers were observed in the glandular mucosa of one male mouse, which may have been a stress-related finding. There was also a dark focus in the cerebrum in one female mouse, which was interpreted to be a spontaneous alteration unassociated with test material administration.

CONCLUSIONS

Under the conditions of this study, the acute oral LD_{50} of AAD-1 in male and female mice is greater than 2000 mg/kg (5000 mg/kg of test substance at 40% purity).

ACKNOWLEDGEMENTS

J. L. Fairchild, K. J. Gallagher	Animal Husbandry, Weights and Data Collection
R. S. Drury	Document Management
T. H. DeLisle, B.S.	Quality Assurance Unit

REFERENCES

Grubbs, F. E. (1969). Procedures for detecting outlying observations in samples. *Technometrics* 11:1-21.

Schafer, B. W. (2007). Determination of the purity and/or identity of Aryloxyalkanoate dioxygenase (AAD-1), Lot# 480-15 (Batch 2), TSN105930, for use in a study. BIOT Number 063610. Dow AgroSciences LLC, Indianapolis, Indiana.

Wilson, D. M., Brooks, K. J., and Golden, R. M. (2004). AAD1 Protein: Class 1 Acute Intraperitoneal (IP) Toxicity Screening Study in CD-1 mice. Report of the Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, Michigan.

AAD-1: 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.

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 2. Individual Animal Detailed Clinical Observations – Males

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 mg/kg				
	5749	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5750	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5751	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5752	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5753	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 3. Individual Animal Detailed Clinical Observations – Females

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 mg/kg				
	5754	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5755	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5756	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5757	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5758	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 4. Individual Animal Clinical Observations – Males

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 mg/kg				
	5749	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5750	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5751	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5752	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5753	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 5. Individual Animal Clinical Observations – Females

Dose	Animal Number	Day Observed First	Last	Observation/Comment
5000 mg/kg				
	5754	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5755	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5756	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5757	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy
	5758	1	15	No Remarkable Observations
		15	15	Disposition, Scheduled Necropsy

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 6. 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

- No Data

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 7. 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

- No Data

AAD-1: 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	5749	30.5	31.2	0.7	32.1	1.6	34.2	3.7
	5750	30.7	30.5	-0.2	30.3	-0.4	32.2	1.5
	5751	30.8	31.4	0.6	32.0	1.2	33.4	2.6
	5752	31.2	30.9	-0.3	31.8	0.6	33.4	2.2
	5753	29.6	29.6	0.0	30.5	0.9	32.2	2.6
	MEAN	30.6	30.7	0.2	31.3	0.8	33.1	2.5
	S.D.	0.6	0.7	0.5	0.9	0.8	0.9	0.8
	N=	5	5	5	5	5	5	5

AAD-1: 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	5754	24.0	24.3	0.3	24.8	0.8	27.0	3.0
	5755	21.6	22.6	1.0	22.7	1.1	23.9	2.3
	5756	22.9	23.2	0.3	24.7	1.8	26.2	3.3
	5757	23.9	24.1	0.2	25.6	1.7	25.1	1.2
	5758	21.8	22.7	0.9	23.2	1.4	23.2	1.4
	MEAN	22.8	23.4	0.5	24.2	1.4	25.1	2.2
	S.D.	1.1	0.8	0.4	1.2	0.4	1.6	0.9
	N=	5	5	5	5	5	5	5

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

TABLE 10. Individual Animal Pathology Reports

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

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
5749	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5750	SCHEDULED NECROPSY	15	(3)	STOMACH; Erosion - Ulcer STOMACH; glandular mucosa; Erosion - Ulcer; multifocal Any remaining protocol required tissues, which have been examined, have no visible lesions
5751	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5752	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5753	SCHEDULED NECROPSY	15	(3)	No Visible Lesions

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TABLE 10. Individual Animal Pathology Reports (continued)

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

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
5754	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5755	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5756	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5757	SCHEDULED NECROPSY	15	(3)	No Visible Lesions
5758	SCHEDULED NECROPSY	15	(3)	BRAIN; cerebrum; Focus; dark; left Any remaining protocol required tissues, which have been examined, have no visible lesions

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

APPENDIX TABLE 1. 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

AAD-1: ACUTE ORAL TOXICITY STUDY IN CRL:CD1(ICR) MICE

APPENDIX A. 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