TRADE SECRET

Study Title

H-28308: 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) (2001)

AUTHOR: Carol Carpenter, B.A.

STUDY COMPLETED ON: May 28, 2008

REVISION 1 COMPLETED ON: July 23, 2008

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers
for Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-25438

WORK REQUEST NUMBER: 17474

SERVICE CODE NUMBER: 834

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

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.

Study Director: Carol Carpenter 23 July 2008
Carol Carpenter, B.A. Date
Senior Staff Toxicologist

QUALITY ASSURANCE STATEMENT

Work Request Number: 17474
Service Code Number: 834

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	January 03, 2008	January 03, 2008	January 03, 2008
Conduct:	January 23, 2008	January 23, 2008	January 23, 2008
Report/Records:	April 03, 2008	April 03, 2008	April 03, 2008
Report Revision 1:	July 17, 2008	July 17, 2008	July 17, 2008

Reported by: Donna M. Johnston 18 July 2008
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

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

Anatomic Pathology Evaluation Reported by: Lisa J. Lewis — 21-July-2008
Lisa J. Lewis
Associate Scientist
Date

Anatomic Pathology Evaluation Reviewed by: Steven R. Frame — 21 July 2008
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P.
Research Fellow and Manager
Date

Reviewed by: Susan M. Munley — 23 July 2008
Susan M. Munley, M.A.
Research Toxicologist
Date

Issued by Study Director: Carol Carpenter — 23 July 2008
Carol Carpenter, B.A.
Senior Staff Toxicologist
Date

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STUDY INFORMATION

Substance Tested:
- FRD-902
- 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionic acid, ammonium salt
- 62037-80-3 (CAS Number)

Haskell Number: 28308

Composition:

86%	HFPO Dimer Acid Ammonium Salt
14.58%	Water
7.0 ppm	Perfluorooctanoic acid

Purity: 86%

Physical Characteristics: Clear and colorless liquid

Study Initiated/Completed: January 3, 2008 / (see report cover page)

Experimental Start/Termination: January 8, 2008 / May 27, 2008

REASON FOR REVISION 1

Reason for revision is to correct the GHS toxicity category.

SUMMARY

A single dose of H-28308 was administered by oral gavage to 1 fasted male rat at a dose of 175 mg/kg, to 2 fasted male rats at a dose of 550 mg/kg, to 4 fasted male rats at a dose of 1750 mg/kg, and to 3 fasted male rats at a dose of 5000 mg/kg. The rats were dosed one at a time at a minimum of 48-hour intervals. 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.

One of the rats dosed at 1750 mg/kg was found dead on the day after dosing, and the 3 rats dosed at 5000 mg/kg were found dead on the day of dosing or the day after dosing. Clinical signs of toxicity were observed in all rats up to the day after dosing and included lethargy, wet fur, stained fur/skin, lung noise, decreased muscle tone, and/or low posture. No body weight losses occurred in the surviving rats. Gross findings were present in 1 rat dosed at 1750 mg/kg and 3 rats dosed at 5000 mg/kg. These included skin stain, lungs expanded, eye discoloration, and/or stomach discoloration. No other gross findings were observed.

Under the conditions of this study, the oral LD_{50} for H-28308 was 1750 mg/kg for male rats. The 95% profile likelihood confidence interval is 1239 to 4450 mg/kg.

In accordance with the provisions of Directive 67/548/EEC, H-28308 is classified as harmful and assigned the symbol Xn and the risk phrase R22 Harmful if swallowed.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, H-28308 is classified in Category 4.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of H-28308 when administered by oral gavage to male rats. The starting dose level of 175 mg/kg was chosen based on the absence of toxicity data for this test substance in male rats.

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* (2001)

B. Test Substance

(Appendix A)

The test substance, H-28308, was supplied by the sponsor. 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

Male 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 DuPont Haskell.

D. Animal Husbandry

1. Housing

All animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

2. Environmental Conditions

Animal rooms were maintained at a temperature of 18-26°C 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. Quarantine

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 DuPont Haskell 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 H-28308 was administered neat by oral gavage to 1 fasted male rat at a dose of 175 mg/kg, to 2 fasted male rats at a dose of 550 mg/kg, to 4 fasted male rats at a dose of 1750 mg/kg, and to 3 fasted male rats at a dose of 5000 mg/kg. The rats were dosed one at a time at a minimum of 48-hour intervals. A software package (A0T425StatPgm)[a] was used to determine the dose progression and the LD_{50}.

