2) OECD 402-OPPTS 870.1200, Acute dermal toxicity (rat), 132-010

ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

STUDY NUMBER: 132-010

SPONSOR

3M Corporate Toxicology
3M Center, Building 220-2E-02
P.O. Box 33220
St. Paul, MN 55133-3320

SPONSOR'S REPRESENTATIVE

Ph.D., DABT, CIH
Senior Laboratory Manager
Telephone:
FAX:

TESTING FACILITY

Primedica Redfield
Mail: P.O. Box 308
Deliveries: 100 East Boone St.
Redfield, AR 72132
Telephone: 501-397-2540
FAX: 501-397-2002

STUDY DATES

Study Initiation: July 26, 2000
Animal Phase Initiation: July 27, 2000
Animal Phase Completion: August 11, 2000
Study Completion: October 10, 2000

GLP COMPLIANCE STATEMENT

Study Number: 132-010

I certify that this study was performed in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice Regulations (21 CFR Part 58) and OECD Regulations [C(81)30 (final)] and that the report accurately reflects the raw data.

10/10/00

John Seng, Ph.D. Date
Study Director
Primedica Redfield

QUALITY ASSURANCE STATEMENT

Study Number: 132-010

This study has been inspected and audited by the Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Food and Drug Administration and OECD Regulations. The following is a record of the dates that audits/inspections were performed and reported by the QAU.

DATE OF AUDIT/INSPECTION	TYPE OF AUDIT/INSPECTION	DATES REPORTED TO STUDY DIRECTOR AND MANAGEMENT
07/26/00	Protocol	07/26/00
07/27/00	Dose Administration	07/27/00
08/10/00	Necropsy	08/10/00
08/17/00-08/18/00	In-Life Raw Data	08/18/00
08/18/00	Formulations Raw Data, Report Tables, Individual Animal Necropsy Records, and Individual Animal Necropsy Observation Tables	08/18/00
09/05/00	Draft Report	09/05/00
10/10/00	Final Report	10/10/00

The report accurately reflects the original data.

APPROVED BY:

Val Gartner 10/10/00

Val Gartner, B.A. Date
Quality Assurance Auditor
Primedica Redfield

TITLE

ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPROVED BY:

John S Seng 10/10/00

John Seng, Ph.D. Date
Study Director
Primedica Redfield

TABLE OF CONTENTS

Page

Cover Page 1
GLP Compliance Statement 2
Quality Assurance Statement 3
Title and Signature Page 4
Table of Contents 5
Study Abstract 6
Objective 7
Materials and Methods 7
Archival Statement 8
Results 9
Conclusion 9
Tables 10
- Table 1 - Summary of Clinical Observations 11
- Table 2 - Summary of Body Weights 14
- Table 3 - Summary of Body Weight Changes 17

Appendices 20
- Appendix 1 - Individual Necropsy Observations 21
- Appendix 2 - Individual Clinical Observations 23
- Appendix 3 - Individual Body Weights 28
- Appendix 4 - Certificate of Analysis 31
- Appendix 5 - Protocol 33
- Appendix 6 - Protocol Deviations 41
- Appendix 7 - Key Personnel 43

ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

STUDY ABSTRACT

The objective of this study was to determine the acute effect(s) of a single dermal application of T-7485 to Sprague-Dawley rats with a fourteen-day recovery.

Healthy male and female rats were individually housed in stainless steel cages. The animals were acclimated for a minimum of seven days prior to Study Day 1. During the acclimation period, the animals were habituated to collars used during the study. The study design was as follows:

Group Number	Group Designation	Dosage Level (mg/kg)	Number of Animals*	
			Males	Females
1	High-dose	2000	5	5
2	Low-dose	500	5	5
3	Mid-dose	1000	5	5

*Because there were no deaths in Group 1, Groups 2 and 3 were not dosed.

A single dosage level of 2000 mg/kg of test material was applied to the Group 1 animals on Study Day 1. The test material was applied uniformly over an area that was approximately 10% of the body surface area. The test material was held in contact with the skin with a porous gauze dressing and non-irritating tape for a 24-hour exposure period. An Elizabethan collar was placed on each animal during the exposure period. After 24 hours, the test material was removed with warm water and gauze.

