TRADE SECRET

Study Title

FRD-902: Acute Oral Toxicity Study in Mice - 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: November 29, 2007

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-24126

WORK REQUEST NUMBER: 17474

SERVICE CODE NUMBER: 835

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, except for the items documented below. The items listed do not impact the validity of the study.

1. The test substance was characterized by the sponsor prior to the initiation of the 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. However, the test substance was characterized in compliance with Good Laboratory Practice Standards soon after the in-life phase of the study. The Certificate of Analysis is included in this report.

2. The dosing preparations used in the study were not analyzed for stability, homogeneity, or accuracy of concentration. The procedures used by trained staff to prepare the dosing preparations ensured:

   - the accuracy of concentration because the test substance was weighed on an analytical balance accurate to 3 decimal places and the vehicle in which the test substance was suspended was accurately measured with graduated pipettes or flasks,
   - homogeneity because the mixture was stirred prior to dosing and while portions were removed for dose administration, and
   - stability because each preparation was formulated daily prior to dosing.

Study Director: Carol Carpenter 29-Nov-2007

Carol Carpenter, B.A. Date
Senior Staff Toxicologist

QUALITY ASSURANCE STATEMENT

Work Request Number: 17474
Service Code Number: 835

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	August 28, 2007	August 28, 2007	August 28, 2007
Conduct:	October 04, 2007	October 04, 2007	October 04, 2007
Report/Records:	November 16, 2007	November 16, 2007	November 16, 2007

Reported by: Donna M. Johnston — 29 Nov. 2007

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 — Date: 27-Nov-2007
Lisa J. Lewis
Associate Scientist

Anatomic Pathology Evaluation Reviewed by: Steven R. Frame — Date: 21 Nov 2007
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P.
Research Fellow and Manager

Reviewed by: Susan M. Munley — Date: 29 Nov 2007
Susan M. Munley, M.A.
Research Toxicologist

Issued by Study Director: Carol Carpenter — Date: 29 Nov 2007
Carol Carpenter, B.A.
Senior Staff Toxicologist

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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: August 23, 2007 / (see report cover page)

Experimental Start/Termination: September 11, 2007 / November 27, 2007

SUMMARY

A single dose of FRD-902 was administered by oral gavage to 1 fasted female mouse at a dose of 175 mg/kg, to 3 fasted female mice at a dose of 550 mg/kg, and to 3 fasted female mice at a dose of 1750 mg/kg. The mice were dosed one at a time at a minimum of 48-hour intervals. The mice were observed for mortality, body weight effects, and clinical signs for up to 14 days after dosing. All mice were necropsied to detect grossly observable evidence of organ or tissue damage.

Death occurred in all 3 mice dosed at 1750 mg/kg. No clinical signs were observed in the mouse dosed at 175 mg/kg or in 2 mice dosed at 550 mg/kg. Wet fur was observed on the day of dosing in 1 mouse dosed at 550 mg/kg. One mouse dosed at 1750 mg/kg was found dead on the day of dosing. No clinical signs were observed in this mouse. Another mouse dosed at 1750 mg/kg exhibited lethargy and low posture and was found dead on the day of dosing. The remaining mouse dosed at 1750 mg/kg exhibited lethargy on the day of dosing and was found dead on the day after dosing. No body weight losses occurred in surviving mice after dosing. No test substance-related gross lesions were found in the study.

Under the conditions of this study, the estimated oral LD_{50} for FRD-902 was 1030 mg/kg for female mice.

In accordance with the provisions of Directive 67/548/EEC, classification is required based on the results of this study. FRD-902 is classified as harmful and assigned the symbol “Xn” and the risk phrase R22 Harmful if swallowed.

INTRODUCTION

The purpose of this study was to assess the acute oral toxicity of FRD-902 when administered by oral gavage to female mice. The starting dose level of 175 mg/kg was chosen based on the absence of toxicity data for this test substance per test guidelines.

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, FRD-902, 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

Female Crl:CDl(ICR) mice were received from Charles River Laboratories, Inc., Raleigh, North Carolina.

The Crl:CDl(ICR) mouse was selected based on consistently acceptable health status and on extensive experience with the strain at Haskell Laboratory.

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

5. Quarantine

Mice were weighed and observed for general health during the 6-day quarantine period.

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 FRD-902, suspended in deionized water in order to deliver an accurate amount, was administered by oral gavage to 1 fasted female mouse at a dose of 175 mg/kg, to 3 fasted female mice at a dose of 550 mg/kg, and to 3 fasted female mice at a dose of 1750 mg/kg. The mice 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 mice were approximately 8 or 9 weeks old on the day of dosing. The mice were fasted approximately 3-4 hours prior to dosing, with food being returned to the mice approximately 0.5-1 hour after dosing. Individual dose volumes were calculated using the fasted body weights obtained prior to dosing. The mice were dosed at a volume of 10 mL per kg of body weight. The dosing mixtures were stirred throughout the dosing procedure.

F. Observations and Body Weights

Observations for mortality and signs of illness, injury, or abnormal behavior were made daily throughout the study. The mice were observed for clinical signs at the beginning of fasting and 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. Mice were weighed on test days 0 (prefast and just before dosing), 7, and 14. On test day 14, the surviving mice were euthanized and necropsied to detect grossly observable evidence of organ or tissue damage. The mice were anesthetized by carbon dioxide and euthanized by exsanguination. The mice 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 all 3 mice dosed at 1750 mg/kg. No other deaths occurred.

