01/07/99
T-7098.1
POSF PK

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-7098.1
PHARMACOKINETIC STUDY OF POSF IN RATS

OR-4

***Study Objective*:**
The objective of this study is to assess the potential for oral absorption, urinary and fecal clearance and biological persistence of perfluorooctane sulfonyl fluoride (POSF) in male Sprague Dawley rats after a single oral dose. The POSF compound is the starting material for the synthesis of a wide variety of perfluorooctane sulfonate (PFOS) based materials. The purpose of this study is to understand the rate of metabolism of POSF to PFOS by the liver. This study will provide data for proper risk characterization of POSF.

Research Client: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55133-3220

Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55133-3220

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55133-3220

Study Director: Andrew M. Seacat, Ph.D.
Sr. Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph.: 651-575-3161 FAX: 651-733-1773

***Study Toxicologist*:** Deanna Nabbefeld, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55133-3220
Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline (Assuming EHS&R approval on Jan. 5th.)*:*
In-Life Start Date: January 11th, 1999
In-Life End Date: February 9th, 1999
Analytical Completion Date: March 22nd, 1999
Final Report Completion Date: April 19th, 1999

Regulatory Compliance:
This study will be performed in the 3M Strategic Toxicology Laboratory under a defined protocol and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure.

Test Material:
Dan Hakes, Product Responsibility Liaison 3M Chemicals Division, will furnish high-purity POSF.

Identification:

Name: Perfluorooctane Sulfonyl Fluoride
Molecular Formula: To be provided.
Lot Number: The lot numbers will be maintained in the raw data.

Purity:

Documentation will be kept in on file.

Stability:

Documentation will be kept on file.

Storage Conditions:

Upon receipt, test material will be stored tightly sealed at room temperature.

Characteristics:

Information on synthesis methods, composition or other characteristics that define the test material will be kept on file.

Animals:

Species: Rat
Strain: Sprague Dawley
Source: Harlan
Age at initiation of treatment: 6-8 weeks
Weight at initiation of treatment: approximately 150-250g
Number and sex: 30 males

Table 1 - Dose Groups

Group	Dose	N	Euthanasia
*1	0 mg/kg	5	day 1 post dose
*2	0 mg/kg	5	day 4 post dose
*3	0 mg/kg	5	day 29 post dose
4	5 mg/kg	5	day 1 post dose
5	5 mg/kg	5	day 4 post dose
6	5 mg/kg	5	day 29 post dose

** Rats in groups 1-3 will be used concomitantly as control animals in studies T-7071.2, Pharmacokinetic Study of M556 in Rats, and T-7099.1, Pharmacokinetic Study of FX-845 in Rats.*

Identification: ear tag with animal number or unique tail mark.
AUA Number: 2154

004424

Husbandry:

Housing:

Three specific rats from groups 3 and 6 will be housed individually in metabolism cages for portions of the study (see Table 2). When not in metabolism cages, these rats will be group housed in standard cages. All other rats will be group housed in standard cages throughout the study.

Diet/Water:

Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet, supplied by Harlan Teklad, Madison, WI, and tap water will be provided to all rats *ad libitum* throughout the study.

Environment:

Environmental controls for the animal room will be set to maintain a temperature of 72 ± 3°F, humidity of 30-70%, a minimum of 10 exchanges of room air per hour and a 12 hour light/dark cycle.

Dose and Dosing Procedures:

Method of administration/Dose preparation:

A single 5mg/kg dose of POSF will be administered via oral gavage to rats in groups 4-6 on day zero of the study. The POSF will be prepared as a 1% (1 mg/ml) uniform suspension in 2% Tween 80 using a 15 ml tissue grinder. A volume of 5 ml suspension / kg body weight will be administered to each rat. Re-suspension of solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing.

A single 5 ml / kg body weight dose of 2% Tween 80 will be administered via oral gavage to rats in groups 1-3 on day zero of the study.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily for mortality and morbidity and notable findings will be recorded. Additional findings will be recorded as they are observed.

Body Weights:

Each animal will be weighed immediately prior to dosing, weekly thereafter and immediately prior to euthanasia.

Specimen Collection:

Frequency (see Table 2):

Urine and feces collections will be made on days 1, 2, 4, 14 and 29 post dose. Necropsies will be performed on days 1, 4 and 29 post dose.