Observations for mortality and moribundity were recorded twice daily (a.m. and p.m.). Clinical observations were recorded predose and approximately hourly for four hours postdose on Study Day 1 and then once daily thereafter for at least 14 days. Treatment with T-7485 produced no test material-related mortalities, but there was one procedural death that resulted in the replacement of a male rat. Clinical observations included red material around the eyes, nose, and mouth. These observations were not present after Study Day 2 and were not considered test material-related.

Body weights were recorded pretest and on Study Days 1, 8, and 15. Treatment with T-7485 produced no adverse effects on body weights or body weight changes. When compared to Study Day 1, Study Days 8 and 15 male mean body weights were increased 17% and 40%, respectively. Likewise, female mean body weights were increased 8% and 19%, respectively. On Study Days 1 through 15, male mean body weight change was increased 96.6 grams while body weight change in the females was increased 31.6 grams.

On Study Day 15, all animals (non-fasted) were humanely euthanized via carbon dioxide asphyxiation and submitted for a complete necropsy examination. There were no adverse gross findings recorded for either male or female rats treated with 2000 mg/kg T-7485 with the exception of one male (this male was replaced) which died due to procedural complications.

Treatment with 2000 mg/kg T-7485 produced no treatment-related adverse clinical observations, mortality, changes in body weight, or gross pathology findings.

OBJECTIVE

The objective of this study was to determine the acute effect(s) of a single dermal application of T-7485 to Sprague-Dawley rats with a fourteen-day recovery.

MATERIALS AND METHODS

TEST AND CONTROL MATERIALS: The test material and vehicle were identified as follows:

Test Material:	T-7485 (Potassium Perfluorobutane Sulfonate, PFBS)
Lot Number:	2
Date Received:	April 6, 2000
Physical Description:	White Powder
Storage:	Room Temperature, Protected From Light
Amount Received:	500 grams
Vehicle:	Carboxymethylcellulose (medium viscosity)
Supplier:	Sigma Chemical Co.
Lot Number:	69H0028

The Sponsor assumed responsibility for characterization (identity, purity, and stability) determinations of the test material. The test material was inventoried when received at Primedica Redfield, and a record of all test material usage was maintained. The Certificate of Analysis for the test material is in Appendix 4.

Doses were prepared by Primedica Redfield. The vehicle was prepared to a concentration of 1% by mixing 10 grams of powdered medium viscosity carboxymethylcellulose with deionized water. The test material was moistened with the vehicle prior to administration to the animals.

TEST ANIMALS: Healthy male and female Crl:CD®(SD) IGS BR stock albino rats were received from Charles River Laboratories, Inc., for use on the study. The animals were individually housed in stainless steel cages. Animal identification consisted of uniquely numbered ear tags and cage cards. On Study Day 1, the animals were approximately eight weeks old. The males weighed 233 to 248 grams, and the females weighed 165 to 170 grams.

Teklad Certified Rodent Diet #8728 and filtered tap water were provided *ad libitum*. The feed and water were routinely analyzed for contaminants. There were no known contaminants in the feed or water that would be expected to affect the results of the study. The results of these analyses are on file at Primedica Redfield.

Environmental controls were set to maintain temperatures of 18° to 26°C (64° to 79°F) with a relative humidity of 30% to 70%. These parameters were recorded at least once daily. A 12:12 hour light:dark cycle and ten or greater air changes per hour were maintained in the animal room.

GROUP DESIGNATION AND TREATMENT: The animals were acclimated for a minimum of seven days prior to Study Day 1 and were examined by the Staff Veterinarian prior to being released for use on the study. During the acclimation period, the animals were habituated to collars used during the study. The study design was as follows:

Group Number	Group Designation	Dosage Level (mg/kg)	Number of Animals	
			Males	Females
1	High-dose	2000	5	5
2	Low-dose	500	5	5
3	Mid-dose	1000	5	5

Hair was removed by clipping the dorsal area of the trunk of all animals at least one hour prior to test material administration and as needed thereafter. The dose site and an area of adjacent untreated skin were marked at the corners with an indelible marker.

A single dosage level of 2000 mg/kg of test material was applied to the Group 1 animals on Study Day 1. The test material was applied uniformly over an area that was approximately 10% of the body surface area. The test material was held in contact with the skin with a porous gauze dressing and non-irritating tape for a 24-hour exposure period. An Elizabethan collar was placed on each animal during the exposure period. After 24 hours, the test material was removed with warm water and gauze.

Because there were no test material-related deaths in Group 1, Groups 2 and 3 were not dosed.

