TRADE SECRET

Study Title

FRD-902: Acute Dermal Toxicity Study in Rats

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.1200 (1998)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 402) (1987)

EEC Methods for the Determination of Toxicity
Method B.3 Directive 92/69/EEC (1992)

AUTHOR: Carol Carpenter, B.A.

STUDY COMPLETED ON: November 28, 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-24113

WORK REQUEST NUMBER: 17474

SERVICE CODE NUMBER: 673

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 item documented below. The item listed does not impact the validity of the study.

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.

Study Director: Carol Carpenter 28-NOV-2007

Carol Carpenter, B.A.
Senior Staff Toxicologist

Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17474
Service Code Number: 673

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Protocol:	August 24, 2007	August 24, 2007	August 24, 2007
Conduct:	September 25, 2007	September 25, 2007	September 25, 2007
Report/Records:	November 16, 2007	November 16, 2007	November 21, 2007

Reported by: Donna M. Johnston 28 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 — 27-Nov-2007
Lisa J. Lewis
Associate Scientist
Date

Anatomic Pathology Evaluation Reviewed by: Steven R. Frame — 28-Nov-2007
Steven R. Frame, D.V.M., Ph.D., Diplomate A.C.V.P.
Research Fellow and Manager
Date

Reviewed by: Susan M. Munley — 21 Nov 2007
Susan M. Munley, M.A.
Research Toxicologist
Date

Issued by Study Director: Carol Carpenter — 28-Nov-2007
Carol Carpenter, B.A.
Senior Staff Toxicologist
Date

TABLE OF CONTENTS

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 12, 2007 / November 28, 2007

SUMMARY

A single dose of FRD-902 was applied to the shaved, intact skin of 5 male and 5 female rats at a dose of 5000 mg/kg of body weight. The application site was covered with a semi-occlusive dressing for 24 hours, after which the test substance was removed. The rats were observed for 14 days following application. The rats were necropsied to detect grossly observable evidence of organ or tissue damage at the end of the 15-day test period.

No deaths occurred. The rats exhibited no clinical signs of systemic toxicity or body weight loss. No erythema or edema was observed on the test site of male rats. All female rats exhibited erythema (score of 2) but no edema on the test site the day after application of the test substance. No erythema was observed in these rats by 2 days after application. Hyperkeratosis was observed on the test site of 8 rats, and ulceration was observed on the test site of 3 rats during the study. All dermal effects cleared by 13 days after application. No gross lesions were present in the rats at necropsy.

Under the conditions of this study, the skin absorption LD_{50} for FRD-902 was greater than 5000 mg/kg of body weight when applied to the skin of male and female rats for 24 hours.

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

INTRODUCTION

The purpose of this study was to determine the median lethal dose (LD_{50}) by skin absorption of FRD-902. The LD_{50} was defined as the calculated dose of the test substance (mg/kg) administered in a single application expected to cause the death of 50% of a given animal population within 14 days following application. If all animals treated at 5000 mg/kg survive the test period, the LD_{50} is assumed to be greater than 5000 mg/kg, and the LD_{50} is not calculated. The LD_{50} will be reported to be greater than 5000 mg/kg.

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.1200: Acute Dermal Toxicity, *Health Effects Test Guidelines* (1998)
- OECD, Section 4 (Part 402): Acute Dermal Toxicity, *Guideline for the Testing of Chemicals* (1987)
- EEC, Method B.3 Directive 92/69/EEC: Acute Dermal Toxicity, *Methods for the Determination of Toxicity* (1992)

B. Test Substance

(Appendix A)

The test substance, FRD-902, was supplied by the sponsor. The test substance was inverted to mix before each amount for dosing was removed. 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

Young adult male and 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 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

The rats were fed PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002. Food and water were available *ad libitum*.

4. Identification

Each rat was assigned an identification number which was recorded on a card affixed to the cage. The identification number was written on each rat's tail with a water-insoluble marker.

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, Observations, Body Weights, and Anatomic Pathology

Approximately 26 hours before dosing, the fur of each rat was closely shaved to expose the back from the scapular to the lumbar region. The test substance was measured for each animal on the day of treatment at a dose of 5000 mg/kg of body weight. The amount of neat test substance designated for each animal was calculated based on body weights collected prior to treatment and the test substance density of 1527.9 mg/mL. Male rats were approximately 9 weeks old, and female rats were approximately 10 weeks old on the day of dosing.

The area to be treated (approximately 5 cm x 7.4 cm) was marked on the dorsal skin of each rat with a water-insoluble marker. The aliquot of test substance designated for an animal was spread evenly, directly on the skin, covering an area of approximately 37 square centimeters.[a] The test substance was covered with a 2-ply gauze patch. The rats were then wrapped with stretch gauze bandage and self-adhesive bandage. This procedure was followed for each of 5 male and 5 female rats. After wrapping, the rats were returned to their cages. The rats were observed for clinical signs prior to and after dosing.