[a] Prepared for U.S. EPA by Westat, May 2001, Updated by U.S. EPA February 2002.

The rats were approximately 9-11 weeks old on the day of dosing. The rats were fasted approximately 16-18 hours prior to dosing, with food being returned to the rats approximately 3-4 hours after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing and the test substance density of 1.528 g/mL. The test substance was stirred throughout the dosing procedure.

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 anesthetized by carbon dioxide and euthanized by exsanguination. The rats that died were also necropsied.

RESULTS AND DISCUSSION

In-life Toxicology

A. Dose Progression and Mortality

The dose progression and mortality are detailed below. Death occurred in one rat dosed at 1750 mg/kg and in all 3 rats dosed at 5000 mg/kg.

AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Test type: Main Test
Limit dose (mg/kg): 5000
Assumed LD_{50} (mg/kg): Default
Assumed sigma (mg/kg): 0.5
Recommended dose progression: 5000, 1750, 550, 175, 55, 17.5, 5.5, 1.75 mg/kg

1. Data

Test Sequence	Animal ID	Dose (mg/kg)	Short-Term Result	Long-Term Result
1	5313	175	O	O
2	5314	550	O	O
3	5576	1750	O	O
4	5577	5000	X	X
5	271	1750	X	X
6	273	550	O	O
7	472	1750	O	O
8	473	5000	X	X
9	546	1750	O	O
10	590	5000	X	X

X = Died, O = Survived
Short-term result = animal response within 48 hours of dosing
Long-term result = animal response at the end of the 14-day observation period

Dose Recommendation: The main test is complete. Stopping criteria met: Likelihood ratio criterion.

2. Summary of Long-Term Results

Dose (mg/kg)	O	X	Total
175	1	0	1
550	2	0	2
1750	3	1	4
5000	0	3	3
All Doses	6	4	10

Statistical estimate based on long-term outcomes: Estimated LD_{50} = 1750 mg/kg (the one dose with partial response). The 95% profile likelihood confidence interval is 1239 to 4450 mg/kg.

B. Body Weights

(Appendices B-C)

No body weight loss occurred in the surviving rats after dosing.

C. Clinical Signs

(Appendix D)

Lethargy was observed on the day of dosing in the rat dosed at 175 mg/kg. The 2 rats dosed at 550 mg/kg exhibited wet fur up to the day after dosing. Three surviving rats dosed at 1750 mg/kg exhibited wet fur and stained fur/skin up to the day after dosing. One of these rats also exhibited low posture on the day of dosing. The remaining rat dosed at 1750 mg/kg exhibited lethargy, stained fur/skin, and wet fur on the day of dosing and was found dead on the day after dosing. Lethargy was observed on the day of dosing in one of the rats dosed at 5000 mg/kg. This rat was found dead on the day of dosing. Another rat dosed at 5000 mg/kg exhibited lung noise, lethargy, decreased muscle tone, stained fur/skin, and wet fur on the day of dosing and was found dead on the day after dosing. The remaining rat dosed at 5000 mg/kg exhibited lethargy and was found dead on the day of dosing.

Anatomic Pathology Evaluation

A. Gross Observations

(Appendix E)

Gross findings were present in 1 rat dosed at 1750 mg/kg and 3 rats dosed at 5000 mg/kg. These included skin stain, lungs expanded, eye discoloration, and/or stomach discoloration. No other gross findings were observed.

CONCLUSIONS

Under the conditions of this study, the oral LD_{50} for H-28308 was 1750 mg/kg for male rats. The 95% profile likelihood confidence interval is 1239 to 4450 mg/kg.

In accordance with the provisions of Directive 67/548/EEC, H-28308 is classified as harmful and assigned the symbol Xn and the risk phrase R22 Harmful if swallowed.

According to the Globally Harmonized System (GHS) of classification and labeling of chemicals and under the conditions of this study, H-28308 is classified in Category 4.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

APPENDICES

Appendix A
Certificate of Analysis

E. I. du Pont de Nemours and Company
Wilmington, DE 19898
USA

CERTIFICATE OF ANALYSIS

This Certificate of Analysis fulfills the requirement for characterization of a test substance prior to a study subject to GLP regulations. It documents the identity and content of the test substance. This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 792).