JUSTIFICATION FOR SPECIES SELECTION, ROUTE OF ADMINISTRATION, AND DOSE LEVEL: Rats are an animal model for acute toxicity studies of this type. The number of animals assigned to the study represented the minimum required to meet the objectives of the study and the OECD guidelines (#402). In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. Dose levels were chosen in accordance with OECD guideline #402.

CLINICAL OBSERVATIONS: Observations for mortality and moribundity were recorded twice daily (a.m. and p.m.). Clinical observations were recorded predose and approximately hourly for four hours postdose on Study Day 1 and then once daily thereafter for at least 14 days.

BODY WEIGHTS: Body weights were recorded pretest and on Study Days 1, 8, and 15. The pretest body weights are not included in this report but are located in the raw data.

NECROPSY: Animals that died prior to scheduled termination were weighed and necropsied as detailed below. On Study Day 15, all animals (non-fasted) were humanely euthanized via carbon dioxide asphyxiation and submitted for a complete necropsy examination. A complete necropsy was defined as examination of the external surface of the body; all orifices; and the cranial, thoracic, and abdominal cavities and their contents. Gross lesions were preserved in neutral buffered formalin. All other tissues were discarded.

HISTOPATHOLOGY: No histopathological examinations were performed.

STATISTICS: Means and standard deviations were calculated for all quantitative data.

ARCHIVAL STATEMENT

All original data and the original final report will be retained at Primedica Redfield's archives, located at 100 East Boone Street, Redfield, Arkansas, for a period of five years after issuance of the final report. Wet tissues, slides, and blocks (if generated) will be retained at Primedica Redfield for one year. After this time, the Sponsor will be contacted for disposition instructions.

RESULTS

CLINICAL OBSERVATIONS: The summary of clinical observations is in Table 1. The individual observations are in Appendix 2.

Treatment with T-7485 produced no test material-related mortalities, but there was one procedural death that resulted in the replacement of a male rat. Clinical observations included red material around the eyes, nose, and mouth. These observations were not present after Study Day 2 and were not considered test material-related.

BODY WEIGHTS AND BODY WEIGHT CHANGES: The summaries of body weights and body weight changes are in Tables 2 and 3, respectively. The individual body weights are in Appendix 3.

Treatment with T-7485 produced no adverse effects on body weights or body weight changes. When compared to Study Day 1, Study Days 8 and 15 male mean body weights were increased 17% and 40%, respectively. Likewise, female mean body weights were increased 8% and 19%, respectively. On Study Days 1 through 15, male mean body weight change was increased 96.6 grams while body weight change in the females was increased 31.6 grams.

NECROPSY: The individual necropsy observations are located in Appendix 1.

There were no adverse gross findings recorded for either male or female rats treated with 2000 mg/kg T-7485 with the exception of one male (this male was replaced) which died due to procedural complications.

CONCLUSION

Treatment with 2000 mg/kg T-7485 produced no treatment-related adverse clinical observations, mortality, changes in body weight, or gross pathology findings.

TABLES

TABLE 1
SUMMARY OF CLINICAL OBSERVATIONS

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 1: CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

DOSE GROUP	1
MG/KG	2000
MORTALITY	0
APPEARS NORMAL, PREDOSE	5/ 5
APPEARS NORMAL, 1 HOUR POSTDOSE	2/ 2
APPEARS NORMAL, 2 HOURS POSTDOSE	2/ 2
APPEARS NORMAL, 3 HOURS POSTDOSE	2/ 2
RED MATERIAL AROUND EYES	3/ 3
RED MATERIAL AROUND EYES, 4 HOURS POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE	2/ 2
RED MATERIAL AROUND NOSE, 1 HOUR POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE, 2 HOURS POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE, 3 HOURS POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE, 4 HOURS POSTDOSE	5/ 5
RED MATERIAL AROUND MOUTH	1/ 1

N/N = NUMBER OF OBSERVATIONS/NUMBER OF ANIMALS.

