1) OECD 423-OPPTS 870.1100,
Acute oral toxicity (rat), 132-002

# ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS

STUDY NUMBER: 132-002



## SPONSOR

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

## SPONSOR'S REPRESENTATIVE

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## 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: May 22, 2000
Animal Phase Initiation: May 25, 2000
Animal Phase Completion: June 8, 2000
Study Completion: October 10, 2000

## GLP COMPLIANCE STATEMENT

Study Number: 132-002

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.

John S Seng 10/10/00

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

## QUALITY ASSURANCE STATEMENT

Study Number: 132-002

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 |
|---|---|---|
| 05/19/00 | Protocol | 05/19/00 |
| 05/22/00 | Randomization | 05/22/00 |
| 05/25/00 | Dose Administration | 05/25/00 |
| 06/02/00 | Amendment #1 | 06/02/00 |
| 06/21/00 | Formulations Raw Data, Individual Animal Necropsy Records, Histopathology, and Gross Pathology | 06/29/00 |
| 06/28/00 | Raw Data | 06/29/00 |
| 06/28/00-06/29/00 | Tables | 06/29/00 |
| 07/11/00 | Draft Report | 07/11/00 |
| 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 ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS

**APPROVED BY:**

John Seng 10/10/00

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

## TABLE OF CONTENTS

## ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS

### STUDY ABSTRACT

The objective of this study was to determine the acute effect(s) of a single oral gavage administration of test material to Sprague-Dawley rats with a fourteen-day recovery.

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 nine weeks old. The males weighed 257 to 280 grams and the females weighed 170 to 194 grams.

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. Fifteen males and fifteen females were randomly assigned to groups such that individual body weights did not exceed ±20% of the mean weight for each sex. The study design was as follows:

| Group Number | Group Designation | Dosage Level (mg/kg) | Dosage Concentration (mg/mL) | Dosage Volume (mL/kg) | Number of Animals | |
|---|---|---|---|---|---|---|
| | | | | | Males | Females |
| 1 | High-dose | 2000 | 200 | 10 | 5 | 5 |
| 2 | Low-dose | 500 | 50 | 10 | 5 | 5 |
| 3 | Mid-dose | 1000 | 100 | 10 | 5 | 5 |

A single dose was administered via oral gavage to fasted animals on Study Day 1. The dose volume was 10 mL/kg. Individual dose volumes were calculated using fasted body weights recorded on Study Day 1. The animals in Group 1 were treated with 2000 mg/kg of test material on Study Day 1 and then held for a 14-day recovery period. Because no deaths occurred, the study was terminated. Groups 2 and 3 were not dosed.

Observations for mortality and moribundity were recorded twice daily (a.m. and p.m.) with no incidence of either occurring throughout the study. Clinical observations were recorded predose and approximately hourly for four hours postdose on the day of dosing then daily thereafter for at least 14 days. Clinical observations included male localized alopecia, urine-stained abdominal fur, and one female had liquid feces at three and four hours postdose. The listed clinical observations did not appear to be related to the test material given that the three observations were unrelated and each occurred in different animals.

Body weights were recorded pretest and on Study Days 1, 8, and 15. Between Study Days 1 and 15, mean body weight increased in males (114.0 ± 15.9 grams) and females (49.4 ± 17.4 grams). During the study, there was no record of body weight loss for either males or females suggesting no adverse effect of a single dose of 2000 mg/kg T-7485 on body weights or body weight changes.

On Study Day 15, all animals (non-fasted) were humanely euthanized via carbon dioxide asphyxiation followed by exsanguination and submitted for a complete necropsy examination. Necropsy examination failed to reveal any adverse findings with the exception of a single male and a single female with dilatation of the right kidney. Dilatation of the kidney is usually induced by hydronephritis of the kidney and this condition is not uncommon in rats. Therefore, these findings are not considered related to the test material.

In conclusion, a single oral (gavage) administration of 2000 mg/kg T-7485 failed to induce adverse clinical observations, mortality, moribundity, changes in body weights or body weight changes, or gross findings in rats.