Haskell Code Number	H-28308
Common Name	HFPO Dimer Acid Ammonium Salt
Purity Percent	86%
Other Components	Water – 14.58% Perfluorooctanoic acid – 7.0 ppm
Date of Analysis	October 4, 2007
Recommended reanalysis interval	1 year
Instructions for storage	NRT&H
Reference	DuPont-24003
Analysis performed at	E. I. DuPont de Nemours and Company DuPont Haskell Laboratories Newark, Delaware USA

Approver:

Peter A. Bloxham, Ph.D.
Senior Research Chemist

18-OCT-2007
Date

Appendix B
Individual Body Weights

Individual Body Weights (g)

Bodyweights (g)

Day numbers relative to Start Date

Group Sex	Animal Number	-1	0	7	14
1m	5313	292.4	267.8	330.9	354.8
2m	5314	324.4	302.4	364.0	387.8
3m	5576	291.0	269.5	316.5	361.3
4m	5577	294.8	271.6	.	.
5m	271	314.5	293.8	.	.
6m	273	361.0	331.2	390.3	406.7
7m	472	332.1	307.9	363.0	404.2
8m	473	346.7	320.8	.	.
9m	546	325.6	302.9	365.9	389.1
10m	590	324.1	302.6	.	.

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg
Group 4 - 5000 mg/kg Group 5 - 1750 mg/kg Group 6 - 550 mg/kg
Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg Group 9 - 1750 mg/kg
Group 10 - 5000 mg/kg

Appendix C
Individual Body Weight Gains

Individual Body Weight Gains (g)

	Days 0-7	Days 7-14	Days 0-14
Male, 175 mg/kg			
5313	63.1	23.9	87.0
Male, 550 mg/kg			
5314	61.6	23.8	85.4
273	59.1	16.4	75.5
Male, 1750 mg/kg			
5576	47.0	44.8	91.8
472	55.1	41.2	96.3
546	63.0	23.2	86.2

Appendix D
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

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

Individual Clinical Observations

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
1m	5313	No Abnormalities Detected		X	X	X	.	.	X	X	X	X	X
		Lethargic		.	.	.	X	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
1m	5313	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Lethargic		.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
2m	5314	No Abnormalities Detected		X	X	X	X	.	X	X	X	X	X
		Wet fur	Inguen	.	.	.	.	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
2m	5314	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Wet fur	Inguen	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
3m	5576	No Abnormalities Detected		X	X	X	.	.	.	X	X	X	X
		Stained skin/fur - brown	Inguen	.	.	.	.	.	X	.	.	.	.
		Stained skin/fur - brown	Perineum	.	.	.	.	.	X	.	.	.	.
		Wet fur	Inguen	.	.	.	X	X	.	.	.	.	.
		Wet fur	Perineum	.	.	.	X	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
3m	5576	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Stained skin/fur - brown	Inguen	.	.	.	.	.	.	.	.	.
		Stained skin/fur - brown	Perineum	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
4m	5577	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.
		Lethargic		.	.	X	.	.	.	.	.	.	.
		Found dead		.	.	.	X	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
4m	5577	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
5m	271	No Abnormalities Detected		X	X	X	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	X	.	.	.	.	.
		Stained skin/fur - brown	Perioral	.	.	.	.	X	.	.	.	.	.
		Wet fur	Inguen	.	.	.	X	X	.	.	.	.	.
		Wet fur	Perineum	.	.	.	X	X	.	.	.	.	.
		Found dead		.	.	.	.	.	X	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
5m	271	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	.	.	.	.	.
		Stained skin/fur - brown	Perioral	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
6m	273	No Abnormalities Detected		X	X	X	X	.	.	X	X	X	X
		Wet fur	Perineum	.	.	.	.	X	X	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
6m	273	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Wet fur	Perineum	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
7m	472	No Abnormalities Detected		X	X	X	.	.	.	X	X	X	X
		Stained skin/fur - yellow	Underbody	.	.	.	.	X	X	.	.	.	.
		Wet fur	Underbody	.	.	.	X	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
7m	472	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Stained skin/fur - yellow	Underbody	.	.	.	.	.	.	.	.	.
		Wet fur	Underbody	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
8m	473	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.
		Breathing - lung noise		.	.	X	X	X	.	.	.	.	.
		Lethargic		.	.	X	X	X	.	.	.	.	.
		Muscle tone decreased		.	.	.	.	X	.	.	.	.	.
		Stained skin/fur - red	Chin	.	.	.	X	X	.	.	.	.	.
		Stained skin/fur - red	Perinasal	.	.	X	X	X	.	.	.	.	.
		Stained skin/fur - red	Perioral	.	.	.	X	X	.	.	.	.	.
		Stained skin/fur - yellow	Abdomen	.	.	.	.	X	.	.	.	.	.
		Stained skin/fur - yellow	Inguen	.	.	.	.	X	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	.	.	.	X	.	.	.	.	.
		Wet fur	Chin	.	.	X	X	X	.	.	.	.	.
		Wet fur	Inguen	.	.	.	X	X	.	.	.	.	.
		Wet fur	Perinasal	.	.	X	X	X	.	.	.	.	.
		Wet fur	Perineum	.	.	.	X	X	.	.	.	.	.
		Wet fur	Perioral	.	.	X	X	X	.	.	.	.	.
		Found dead		.	.	.	.	.	X	.	.	.	.

