TRADE SECRET

Study Title

FRD-902: Acute Dermal Irritation Study in Rabbits

TEST GUIDELINES: U.S. EPA Health Effects Test Guidelines
OPPTS 870.2500 (1998)

OECD Guideline for the Testing of Chemicals
Section 4 (Part 404) (2002)

EEC Methods for the Determination of Toxicity
Method B.4 Directive 92/69/EEC (1992)

AUTHOR: Carol Carpenter, B.A.

STUDY COMPLETED ON: November 21, 2007

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-24030

WORK REQUEST NUMBER: 17474

SERVICE CODE NUMBER: 1008

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 21-Nov-2007

Carol Carpenter, B.A.
Senior Staff Toxicologist

Date

QUALITY ASSURANCE STATEMENT

Work Request Number: 17474
Service Code Number: 1008

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 20, 2007	September 20, 2007	September 20, 2007
Report/Records:	November 20, 2007	November 20, 2007	November 20, 2007

Reported by: Donna M. Johnston 21 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.

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

Issued by Study Director: Carol Carpenter 21 Nov 2007
Carol Carpenter, B.A. Date
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 / September 21, 2007

SUMMARY

FRD-902 was applied as a single 0.5-mL dermal dose to the shaved intact skin of a single New Zealand White rabbit. Since no corrosion occurred, 2 additional rabbits were treated. The test substance was applied to a 6 cm^2 area of skin. The application area was covered with a 2-ply gauze square which was held in place with non-irritating tape and covered with porous tape for a semi-occlusive dressing. The rabbits were exposed to the test substance for 4 hours after which the test substance was removed. Test sites were evaluated and scored by the method of Draize for signs of dermal irritation approximately 60 minutes, and 24, 48/50, and 72 hours after test substance removal. The rabbit that was initially treated was also examined immediately after test substance removal.

Erythema (score of 1 or 2) but no edema was observed in the 3 rabbits on the day of dosing. No dermal irritation was observed at 24, 48/50, or 72 hours after removal of the test substance. No clinical signs were observed, and no body weight loss occurred.

Under the conditions of this study, FRD-902 produced erythema, which cleared by 24 hours, when applied to the skin of rabbits.

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

INTRODUCTION

A. Objective

The objective of this study was to evaluate the skin irritation/corrosive potential and the reversibility of dermal effects of FRD-902 following a 4-hour dermal exposure in albino rabbits. The results of the study were used to determine the appropriate toxicity classification and labeling requirements in accordance with EEC.

B. Weight of the Evidence Analysis

Data relevant to the potential dermal irritation/corrosivity of the test substance was evaluated in a weight-of-the-evidence analysis prior to conducting this study. The pH of the test substance was not less than or equal to 2.0 nor greater than or equal to 11.5. There are no internationally validated and accepted *in vitro* or *ex vivo* tests available for skin irritation.

C. Principles of the Methodology

Dermal erythema and edema are evaluated and scored at approximately 60 minutes, and 24, 48/50, and 72 hours following the removal of the test substance at the end of the 4-hour exposure in each of 3 rabbits. The rabbit initially treated is also examined immediately after test substance removal. The reversibility of any dermal effects is assessed for up to 14 days, if necessary. Dermal effects are quantified according to the Draize Scale (Table 1).

MATERIALS AND METHODS

A. Test Guidelines

The study design complied with the following test guidelines:

- U.S. EPA, OPPTS 870.2500: Acute Dermal Irritation, *Health Effects Test Guidelines* (1998)
- OECD, Section 4 (Part 404): Acute Dermal Irritation/Corrosion, *Guideline for the Testing of Chemicals* (2002)
- EEC, Method B.4 Directive 92/69/EEC: Acute Toxicity (Dermal Irritation), *Methods for the Determination of Toxicity* (1992)

Rabbits 275 and 276 were examined at approximately 50 hours (rather than 48 hours) after test substance removal. This deviation did not affect the validity of the study because the difference in time is minimal.

B. Test Substance

(Appendix A)

The test substance, FRD-902, was supplied by the sponsor. The pH of the test substance is 8. 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 New Zealand White rabbits were received from Covance Research Products, Denver, Pennsylvania.

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 16-22ºC 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 rabbits were offered approximately 125 grams of PMI® Nutrition International, LLC Certified Rabbit LabDiet® 5322 daily during the study. Water was available *ad libitum*.

4. Identification

Each rabbit was assigned an identification number which was recorded on a card affixed to the cage. The identification number was written on the inside of each rabbit's ear with a water insoluble marker.

5. Quarantine

Rabbits were weighed and observed for general health during the quarantine period (at least 7 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, and Body Weights

One rabbit was initially treated. Since no skin corrosion occurred, 2 additional rabbits were treated to complete the test.

Approximately 23/24 hours prior to treatment, the fur of 3 male New Zealand White rabbits was closely shaved to expose the skin from the scapular to the lumbar region of the back. The area to be treated (approximately 6 cm^2) was marked on each rabbit's back with a water-insoluble marker. An aliquot of 0.5 mL of FRD-902 was applied to the test site and covered with a 2-ply gauze pad. The pad was held in place with non-irritating tape. The trunk of each rabbit was wrapped with porous tape. The tape was further secured with waterproof tape. The rabbits were returned to their cages after treatment. No other substances were tested on these rabbits.

Approximately 4 hours after application of the test substance, the rabbits were removed from their cages and the wrappings and gauze pads were removed. The test sites were gently washed with warm water to remove excess test substance and gently patted dry. The rabbits were then returned to their cages.