Approximately 24 hours after treatment, the rats were removed from their cages, and the wrappings were removed. Excess test substance was washed from the dorsal skin of each rat with warm water, and the skin was dried. The rats were observed for clinical signs of toxicity and dermal response and returned to their cages. Dermal effects were scored according to the Draize Scale (Table 1). A glossary of dermal effects and abbreviations are presented in Table 2. Observations for mortality and signs of illness, injury, and abnormal behavior were made daily throughout the study. Observations for clinical signs of toxicity and dermal irritation were made daily throughout the study (weekends excluded for dermal irritation). The rats were weighed prior to treatment (test day 0) and on test days 7 and 14. The rats were reshaved as needed during the study. All rats were euthanized at the end of the 15-day test period and examined to detect grossly observable evidence of organ or tissue damage. The rats were anesthetized by carbon dioxide and euthanized by exsanguination.

a Thirty-seven square centimeters is equal to approximately 10 percent of the total body surface area of rats in the 200 - 300 g body weight range.

RESULTS AND DISCUSSION

In-Life Toxicology

A. Dose Information and Mortality

The dose regimen and the mortality during the test period are summarized in the following table.

Dose (mg/kg)	Average Amount (mL)	Average Initial Body Weight (g)	Mortality
Male 5000	0.97	295.7	0/5
Female 5000	0.77	236.0	0/5

No deaths occurred.

B. Clinical Observations, Body Weights, and Skin Responses

(Appendices B-D)

The rats exhibited no clinical signs of systemic toxicity during the study. Four rats exhibited wet fur (perineum, inguen) and yellow-stained fur/skin (perineum, inguen) after test substance removal. These clinical signs are commonly seen in wrapped rats and, therefore, are not considered test substance related. High posture observed in a rat on test day 4 was not considered test substance related because it was only observed in a single animal. Hair loss observed in 1 rat was considered incidental. The rats exhibited no body weight losses. No erythema or edema was observed on the test site of male rats. All female rats exhibited erythema (score of 2) but no edema on the test site the day after application of the test substance. No erythema was observed in these rats by 2 days after application. Hyperkeratosis was observed on the test site of 8 rats, and ulceration was observed on the test site of 3 rats during the study. All dermal effects cleared by 13 days after application.

Anatomic Pathology Evaluation

A. Gross Observations

(Appendix E)

No gross lesions were present in the rats at necropsy.

CONCLUSIONS

Under the conditions of this study, the skin absorption LD_{50} for FRD-902 was greater than 5000 mg/kg of body weight when applied to the skin of male and female rats for 24 hours.

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

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.

TABLES

Table 1
Draize[a] Scale for Scoring Primary Skin Irritation

Evaluation of Skin Reactions	Score
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1 (Slight)
Well-defined erythema	2 (Mild)
Moderate to severe erythema	3 (Moderate)
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4 (Severe)
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1 (Slight)
Slight edema (edges of area well defined by definite raising)	2 (Mild)
Moderate edema (raised approximately 1.0 mm)	3 (Moderate)
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4 (Severe)

a Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

Table 2
Glossary of Dermal Effects

Blanching	white appearance to skin
Corrosion	area of rough/hard/dry black or dark colored skin that may crater
Desquamation	dry, flaking of the skin
Epidermal Scaling	platelike areas of the top layer of skin that have separated from but are still attached to viable skin
Eschar	scab on the skin that is more superficial than necrosis
Fissuring	a split or cleft in the top layer of skin without bleeding
Fissuring with Bleeding	a split or cleft in the skin with bleeding
Hyperkeratosis	thick, dry discoloration (usually but not limited to brown or white in color) of the top layer of skin
Sloughing	peeling of the top layer of skin, and epidermal scaling that has detached
Thickening	skin is firm and/or dense to the touch
Ulceration	open sore

Abbreviations and Symbols

-	=	No Effect	H	=	Hyperkeratosis
B	=	Blanching	L	=	Sloughing
C	=	Eschar	N	=	Necrosis
D	=	Desquamation	R	=	Raw Areas
Fi	=	Fissuring	S	=	Epidermal Scaling
G	=	Fissuring with Bleeding	T	=	Thickening
--	=	Not Evaluated	X	=	Test Substance Adhered to Skin

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

EXPLANATORY NOTES

SYMBOLS:

S.D. - Standard Deviation
N - Number of Animals

Individual Body Weights (g)

Day numbers relative to Start Date

Group Sex	Animal Number	0	7	14
1m	3416	298.7	338.6	371.8
	3417	301.6	326.1	353.6
	3418	289.7	324.7	361.8
	3419	297.6	340.7	372.4
	3420	291.1	320.1	353.6