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 1: CLINICAL OBSERVATIONS - SUMMARY - FEMALE RATS

DOSE GROUP	1
MG/KG	2000
MORTALITY	0
APPEARS NORMAL, PREDOSE	5/ 5
APPEARS NORMAL, 1 HOUR POSTDOSE	2/ 2
APPEARS NORMAL, 2 HOURS POSTDOSE	1/ 1
APPEARS NORMAL, 3 HOURS POSTDOSE	1/ 1
RED MATERIAL AROUND EYES	3/ 3
RED MATERIAL AROUND EYES, 1 HOUR POSTDOSE	1/ 1
RED MATERIAL AROUND EYES, 2 HOURS POSTDOSE	1/ 1
RED MATERIAL AROUND EYES, 3 HOURS POSTDOSE	1/ 1
RED MATERIAL AROUND EYES, 4 HOURS POSTDOSE	2/ 2
RED MATERIAL AROUND NOSE	3/ 3
RED MATERIAL AROUND NOSE, 1 HOUR POSTDOSE	2/ 2
RED MATERIAL AROUND NOSE, 2 HOURS POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE, 3 HOURS POSTDOSE	3/ 3
RED MATERIAL AROUND NOSE, 4 HOURS POSTDOSE	5/ 5
RED MATERIAL AROUND MOUTH	1/ 1

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF ANIMALS WITH THE OBSERVATION.

TABLE 2
SUMMARY OF BODY WEIGHTS

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 2: BODY WEIGHTS - SUMMARY - MALE RATS

DOSE GROUP MG/KG			2000
RATS - TESTED			5
BODY WEIGHT (G)			
SD	1	MEAN±S.D.	240.0 ± 5.8
SD	8	MEAN±S.D.	281.0 ± 6.0
SD	15	MEAN±S.D.	336.6 ± 21.6

SD = STUDY DAY

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 2: BODY WEIGHTS - SUMMARY - FEMALE RATS

DOSE GROUP MG/KG			2000
RATS - TESTED			5
BODY WEIGHT (G)			
SD	1	MEAN±S.D.	167.6 ± 2.1
SD	8	MEAN±S.D.	181.6 ± 5.7
SD	15	MEAN±S.D.	199.2 ± 3.4

SD = STUDY DAY

TABLE 3
SUMMARY OF BODY WEIGHT CHANGES

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 3: BODY WEIGHT CHANGES - SUMMARY - MALE RATS

DOSE GROUP MG/KG			2000
RATS - TESTED			5
BODY WEIGHT CHANGE (G)			
SD	1 - 8	MEAN±S.D.	+41.0 ± 4.8
SD	8 - 15	MEAN±S.D.	+55.6 ± 17.8
SD	1 - 15	MEAN±S.D.	+96.6 ± 17.7

SD = STUDY DAY

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

TABLE 3: BODY WEIGHT CHANGES - SUMMARY - FEMALE RATS

DOSE GROUP MG/KG			2000
RATS - TESTED			5
BODY WEIGHT CHANGE (G)			
SD	1 - 8	MEAN±S.D.	+14.0 ± 5.8
SD	8 - 15	MEAN±S.D.	+17.6 ± 4.6
SD	1 - 15	MEAN±S.D.	+31.6 ± 2.2

SD = STUDY DAY

APPENDICES

APPENDIX 1
INDIVIDUAL NECROPSY OBSERVATIONS

APPENDIX 1
INDIVIDUAL NECROPSY OBSERVATIONS

GROUP 1 - 2000 mg/kg

Animal #6981	-	Male	-	No adverse findings.
Animal #6983	-	Male	-	No adverse findings.
			-	Died Following Post-Dose Procedure.
Animal #6985	-	Male	-	No adverse findings.
Animal #6991	-	Male	-	No adverse findings.
Animal #6993	-	Male	-	No adverse findings.
Animal #6995	-	Male	-	No adverse findings.
Animal #6988	-	Female	-	No adverse findings.
Animal #6990	-	Female	-	No adverse findings.
Animal #6992	-	Female	-	No adverse findings.
Animal #6994	-	Female	-	No adverse findings.
Animal #6996	-	Female	-	No adverse findings.