Approximately 60 minutes after removal of the test patches, the test sites were evaluated for erythema, edema, and other evidence of dermal effects and were scored according to the Draize Scale (Table 1). The rabbit that was initially treated was also examined immediately after test substance removal. Additional evaluations were made at approximately 24, 48/50-, and 72 hours after removal of the patches. The adjacent areas of untreated skin were used for comparison. A glossary of dermal effects and abbreviations/symbols is presented in Table 2. Additionally, the rabbits were examined for clinical signs of toxicity at each observation period. The rabbits were weighed on the day of treatment and at the last dermal evaluation.

F. Data Analysis and Interpretation of Results

Mean values for each lesion (erythema and edema) were calculated for each animal separately from numerical scores obtained at the 24-, 48/50-, and 72-hour observations. The results were

interpreted according to 67/548/EEC relative to the general classification and labeling requirements for dangerous substances.

Corrosive

The test substance will be considered to be "CORROSIVE" and will require the symbol "C" and the indication of danger "CORROSIVE" if it produces full thickness destruction of the skin tissue on at least 1 animal during the skin irritation test.

Risk phrases will be assigned in accordance with the following criteria.

R35 "CAUSES SEVERE BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 3 minutes exposure or if this result can be predicted.

R34 "CAUSES BURNS"

If, when applied to healthy intact animal skin, full thickness destruction of skin tissue occurs as a result of up to 4 hours exposure or if this can be predicted.

Irritant

The test substance will be classified as "IRRITANT" and will require the symbol "Xi" and the indication of danger "IRRITANT" in accordance with the criteria given below.

In addition, the following risk (R) phrase will be assigned to substances, if appropriate, according to the criteria indicated below:

R38 "IRRITATING TO SKIN"

If, when applied to healthy intact animal skin for up to 4 hours, significant inflammation is caused and is present 24 hours or more after the end of the exposure period. Inflammation is significant if the mean values of the scores for either erythema and eschar formation or edema formation corresponds to one or more of the following mean values calculated for each animal separately and has been observed in 2 or more animals:

- Erythema and eschar formation 2.0 or more
- Edema 2.0 or more

All scores at each of the reading times (24, 48, and 72 hours) for an effect should be used in calculating the respective mean values.

An R38 "IRRITATING TO SKIN" phrase should also be assigned if:

The inflammation persists in at least 2 animals at the end of the observation time. Particular effects such as hyperplasia, scaling, discoloration, fissures, scabs, and alopecia should be taken into account.

RESULTS AND DISCUSSION

Erythema (score of 1 or 2) but no edema was observed in the 3 rabbits on the day of dosing. No dermal irritation was observed at 24, 48/50, or 72 hours after removal of the test substance. No clinical signs were observed, and no body weight loss occurred.

The dermal scores from individual animals with respect to observation time are presented in Table 3. Summary of mean scores calculated in accordance with EEC are presented in Table 4. Incidences of dermal irritation responses (scores) are summarized for each observation period in Table 5. Individual rabbit body weights and clinical signs of toxicity are presented in Table 6.

CONCLUSIONS

Under the conditions of this study, FRD-902 produced erythema, which cleared by 24 hours, when applied to the skin of rabbits.

In accordance with the provisions of Directive 67/548/EEC, classification 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 Skin Irritation

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

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
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
Desquamation	dry, flaking of the skin
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

Table 3
Dermal Responses Observed in Individual Rabbits

ERYTHEMA

RABBIT NUMBER	EVALUATION AFTER REMOVAL OF TEST SUBSTANCE				
	0 MINUTES	60 MINUTES	24 HOURS	48/50 HOURS	72 HOURS
264[a]	2	2	0	0	0
275	--	2	0	0	0
276	--	1	0	0	0

EDEMA

RABBIT NUMBER	EVALUATION AFTER REMOVAL OF TEST SUBSTANCE				
	0 MINUTES	60 MINUTES	24 HOURS	48/50 HOURS	72 HOURS
264[a]	0	0	0	0	0
275	--	0	0	0	0
276	--	0	0	0	0

Symbols are defined in Table 2.

a Initial rabbit tested

Table 4
Summary of Mean Scores for Dermal Responses

RABBIT NUMBER	ERYTHEMA[a]	EDEMA[a]
264	0	0
275	0	0
276	0	0

a Calculated from the 24-, 48/50-, and 72-hour dermal responses (EEC).

Table 5
Incidences of Dermal Responses (Scores)

ERYTHEMA

SCORE	0 MINUTES	60 MINUTES	24 HOURS	48/50 HOURS	72 HOURS
0	0/1	0/3	3/3	3/3	3/3
1	0/1	1/3	0/3	0/3	0/3
2	1/1	2/3	0/3	0/3	0/3
3	0/1	0/3	0/3	0/3	0/3
4	0/1	0/3	0/3	0/3	0/3

EDEMA

SCORE	0 MINUTES	60 MINUTES	24 HOURS	48/50 HOURS	72 HOURS
0	1/1	3/3	3/3	3/3	3/3
1	0/1	0/3	0/3	0/3	0/3
2	0/1	0/3	0/3	0/3	0/3
3	0/1	0/3	0/3	0/3	0/3
4	0/1	0/3	0/3	0/3	0/3

Table 6
Body Weights and Clinical Signs of Toxicity Observed in Rabbits

RABBIT NUMBER	SEX	INITIAL WEIGHT (g)	FINAL WEIGHT (g)
264	Male	3033	3142
275	Male	3167	3327
276	Male	3044	3157

No clinical signs of toxicity were observed in any of the rabbits during the study.

APPENDIX

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