## OBJECTIVE

The objective of this study was to determine the acute effect(s) of a single oral gavage administration of test material 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 on the day of use. The vehicle was prepared to a concentration of 1% by mixing 10 grams of powdered medium viscosity carboxymethylcellulose with deionized water using a Waring blender. Additional deionized water was added to yield 1000 mL of prepared vehicle. The vehicle was stored refrigerated when not in use. The test material was dissolved in the prepared vehicle to the appropriate concentration and stirred on a magnetic stir plate.

A 2 mL sample of the formulated test material was retained and frozen at approximately -20°C.

**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 nine weeks old. The males weighed 257 to 280 grams and the females weighed 170 to 194 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. Fifteen males and fifteen females were randomly assigned to groups such that individual body weights did not exceed ±20% of the mean weight for each sex.

The study design was as follows:

| Group Number | Group Designation | Dosage Level (mg/kg) | Dosage Concentration (mg/mL) | Dosage Volume (mL/kg) | Number of Animals | |
|---|---|---|---|---|---|---|
| | | | | | Males | Females |
| 1 | High-dose | 2000 | 200 | 10 | 5 | 5 |
| 2 | Low-dose | 500 | 50 | 10 | 5 | 5 |
| 3 | Mid-dose | 1000 | 100 | 10 | 5 | 5 |

A single dose was administered via oral gavage to fasted animals on Study Day 1. The dose volume was 10 mL/kg. Individual dose volumes were calculated using fasted body weights recorded on Study Day 1. The animals in Group 1 were treated with 2000 mg/kg of test material on Study Day 1 and then held for a 14-day recovery period. Because no deaths occurred, the study was terminated. 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 (#401). In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. Dose levels were chosen in accordance with OECD guideline #401.

**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 the day of dosing then 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:** On Study Day 15, all animals (non-fasted) were humanely euthanized via carbon dioxide asphyxiation followed by exsanguination 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:** Observations for mortality and moribundity were recorded twice daily (a.m. and p.m.) with no incidence of either occurring throughout the study. Clinical observations were recorded predose and approximately hourly for four hours postdose on the day of dosing then daily thereafter for at least 14 days. Clinical observations included male localized alopecia, urine-stained abdominal fur, and one female had liquid feces at three and four hours postdose. The listed clinical observations did not appear to be related to the test material given that the three observations were unrelated and each occurred in different animals. The summary of clinical observations is in Table 1. The individual clinical observations are in Appendix 2.

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

Between Study Days 1 and 15, mean body weight increased in males (114.0 ± 15.9 grams) and females (49.4 ± 17.4 grams). During the study, there was no record of body weight loss for either males or females suggesting no adverse effect of a single dose of 2000 mg/kg T-7485 on body weights or body weight changes.

**NECROPSY:** On Study Day 15, all animals (non-fasted) were humanely euthanized via carbon dioxide asphyxiation followed by exsanguination and submitted for a complete necropsy examination. The individual necropsy observations are located in Appendix 1.

Necropsy examination failed to reveal any adverse findings with the exception of a single male and a single female with dilatation of the right kidney. Dilatation of the kidney is usually induced by hydronephritis of the kidney and this condition is not uncommon in rats. Therefore, these findings are not considered related to the test material.

## CONCLUSION

In conclusion, a single oral (gavage) administration of 2000 mg/kg T-7485 failed to induce adverse clinical observations, mortality, moribundity, body weights or body weight changes, or gross findings in rats.

## TABLES

## TABLE 1
## SUMMARY OF CLINICAL OBSERVATIONS

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 1: CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

| DOSE GROUP<br>(MG/KG) | 1<br>2000 |
|---|---|
| MORTALITY | 0 |
| LOCALIZED ALOPECIA, LIMBS | 11/ 1 |
| URINE-STAINED ABDOMINAL FUR, 4 HOURS POSTDOSE | 1/ 1 |

N/N = NUMBER OF OBSERVATIONS/NUMBER OF ANIMALS

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 1: CLINICAL OBSERVATIONS - SUMMARY - FEMALE RATS

| DOSE GROUP (MG/KG) | 1 2000 |
|---|---|
| MORTALITY | 0 |
| LIQUID FECES, 3 HOURS POSTDOSE | 1/ 1 |
| LIQUID FECES, 4 HOURS POSTDOSE | 1/ 1 |