APPENDIX 2
INDIVIDUAL CLINICAL OBSERVATIONS

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS FOR STUDY DAY 1 - INDIVIDUAL - MALE RATS

STUDY DAY 1 DOSE GROUP 1 2000 MG/KG

RAT #	PREDOSE	1 HOUR POSTDOSE	2 HOURS POSTDOSE	3 HOURS POSTDOSE	4 HOURS POSTDOSE
6981	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	RED MATERIAL AROUND EYES RED MATERIAL AROUND NOSE
6985	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	RED MATERIAL AROUND EYES RED MATERIAL AROUND NOSE
6991	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND EYES RED MATERIAL AROUND NOSE
6993	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE
6995	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS - INDIVIDUAL - MALE RATS

DOSE GROUP 1		2000 MG/KG
RAT #		DESCRIPTION
6981	SD(2)	RED MATERIAL AROUND NOSE
	SD(2)	RED MATERIAL AROUND MOUTH
6985	SD(2)	RED MATERIAL AROUND EYES
6991	SD(2)	RED MATERIAL AROUND EYES
6993	SD(2)	RED MATERIAL AROUND NOSE
6995	SD(2)	RED MATERIAL AROUND EYES

SD = STUDY DAY

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS FOR STUDY DAY 1 - INDIVIDUAL - FEMALE RATS

STUDY DAY 1 DOSE GROUP 1 2000 MG/KG

RAT #	PREDOSE	1 HOUR POSTDOSE	2 HOURS POSTDOSE	3 HOURS POSTDOSE	4 HOURS POSTDOSE
6988	APPEARS NORMAL	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE
6990	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE
6992	APPEARS NORMAL	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND NOSE	RED MATERIAL AROUND EYES
					RED MATERIAL AROUND NOSE
6994	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	APPEARS NORMAL	RED MATERIAL AROUND NOSE
6996	APPEARS NORMAL	RED MATERIAL AROUND EYES	RED MATERIAL AROUND EYES	RED MATERIAL AROUND EYES	RED MATERIAL AROUND EYES
					RED MATERIAL AROUND NOSE

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS - INDIVIDUAL - FEMALE RATS

DOSE GROUP 1		2000 MG/KG
RAT #		DESCRIPTION
6988		NO ADVERSE FINDINGS
6990	SD(2)	RED MATERIAL AROUND NOSE
	SD(2)	RED MATERIAL AROUND MOUTH
6992	SD(2)	RED MATERIAL AROUND EYES
	SD(2)	RED MATERIAL AROUND NOSE
6994	SD(2)	RED MATERIAL AROUND EYES
	SD(2)	RED MATERIAL AROUND NOSE
6996	SD(2)	RED MATERIAL AROUND EYES

SD = STUDY DAY

APPENDIX 3
INDIVIDUAL BODY WEIGHTS

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 3: BODY WEIGHTS - INDIVIDUAL - MALE RATS

SD	1	8	15
RAT #	DOSE GROUP 1	2000 MG/KG	
6981	248.	290.	368.
6985	241.	277.	348.
6991	236.	284.	332.
6993	242.	279.	317.
6995	233.	275.	318.

SD = STUDY DAY
All weights were reported in grams (G).

PROTOCOL 132-010: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

APPENDIX 3: BODY WEIGHTS - INDIVIDUAL - FEMALE RATS

SD	1	8	15
RAT #	DOSE GROUP 1	2000 MG/KG	
6988	169.	178.	200.
6990	168.	180.	201.
6992	165.	187.	198.
6994	170.	188.	203.
6996	166.	175.	194.

SD = STUDY DAY
All weights were reported in grams (G).

APPENDIX 4
CERTIFICATE OF ANALYSIS

Certificate Of Analysis

$C_4F_9SO_3^-K^+$ (PFBS), Lot 2

March 10, 2000

This sample was analyzed using LC/MS, ^{1}H-NMR, ^{19}F-NMR, and elemental analyses techniques. The results of these tests show the sample to contain the following weight percent composition:

	0.13 %
$C_4F_9SO_3^-K^+$	99.82 %
	0.04 %

Additionally, the isomer distribution of the sample was determined using ^{19}F-NMR techniques and found to contain the following weight percent composition:

$CF_3(CF_2)_3$-$SO_3^-K^+$	97.86 %
	1.96 %

APPENDIX 5
PROTOCOL

Primedica Redfield Protocol

Protocol Number: 132-010

ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

PROPOSAL NO.: 132-010

OBJECTIVE: Determine the acute effect(s) of a single dermal application of T-7485 to Sprague-Dawley rats with a fourteen-day recovery.