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 Seq.	Animal ID	Dose (mg/kg)	Short-Term Result	Long-Term Result
1	3315	175	O	O
2	3316	550	O	O
3	3357	1750	X	X
4	3358	550	O	O
5	3394	1750	X	X
6	3395	550	O	O
7	3396	1750	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: 5 reversals in 6 tests.

2. Summary of Long-Term Results

Dose (mg/kg)	O	X	Total
175	1	0	1
550	3	0	3
1750	0	3	3
All Doses	4	3	7

Statistical estimate based on long term outcomes: estimated LD_{50} = 1030 mg/kg (based on an assumed sigma of 0.5). Approximate 95% confidence interval is 550 to 1750 mg/kg.

B. Body Weights

(Appendices B-C)

No body weight loss occurred in surviving mice after dosing.

C. Clinical Signs

(Appendix D)

No clinical signs were observed in the mouse dosed at 175 mg/kg or in 2 mice dosed at 550 mg/kg. Wet fur was observed on the day of dosing in 1 mouse dosed at 550 mg/kg. One mouse dosed at 1750 mg/kg was found dead on the day of dosing. No clinical signs were observed in this mouse. Another mouse dosed at 1750 mg/kg exhibited lethargy and low posture and was found dead on the day of dosing. The remaining mouse dosed at 1750 mg/kg exhibited lethargy on the day of dosing and was found dead on the day after dosing.

Anatomic Pathology Evaluation

A. Gross Observations

(Appendix E)

No test substance-related gross lesions were found in the study. The only gross lesions observed, lungs, discoloration and ovaries, cyst in mouse 3316 and skin stain in mice 3394 and 3396, were nonspecific and not indicative of target organ toxicity.

CONCLUSIONS

Under the conditions of this study, the estimated oral LD_{50} for FRD-902 was 1030 mg/kg for female mice.

In accordance with the provisions of Directive 67/548/EEC, classification is required based on the results of this study. FRD-902 is classified as harmful and assigned the symbol “Xn” and the risk phrase R22 Harmful if swallowed.

RECORDS AND SAMPLE STORAGE

A sample of the test substance was collected for archive purposes and retained at DuPont Haskell, Newark, Delaware. 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)

Day numbers relative to Start Date

Group Sex	Animal Number	0 b	0 f	7	14
1f	3315	25.6	24.8	26.1	26.9
2f	3316	25.1	24.0	24.6	25.8
3f	3357	25.7	24.4	--	--
4f	3358	27.0	25.9	27.2	27.8
5f	3394	23.7	23.0	--	--
6f	3395	26.0	25.1	27.7	28.9
7f	3396	27.4	26.4	--	--

b prefast
f postfast
-- not weighed

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

Appendix C
Individual Body Weight Gains

Individual Body Weight Gains (g)

	Days 0-7	Days 7-14	Days 0-14
Female, 175 mg/kg			
3315	1.3	0.8	2.1
Female, 550 mg/kg			
3316	0.6	1.2	1.8
3358	1.3	0.6	1.9
3395	2.6	1.2	3.8

Appendix D
Individual Clinical Observations and Mortality Records

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS

EXPLANATORY NOTES

ABBREVIATIONS:

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
1f	3315	No Abnormalities Detected		X	X	X	X	X	X	X	X	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
1f	3315	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
2f	3316	No Abnormalities Detected		X	X	X	X	X	X	X	X	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
2f	3316	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
3f	3357	No Abnormalities Detected		X	X	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
3f	3357	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
4f	3358	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
4f	3358	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 - 550 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
5f	3394	No Abnormalities Detected		X	X	X	.	.	.	.	.	.	.
		Lethargic		.	.	.	X	.	.	.	.	.	.
		Posture - low		.	.	.	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
5f	3394	No Abnormalities Detected		.	.	.	.	.	.	.	.	.
		Lethargic		.	.	.	.	.	.	.	.	.
		Posture - low		.	.	.	.	.	.	.	.	.
		Found dead		.	.	.	.	.	.	.	.	.

X = Present

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
6f	3395	No Abnormalities Detected		X	X	X	X	X	X	X	X	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
6f	3395	No Abnormalities Detected		X	X	X	X	X	X	X	X	X
		Scheduled sacrifice		.	.	.	.	.	.	.	.	X

X = Present

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

Individual Clinical Observations

Group Sex	Animal Number	Clinical Sign	Site	Day numbers relative to Start Date: 0 A	0 B	0 Ts1	0 Ts2	0 Ts3	1 A	2 A	3 A	4 A	5 A
7f	3396	No Abnormalities Detected		X	X	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
7f	3396	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 - 550 mg/kg
Group 5 - 1750 mg/kg Group 6 - 550 mg/kg Group 7 - 1750 mg/kg

Appendix E
Individual Animal Gross Observations

Individual Gross Observations in Female Mice

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3315	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3316	SACRIFICE BY DESIGN	14	(2)	LUNGS; Discoloration; dark; diffuse OVARIES; left; Cyst; clear: 4mm dia Any remaining protocol required tissues, which have been examined, have no visible lesions

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3357	FOUND DEAD	0	(0)	No Visible Lesions

Group: 4 Dose: 550 mg/kg Sex: Female

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3358	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

Individual Gross Observations in Female Mice

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3394	FOUND DEAD	0	(0)	SKIN; inguen; Stain; yellow Any remaining protocol required tissues, which have been examined, have no visible lesions

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3395	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

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

Animal Ref.	Mode Of Death	Death Day	(Week)	Observation(s)
3396	FOUND DEAD	1	(0)	SKIN; inguen; Stain; yellow SKIN; nose; Stain; red Any remaining protocol required tissues, which have been examined, have no visible lesions