N/N = NUMBER OF OBSERVATIONS/NUMBER OF ANIMALS

## TABLE 2
## SUMMARY OF BODY WEIGHTS

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 2: BODY WEIGHTS - SUMMARY - MALE RATS

| DOSE GROUP (MG/KG) | | | 1 2000 |
|---|---|---|---|
| RATS - TESTED | | | 5 |
| BODY WEIGHT (G) | | | |
| SD 1 | | MEAN±S.D. | 271.6 ± 8.7 |
| SD 8 | | MEAN±S.D. | 341.8 ± 19.9 |
| SD 15 | | MEAN±S.D. | 385.6 ± 22.4 |

SD = STUDY DAY

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 2: BODY WEIGHTS - SUMMARY - FEMALE RATS

| DOSE GROUP (MG/KG) | | | 1 2000 |
|---|---|---|---|
| RATS - TESTED | | | 5 |
| BODY WEIGHT (G) | | | |
| SD 1 | | MEAN±S.D. | 183.6 ± 8.7 |
| SD 8 | | MEAN±S.D. | 215.8 ± 14.0 |
| SD 15 | | MEAN±S.D. | 233.0 ± 21.4 |

SD = STUDY DAY

## TABLE 3
## SUMMARY OF BODY WEIGHT CHANGES

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 3: BODY WEIGHT CHANGES - SUMMARY - MALE RATS

| DOSE GROUP | | 1 |
|---|---|---|
| (MG/KG) | | 2000 |
| RATS - TESTED | | 5 |
| BODY WEIGHT CHANGES (G) | | |
| SD 1 - 8 | MEAN±S.D. | +70.2 ± 13.7 |
| SD 8 - 15 | MEAN±S.D. | +43.8 ± 4.2 |
| SD 1 - 15 | MEAN±S.D. | +114.0 ± 15.9 |

SD = STUDY DAY

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

TABLE 3: BODY WEIGHT CHANGES - SUMMARY - FEMALE RATS

| DOSE GROUP (MG/KG) | | 1 2000 |
|---|---|---|
| RATS - TESTED | | 5 |
| BODY WEIGHT CHANGE (G) | | |
| SD 1 - 8 | MEAN±S.D. | +32.2 ± 6.6 |
| SD 8 - 15 | MEAN±S.D. | +17.2 ± 12.1 |
| SD 1 - 15 | MEAN±S.D. | +49.4 ± 17.4 |

SD = STUDY DAY

# APPENDICES

# APPENDIX 1
# INDIVIDUAL NECROPSY OBSERVATIONS

## APPENDIX 1
## INDIVIDUAL NECROPSY OBSERVATIONS

### GROUP 1 - 2000 MG/KG

| | | | | |
|---|---|---|---|---|
| Animal #6209 | - | Male | - | No adverse findings. |
| Animal #6211 | - | Male | - | No adverse findings. |
| Animal #6213 | - | Male | - | No adverse findings. |
| Animal #6215 | - | Male | - | No adverse findings. |
| Animal #6217 | - | Male | - | Kidneys, Dilatation, Right, Pelvis. |
| Animal #6210 | - | Female | - | No adverse findings. |
| Animal #6212 | - | Female | - | No adverse findings. |
| Animal #6214 | - | Female | - | No adverse findings. |
| Animal #6216 | - | Female | - | No adverse findings. |
| Animal #6218 | - | Female | - | Kidneys, Dilatation, Right, Pelvis. |

# APPENDIX 2
# INDIVIDUAL CLINICAL OBSERVATIONS

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 2: TIMED CLINICAL OBSERVATIONS - INDIVIDUAL - MALE RATS

| RATS # | PREDOSE | APPROXIMATELY 1 HOUR POSTDOSE | APPROXIMATELY 2 HOURS POSTDOSE | APPROXIMATELY 3 HOURS POSTDOSE | APPROXIMATELY 4 HOURS POSTDOSE |
|---|---|---|---|---|---|
| STUDY DAY 1 DOSE GROUP 1 2000 MG/KG | | | | | |
| 6209 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6211 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | URINE-STAINED ABDOMINAL FUR |
| 6213 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6215 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6217 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS - INDIVIDUAL - MALE RATS

| DOSE GROUP 1 | | 2000 MG/KG |
|---|---|---|
| RAT # | | DESCRIPTION |
| 6209 | | NO ADVERSE FINDINGS |
| 6211 | | NO ADVERSE FINDINGS |
| 6213 | | NO ADVERSE FINDINGS |
| 6215 | SD( 4 ) | LOCALIZED ALOPECIA, LIMBS |
| | SD( 6- 15) | LOCALIZED ALOPECIA, LIMBS |
| 6217 | | NO ADVERSE FINDINGS |

SD = STUDY DAY
Animals with NO ADVERSE FINDINGS were normal until their scheduled sacrifice.