LOCATION OF STUDY AND CONDITIONS OF TESTING:

It is the intention of 3M Corporate Toxicology, through the conduct of this study, to generate animal safety data that may be submitted to regulatory authorities. Primedica Redfield, 100 East Boone Street, Redfield, Arkansas, 72132, is accredited by AAALAC and licensed by the United States Department of Agriculture to conduct research in laboratory animals. All the conditions of testing will conform to the Animal Welfare Act (CFR 9) and its amendments. Primedica Redfield will follow all requirements specified in this approved protocol and all applicable governmental regulations regarding Good Laboratory Practices as well as Primedica Redfield's Standard Operating Procedures. Changes in the protocol may be made by consultation with and approval from 3M Corporate Toxicology followed by written verification of the change. 3M Corporate Toxicology reserves the right to inspect facilities and procedures used for this study by means of announced or unannounced site visits. Primedica Redfield will notify 3M Corporate Toxicology promptly by telephone prior to release of any data for review.

SPONSOR:	3M Corporate Toxicology 3M Center, Building 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3320
SPONSOR'S REPRESENTATIVE:	Ph.D., DABT, CIH Senior Laboratory Manager Telephone: FAX:
TESTING FACILITY:	Primedica Redfield Mail: P.O. Box 308 Deliveries: 100 East Boone St. Redfield, AR 72132 Telephone: 501-397-2540 FAX: 501-397-2002
PERSONNEL:	Study Director: John Seng, Ph.D. Principle Investigator: Karen Tranter, B.A., LATG Veterinarian: Allan Manus, D.V.M., M.Sc., ACLAM Pathologist: TBD
PROPOSED STUDY DATES:	Experimental Start Date: July 27, 2000 Experimental Termination Date: August 10, 2000 Draft Report Date: September 7, 2000

1. REGULATORY COMPLIANCE AND QUALITY ASSURANCE

This study will be conducted in accordance with the following Good Laboratory Practice Regulations/Standards/Guidelines:

☒ 21 CFR 58
☒ C(81)30 (Final) (OECD)

The Quality Assurance Unit, in accordance with Primedica Redfield's Standard Operating Procedures (SOPs), will audit the protocol, study conduct, and the final report.

2. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE REVIEW

The protocol will be reviewed and approved by Primedica Redfield's Institutional Animal Care and Use Committee (IACUC) for compliance with regulations prior to study initiation.

In the opinion of the Sponsor, indicated by the signature on this protocol, the study does not unnecessarily duplicate any previous work.

3. MATERIALS

TEST MATERIAL	T-7485 (Potassium perfluorobutane sulfonate, PFBS)
IDENTIFICATION	The lot number will be listed in the raw data and final report.
PHYSICAL DESCRIPTION	White powder
VEHICLE	1% carboxymethylcellulose (aqueous, medium viscosity)
IDENTIFICATION	The lot number will be listed in the raw data and final report.
PHYSICAL DESCRIPTION	White powder

STORAGE CONDITIONS

The bulk vehicle and test material will be stored in its original container and will be stored at room temperature.

ANALYTICAL CHEMISTRY

The Sponsor assumes responsibility for characterization (identity, purity, and stability) of the bulk test material (including under test conditions). Information on the composition and method of synthesis of the bulk test material will be held by the Sponsor. The doses, unless otherwise stated, will be calculated assuming the test material to be 100% pure.

DOSE PREPARATION AND CONCENTRATION

Doses will be weighed by Primedica Redfield on the day of use. The carboxymethylcellulose will be prepared at 1% in deionized water and may be prepared prior to Study Day 1.

INVENTORY

The control and test materials will be inventoried when received at the testing laboratory, and a record of all test material use will be maintained.

TEST MATERIAL RETENTION

Unused test material may be returned to the Sponsor or designee at the termination of the study, or retained for use on future studies. The Sponsor will be notified in advance of shipping, and a transmittal letter will accompany the shipment. The material will be packed in a suitable container to maintain the conditions specified by the Sponsor during transit plus an adequate margin of safety for any transit delays.

SAFETY PRECAUTIONS

General safety precautions as required by Primedica Redfield's policies and procedures will be followed. The Sponsor's Representative will be notified of any personnel exposures requiring a physician's examination or care.

4. *ANIMALS*

Species:	Rat
Strain/Source:	Crl:CD®(SD) albino rats, Charles River Laboratories, Inc.
Age at Initiation:	Six to eight weeks
Number and Sex:	15 male and 15 female (female nulliparous and non pregnant)
Identification:	Uniquely numbered ear tag and cage card

ANIMAL HUSBANDRY

HOUSING

The animals will be individually housed in stainless steel cages. The cages conform to standards set forth in the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, D.C., 1996.