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
8m	473	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Breathing - lung noise		.	.	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	.	.	.	.	.
		Muscle tone decreased		.	.	.	.	.	.	.	.	.
		Stained skin/fur - red	Chin	.	.	.	.	.	.	.	.	.
		Stained skin/fur - red	Perinasal	.	.	.	.	.	.	.	.	.
		Stained skin/fur - red	Perioral	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Abdomen	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Inguen	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	.	.	.	.	.	.	.	.
		Wet fur	Chin	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	.	.	.	.	.	.	.	.
		Wet fur	Perinasal	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	.	.	.	.	.	.	.	.
		Wet fur	Perioral	.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
9m	546	No Abnormalities Detected		X	X	X	.	.	.	X	X	X	X
		Posture - low		.	.	.	.	X	.	.	.	.	.
		Stained skin/fur - brown	Inguen	.	.	.	.	.	X	.	.	.	.
		Stained skin/fur - brown	Perineum	.	.	.	.	.	X	.	.	.	.
		Wet fur	Inguen	.	.	.	X	X	.	.	.	.	.
		Wet fur	Perineum	.	.	.	X	X	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
9m	546	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Posture - low		.	.	.	.	.	.	.	.	.
		Stained skin/fur - brown	Inguen	.	.	.	.	.	.	.	.	.
		Stained skin/fur - brown	Perineum	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Individual Clinical Observations – (Continued)

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	Site	-1 A	0 A	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
10m	590	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.
		Lethargic		.	.	X	.	.	.	.	.	.	.
		Found dead		.	.	.	X	.	.	.	.	.	.

(continued)

Group Sex	Animal Number	Clinical Sign	Site	6 A	7 A	8 A	9 A	10 A	11 A	12 A	13 A	14 A
10m	590	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

Nominal Dose: Group 1 - 175 mg/kg Group 2 - 550 mg/kg Group 3 - 1750 mg/kg Group 4 - 5000 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg Group 8 - 5000 mg/kg
Group 9 - 1750 mg/kg Group 10 - 5000 mg/kg

Appendix E
Individual Animal Gross Observations

Individual Gross Observations in Male Rats

Group: 1 Dose: 175 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
5313	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 2 Dose: 550 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
5314	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 3 Dose: 1750 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
5576	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 4 Dose: 5000 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
5577	FOUND DEAD	0	(0)	LUNGS; Expanded STOMACH; glandular; Discoloration; black; diffuse: raised Any remaining protocol required tissues, which have been examined, have no visible lesions

Group: 5 Dose: 1750 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
271	FOUND DEAD	1	(0)	SKIN; nose; Stain; red Any remaining protocol required tissues, which have been examined, have no visible lesions

Group: 6 Dose: 550 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
273	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 7 Dose: 1750 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
472	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 8 Dose: 5000 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
473	FOUND DEAD	1	(0)	EYES; right; Discoloration; cloudy SKIN; perineum; Stain; yellow SKIN; nose; Stain; red STOMACH; glandular; Discoloration; black Any remaining protocol required tissues, which have been examined, have no visible lesions

Group: 9 Dose: 1750 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
546	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Group: 10 Dose: 5000 mg/kg Sex: Male

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
590	FOUND DEAD	0	(0)	LUNGS; Expanded SKIN; face; Stain; brown STOMACH; glandular; Discoloration; dark: raised, half glandular section effected. Any remaining protocol required tissues, which have been examined, have no visible lesions