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 2: TIMED CLINICAL OBSERVATIONS - INDIVIDUAL - FEMALE RATS

| RATS # | PREDOSE | APPROXIMATELY 1 HOUR POSTDOSE | APPROXIMATELY 2 HOURS POSTDOSE | APPROXIMATELY 3 HOURS POSTDOSE | APPROXIMATELY 4 HOURS POSTDOSE |
|---|---|---|---|---|---|
| STUDY DAY 1 DOSE GROUP 1 2000 MG/KG | | | | | |
| 6210 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6212 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6214 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6216 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL |
| 6218 | APPEARS NORMAL | APPEARS NORMAL | APPEARS NORMAL | LIQUID FECES | LIQUID FECES |

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 2: CLINICAL OBSERVATIONS - INDIVIDUAL - FEMALE RATS

| DOSE GROUP 1 | 2000 MG/KG |
|---|---|
| RAT # | DESCRIPTION |
| 6210 | NO ADVERSE FINDINGS |
| 6212 | NO ADVERSE FINDINGS |
| 6214 | NO ADVERSE FINDINGS |
| 6216 | NO ADVERSE FINDINGS |
| 6218 | NO ADVERSE FINDINGS |

Animals with NO ADVERSE FINDINGS were normal until their scheduled sacrifice.

## APPENDIX 3
## INDIVIDUAL BODY WEIGHTS

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 3: BODY WEIGHTS - INDIVIDUAL - MALE RATS

| SD | 1 | 8 | 15 |
|---|---|---|---|
| RAT | DOSE GROUP 1 | 2000 MG/KG | |
| 6209 | 257. | 313. | 352. |
| 6211 | 280. | 346. | 390. |
| 6213 | 272. | 341. | 391. |
| 6215 | 273. | 340. | 381. |
| 6217 | 276. | 369. | 414. |

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

PROTOCOL 132-002: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE DAWLEY RATS

APPENDIX 3: BODY WEIGHTS - INDIVIDUAL - FEMALE RATS

| SD | 1 | 8 | 15 |
|---|---|---|---|
| RAT | DOSE GROUP 1 | 2000 MG/KG | |
| 6210 | 170. | 199. | 225. |
| 6212 | 187. | 224. | 241. |
| 6214 | 184. | 209. | 208. |
| 6216 | 183. | 212. | 226. |
| 6218 | 194. | 235. | 265. |

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 AND AMENDMENT

## Primedica Redfield Protocol

*Protocol Number: 132-002*

**ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS**

**PROTOCOL NO.: 132-002**

**OBJECTIVE:** Determine the acute effect(s) of a single oral gavage administration of test material to Sprague-Dawley rats with a fourteen-day recovery.

**LOCATION OF STUDY AND CONDITIONS OF TESTING:**

*It is the intention of 3M Pharmaceuticals, 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 Pharmaceuticals followed by written verification of the change. 3M Pharmaceuticals 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 Pharmaceuticals promptly by telephone prior to release of any data for review 5/26/2000

**SPONSOR:**
3M ~~Pharmaceuticals~~ Corporate Toxicology
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**SPONSOR'S REPRESENTATIVE:**
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**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
Head Technician: Jennifer Dedman, B.S.
Pathologist: TBD

**PROPOSED STUDY DATES:**
Experimental Start Date: May 25, 2000
Experimental Termination Date: June 8, 2000
Draft Report Date: July 14, 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 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 prepared 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 test material container(s) will be inventoried when received at the testing laboratory, and a record of all test material use will be maintained. The technician dosing the animals will: 1) Record the weight or volume of the test material container immediately before and after dosing; 2) Compute and record the amount used and compare it to the theoretical amount immediately after dosing; 3) Keep the formulated test material use log in the animal study books at all times; 4) Assure the dose used is within ±10% of theoretical.