FEED

Teklad Certified Rodent Diet #8728 will be provided *ad libitum*. This diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of the manufacturer's analyses are on file at Primedica Redfield.

WATER

Filtered tap water will be provided *ad libitum*. Samples of the water are analyzed for total dissolved solids, hardness, specified microbiological content, and for environmental contaminants. Results of these analyses are on file at Primedica Redfield.

CONTAMINANTS

There are no known contaminants in the feed or water that would be expected to interfere with this study.

ENVIRONMENT

Environmental controls are set to maintain a temperature of 18° to 26°C (64° to 79°F) with a relative humidity of 30% to 70%. These parameters are recorded at least once daily. A 12:12 hour light:dark cycle is maintained.

ACCLIMATION

Animals will be acclimated for a minimum of seven days prior to the study start. An adequate number of extra animals will be purchased so that no animal in obviously poor health is placed on test. At the request of the Study Director, excess animals may be used as replacement animals. Animals will be examined by the Staff Veterinarian prior to release of that shipment of animals for use on the study. During pretest the animals will be habituated to the collars used during the study at least three times increasing the minimum time period to at least six hours.

JUSTIFICATION FOR SPECIES SELECTION AND NUMBER OF ANIMALS

Rats are an animal model for acute toxicity studies of this type. The number of animals assigned to this study represents the minimum required to meet the objective(s) of the study and the OECD guidelines (#402).

5. *EXPERIMENTAL METHODOLOGY*

STUDY DESIGN

Group Number	Group Designation	Dosage Level (mg/kg)	Number of Animals	
			Males	Females
1	High-dose	2000	5	5
2	Low-dose	500	5	5
3	Mid-dose	1000	5	5

ASSIGNMENT TO GROUPS

For the first phase (Group 1), no random specific procedure will be used. If it is necessary to treat Groups 2 and 3, animals will be randomly assigned to groups by a computerized weight-ordered distribution such that individual body weights will not exceed ±20% of the mean weight for each sex. Only naïve animals will be used.

PREPARATION OF THE DOSE SITE

Hair will be removed from the dorsal area of the trunk of the test animals by clipping at least one hour prior to test material administration and as needed thereafter. Care will be taken to avoid abrading the skin, which could alter its permeability. The dose site and an area of adjacent untreated skin will be marked at the corners with an indelible marker at the time of dosing. The location of the test and control sites will be documented in the raw data.

FREQUENCY AND DURATION OF ADMINISTRATION

A single dosage level of 2000 mg/kg of test material will be applied to Group 1 animals on Study Day 1. The test material will be wetted to a paste with medium viscosity carboxymethylcellulose and applied uniformly over an area that is approximately 10% of the body surface area. Test material will be held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour period. The test material will be further covered in a suitable manner to ensure that the animals cannot ingest the test material. Elizabethan collars will be applied and worn during the exposure period. After the 24-hour exposure period the test material will be removed with warm water and gauze, and

the animals observed at least 14 days. If deaths occur, then a full study will follow and consist of applying 1000 and 500 mg/kg of test material with a 14-day recovery. If no deaths occur, the study will be terminated. Individual doses will be calculated using the body weights collected on the day of dosing.

JUSTIFICATION FOR ROUTE OF ADMINISTRATION AND DOSE LEVEL

In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is useful where exposure by the dermal route is likely. Dose levels were chosen by the OECD guideline #402.

ANTEMORTEM OBSERVATIONS

CLINICAL OBSERVATIONS

Observations will be performed and recorded twice daily (a.m. and p.m.) for moribundity and mortality.

Clinical observations will be recorded at least once each day and when a change is noted. Observations will be recorded predose and approximately hourly for four hours postdose on the first day of dosing then daily thereafter for at least 14 days. Observations should include changes in the skin and fur, eyes, and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity, and behavior pattern. Particular attention should be directed to observation of tremors, convulsions, salivation, diarrhea (liquid feces), lethargy, and coma.

BODY WEIGHTS

Body weights will be recorded pretest, on Study Day 1, approximately weekly, and at termination.

POSTMORTEM OBSERVATIONS

MORIBUND ANIMALS AND ANIMALS FOUND DEAD

Animals unlikely to survive until the next scheduled observation will be weighed, euthanized, and necropsied. Animals found dead will be weighed and necropsied. In either case, a complete necropsy will be performed as detailed below. Euthanasia of moribund animals will be authorized by the Study Director or Staff Veterinarian with the concurrence of the Study Director.