Day numbers relative to Start Date

Group Sex	Animal Number	0	7	14
1f	3421	233.0	250.3	261.7
	3422	234.6	266.7	305.0
	3423	242.1	249.8	264.1
	3424	233.6	240.8	254.6
	3425	236.8	243.1	258.2

Nominal Dose: Group 1 – 5000 mg/kg

Appendix C
Individual Clinical Observations and Mortality Records

Individual Clinical Observations and Mortality in Male Rats

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1m	3416	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3417	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Hyperkeratosis	.	.	X	.	.	X	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3418	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Hyperkeratosis	.	.	X	.	.	X	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3419	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Hyperkeratosis	.	.	.	.	.	X	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3420	No Abnormalities Detected	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Ulceration	.	.	.	.	.	X	X	X	X	X	.	.	X	.	.
		Hyperkeratosis	.	.	X	.	.	X	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 – 5000 mg/kg

Individual Clinical Observations and Mortality in Female Rats

Day numbers relative to Start Date

Group Sex	Animal Number	Clinical Sign		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1f	3421	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Stained skin/fur - yellow	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3422	No Abnormalities Detected		X	X	.	.	.	.	.	.	X	X	.	.	X	X	X
		Hair loss	Forelimb bilateral	.	.	X	X	X	X	X	X	X	X	X	X	X	X	X
		Stained skin/fur - yellow	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Hyperkeratosis		.	.	X	.	.	X	X	X	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3423	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Hyperkeratosis		.	.	X	.	.	X	X	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	3424	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
		Stained skin/fur - yellow	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ulceration		.	.	.	.	.	.	X	X	X	X	.	.	X	.	.
		Hyperkeratosis		.	.	X	.	.	X	.	.	.	.	.	.	.	.	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
1f	3425	No Abnormalities Detected		X	X	X	X	.	X	X	X	X	X	X	X	X	X	X
		Posture - high		.	.	.	.	X	.	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Stained skin/fur - yellow	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Inguen	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Wet fur	Perineum	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Ulceration		.	.	.	.	.	.	X	X	X	.	.	.	.	.	.
		Hyperkeratosis		.	.	X	.	.	X	X	.	.	X	.	.	X	X	.
		Scheduled sacrifice		.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

X = Present

Nominal Dose: Group 1 – 5000 mg/kg

Appendix D
Individual Erythema and Edema Scores

Individual Erythema Scores

Day numbers relative to Start Date

Group Sex	Animal Number	1	2	5	6	7	8	9	12	13	14
1m	3416	0	0	0	0	0	0	0	0	0	0
	3417	0	0	0	0	0	0	0	0	0	0
	3418	0	0	0	0	0	0	0	0	0	0
	3419	0	0	0	0	0	0	0	0	0	0
	3420	0	0	0	0	0	0	0	0	0	0

Day numbers relative to Start Date

Group Sex	Animal Number	1	2	5	6	7	8	9	12	13	14
1f	3421	2	0	0	0	0	0	0	0	0	0
	3422	2	0	0	0	0	0	0	0	0	0
	3423	2	0	0	0	0	0	0	0	0	0
	3424	2	0	0	0	0	0	0	0	0	0
	3425	2	0	0	0	0	0	0	0	0	0

Nominal Dose: Group 1 – 5000 mg/kg

Individual Edema Scores

Day numbers relative to Start Date

Group Sex	Animal Number	1	2	5	6	7	8	9	12	13	14
1m	3416	0	0	0	0	0	0	0	0	0	0
	3417	0	0	0	0	0	0	0	0	0	0
	3418	0	0	0	0	0	0	0	0	0	0
	3419	0	0	0	0	0	0	0	0	0	0
	3420	0	0	0	0	0	0	0	0	0	0

Day numbers relative to Start Date

Group Sex	Animal Number	1	2	5	6	7	8	9	12	13	14
1f	3421	0	0	0	0	0	0	0	0	0	0
	3422	0	0	0	0	0	0	0	0	0	0
	3423	0	0	0	0	0	0	0	0	0	0
	3424	0	0	0	0	0	0	0	0	0	0
	3425	0	0	0	0	0	0	0	0	0	0

Nominal Dose: Group 1 – 5000 mg/kg

Appendix E
Individual Animal Gross Observations

Individual Animal Gross Observations in Rats

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

Animal Ref.	Mode Of Death	Death Day	Death (Week)	Observation(s)
3416	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3417	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3418	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3419	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3420	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions

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

Animal Ref.	Mode Of Death	Death Day	Death (Week)	Observation(s)
3421	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3422	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3423	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3424	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions
3425	SACRIFICE BY DESIGN	14	(2)	No Visible Lesions