*ANALYTICAL SAMPLES*

Samples of the formulated test materials will be retained and frozen at approximately -20°C for possible analysis. If no analysis is performed, the samples will be discarded upon finalization of the study report, or as authorized by the Sponsor.

*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) BR VAF®/Plus out-bred 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.

*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 (#401).

5. ***EXPERIMENTAL METHODOLOGY***

*STUDY DESIGN*

| Group Number | Group Designation | Dosage Level (mg/kg) | Dosage Concentration (mg/mL) | Dosage Volume (mL/kg) | Number of Animals | |
|---|---|---|---|---|---|---|
| | | | | | Males | Females |
| 1 | High-dose | 2000 | 200 | 10 | 5 | 5 |
| 2 | Low-dose | 500 | 50 | 10 | 5 | 5 |
| 3 | Mid-dose | 1000 | 100 | 10 | 5 | 5 |

*ASSIGNMENT TO GROUPS*

Animals will be randomly assigned to groups by a weight ordered distribution such that individual body weights will not exceed ±20% of mean weight for each sex.

*FREQUENCY AND DURATION OF ADMINISTRATION*

A single dose will be administered via oral gavage to fasted animals (feed withheld over-night). The dose volume will be 10 mL/kg. The first day of dosing on the study will be Study Day 1. The animals in Group 1 will be treated with 2000 mg/kg of test material on Study Day 1 with a 14-day recovery period. If deaths occur, then rats will be dosed at 1000 and 500 mg/kg of test material with a 14-day recovery. If no deaths occur, the study will be terminated. Individual dose volumes will be calculated using the fasted 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 oral toxicity is usually an initial step. Dose levels were chosen by the OECD guideline #401.

*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. Observations will be recorded predose and approximately hourly for four hours postdose on the 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, and 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*

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*

Starting on Study Day 15, the animals 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 approved 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.**

Fran Pattillo 5-22-00

Fran Pattillo, M.S., RQAP-GLP Date
Manager of Quality Assurance
Primedica Redfield

***10. APPROVALS***

May 26, 2000

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

John Seng 5/22/00

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

## Primedica Redfield Protocol Amendment

*Protocol Number: 132-002*

STUDY NUMBER: 132-002

AMENDMENT: 1

STUDY TITLE: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS

DATE ISSUED: June 9, 2000

**AMENDMENT # 1**

***ITEM 1:*** **Protocol**

CHANGED FROM: 3M Pharmaceuticals

CHANGED TO: 3M Corporate Toxicology

REASON: Sponsor requested clarification.

***ITEM 2:*** **Page 1, SPONSOR'S REPRESENTATIVE**

CHANGED FROM: Telephone:

CHANGED TO: Telephone:

REASON: Correction of telephone number.

***ITEM 3:*** **Page 6, Section 5, Necropsy**

CHANGED FROM: Starting on Study Day 15, the animals will be sacrificed rotating sequentially through groups for each sex.

CHANGED TO: Starting on Study Day 15, the animals (non-fasted) will be sacrificed rotating sequentially through groups for each sex.

REASON: Fasting of the animals will not be necessary.

**APPROVALS:**

6/14/00

Ph.D., DABT, CIH Date

Senior Laboratory Manager
3M Corporate Toxicology

6/9/00

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

1

7.7485.3

# Primedica Redfield Protocol Amendment

*Protocol Number: 132-002*

STUDY NUMBER: 132-002

AMENDMENT: 2

STUDY TITLE: ACUTE ORAL TOXICITY STUDY OF T-7485 ADMINISTERED TO SPRAGUE-DAWLEY RATS

DATE ISSUED: October 18, 2000

**AMENDMENT # 2**

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

CHANGED FROM: Three copies (one bound, two unbound) of the approved 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

10/18/00

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: 132-002

| | |
|---|---|
| Study Director: | John Seng, Ph.D. |
| Study Coordinator: | Karen Tranter, B.A., LATG |
| Head Technician: | Jennifer Dedman, 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.<br>Tammie Hughey, Ad. Asst. |