EUTHANASIA

All animals surviving to the end of the study will be humanely euthanized via carbon dioxide asphyxiation. Euthanasia will be performed in accordance with accepted American Veterinary Medical Association (AVMA) guidelines [J. Amer. Vet. Med. Assoc., 202:229-249, 1993].

NECROPSY

On Study Day 15, the animals (non-fasted) will be sacrificed rotating sequentially through groups for each sex. All animals that die during the study, or are killed at study termination, will be subjected to a complete necropsy examination. A complete necropsy is defined as examination of the external surface of the body, all orifices, and the cranial, thoracic, and

abdominal cavities and their contents. Gross lesions will be preserved in a suitable fixative for possible histopathology; all other tissues will be discarded. Histopathology will be provided at additional expense to the Sponsor.

6. ***STATISTICAL ANALYSIS***

Means and standard deviations will be calculated for all quantitative data.

7. ***REPORT***

An audited draft report will be sent to the Sponsor. Revisions to the initial draft report will be provided to the Sponsor by mail or fax transmission as printed copies of the corrected pages only. Additional revisions or complete copies of the revised draft reports will be provided at additional expense to the Sponsor.

Three copies (one bound, two unbound) of the final report will be submitted to the Sponsor approximately two weeks after approval of the draft report. The final report will include all elements required/recommended by the regulations and guidelines.

The report will include, but is not limited to, those items listed below:

- Descriptive text of the study objective
- Summary
- Test material identification (Certificate of Analysis, stability data, and analytical analysis if any are performed)
- Methods
- Results and conclusions
- Body weights
- Body weight changes
- Necropsy report
- A copy of the protocol, all protocol amendments, and all protocol deviations that may affect the integrity of the study

8. ***MAINTENANCE OF RAW DATA AND RECORDS***

Original data or copies thereof, will be available at Primedica Redfield to facilitate auditing the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data and the original final report will be retained in the archives of the laboratory for at least five years. Wet tissues, slides, and blocks (if generated) will be retained at Primedica Redfield for one year. After one year the storage of the wet tissues, slides, and blocks will be negotiated with the Sponsor. After five years the storage of the paper data will be negotiated with the Sponsor. The Sponsor will be notified prior to disposal of any original study data.

9. QUALITY ASSURANCE REVIEW

This is to certify that this protocol has been reviewed by Quality Assurance.

Val Gartner 7/26/00
Val Gartner, B.A. Date
Quality Assurance Auditor
Primedica Redfield

10. APPROVALS

7/31/00
Ph.D., DABT, CIH Date
Senior Laboratory Manager

7/26/00
John Seng, Ph.D. Date
Study Director
Primedica Redfield

STUDY NUMBER: 132-010

AMENDMENT: 1

STUDY TITLE: ACUTE DERMAL TOXICITY STUDY OF T-7485 APPLIED TO SPRAGUE-DAWLEY RATS

DATE ISSUED: October 20, 2000

AMENDMENT # 1

***ITEM 1*:** **Page 6, Section 7, Report**

CHANGED FROM: Three copies (one bound, two unbound) of the final report will be submitted to the Sponsor approximately two weeks after approval of the draft report.

CHANGED TO: Three copies (two bound, one unbound) of the approved final report will be submitted to the Sponsor approximately two weeks after approval of the draft report.

REASON: Sponsor request.

APPROVALS:

10/24/00

Ph.D., DABT, CIH Date
Senior Laboratory Manager
3M Corporate Toxicology

10/20/00

John Seng, Ph.D. Date
Study Director
Primedica Redfield

APPENDIX 6
PROTOCOL DEVIATIONS

PROTOCOL DEVIATIONS

There were no protocol deviations that adversely affected the integrity of the study.

APPENDIX 7
KEY PERSONNEL

KEY PERSONNEL

Study Number: 132-010

Study Director:	John Seng, Ph.D.
Study Coordinator:	Karen Tranter, B.A., LATG
Head Technician:	Martha Downing, B.S.
Veterinarian:	Allan Manus, D.V.M., M.Sc., ACLAM
Quality Assurance:	Val Gartner, B.A.
Report Coordination:	Amy Babb, B.S.
Report Preparation:	Tammy Peckham, Ad. Asst. Tammie Hughey, Ad. Asst.