Table 2 - Schedule

Jan 10	**Jan 11** **day 0** DOSING	**Jan 12** **day 1 PD** Collection Dy 1 PD sac	**Jan 13** **day 2 PD** Collection Switch to reg cages.	**Jan 14** **day 3 PD** Switch to met cages.	**Jan 15** **day 4 PD** Collection Switch to reg cages. Dy 4 PD sac.	**Jan 16** **day 5 PD**
Jan 17 **day 6 PD**	**Jan 18** **day 7 PD**	**Jan 19** **day 8 PD**	**Jan 20** **day 9 PD**	**Jan 21** **day 10 PD**	**Jan 22** **day 11 PD**	**Jan 23** **day 12 PD**
Jan 24 **day 13 PD**	**Jan 25** **day 14 PD** Switch to met cages.	**Jan 26** **day 15 PD** Collection Switch to reg. cages.	**Jan 27** **day 16 PD**	**Jan 28** **day 17 PD**	**Jan 29** **day 18 PD**	**Jan 30** **day 19 PD**
Jan 31 **day 20 PD**	**Feb 1** **day 21 PD**	**Feb 2** **day 22 PD**	**Feb 3** **day 23 PD**	**Feb 4** **day 24 PD**	**Feb 5** **day 25 PD**	**Feb 6** **day 26 PD**
Feb 7 **day 27 PD**	**Feb 8** **day 28 PD** switch to met cages	**Feb 9** **day 29 PD** collection Dy 29 PD sac				

Method of Specimen Collection:

Urine and feces will be collected from each metabolism cage at the designated times. The initial volume of urine will be recorded, the sides of the urine collection apparatus will be washed with 10-20 ml deionized water and the final volume of urine will be brought to 45 ml with additional deionized water. Daily feces weight will be recorded for each animal. At the designated times, animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, blood (≈ 6 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. Blood samples will be allowed to clot for a period of 15 to 30 minutes at room temperature, and the clot will be spun down in a centrifuge at 1100 x g for 5 minutes. The serum will be transferred to labeled 1.5 ml microfuge tubes and centrifuged again at 2000 x g to remove any remaining red blood cells. Each sera sample will then be transferred to a separate labeled polypropylene microfuge tube and flash-frozen in liquid nitrogen. Liver, kidneys and subcutaneous fat from each animal will be removed, weighed, flash frozen in liquid nitrogen and placed individually into labeled sterile sample bags. The remainder of each carcass will be placed in a labeled ziplock bag and frozen (-70 °C) until analysis.

Specimen Handling:

Specimens will temporarily be stored in a freezer set to maintain -60 to -80°C. For metabolite analysis, these specimens will be packed in dry ice and shipped to:

Kris Hansen, Ph.D.
3M Environmental Technology and Safety Services
935 Bush Avenue
St. Paul, MN 55133-3331
Ph: 612-778-6081, FAX: 612-778-6176.

The 3M Environmental Laboratory will manage the extraction and analysis of sera, liver, urine and feces for the parent compound, POSF, and its presumed metabolite, PFOS. All tissue samples will be retained for possible future analysis of total organic fluorine (TOF) if deemed necessary by the Study Director. 3M Environmental Laboratory or its designee would perform this analysis. All results will be provided for inclusion in the final report.

The number, type and date of collection of specimens to be generated for analysis are as follows:

Table 3 - Specimens

Specimens	day 1 post dose	day 2 post dose	day 4 post dose	day 15 post dose	day 29 post dose	Total
Serum	10		10		10	30
Liver	10		10		10	30
Kidneys	10		10		10	30
Subcutaneous fat	10		10		10	30
Carcass	10		10		10	30
Urine	6	6	6	6	6	30
Feces	6	6	6	6	6	30

Data Analysis:
Data collected on tissue levels of parent compound and identifiable metabolites will be analyzed for toxicokinetic parameters and for statistically significant differences between groups using Students T-test and/or ANOVA.

Responsibilities:
- Deanna Nabbefeld and Andrew Seacat will be responsible for dosing the animals, collecting in-life specimens, performing the necropsy and collecting and sending tissue samples for analysis.
- Kris Hansen, 3M Environmental, will be responsible for analytical analysis.
- Andrew Seacat will draft a final report and ensure the report receives appropriate 3M review before a final report is issued.

Signatures:

Andrew Seacat 1/8/99

Dr. Andrew Seacat — Date
Senior Research Toxicologist
Study Director

Deanna Nabbefeld 1/8/99

Deanna Nabbefeld, MS — Date
Advanced Toxicologist
Study Toxicologist

Sponsor Representative — Date

004428