

PHARMACOKINETIC STUDY OF POSF IN RATS – STUDY OUTLINE

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.

GLP Status: 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 Animals: Male, Sprague-Dawley rats, 150-250 g, obtained from Harlan.

Dose Groups: 0 and 5 mg/kg according to the following schedule:

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

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

Compound Administration: Oral gavage.

Clinical Observations: Once daily.

Sample Collection: Urine and feces will be collected three specific rats from groups 3 and 6 on days 1, 2, 4, 15 and 29-post dose. These animals will be placed in metabolism cages for 24 hours for each for each collection. Necropsies will occur on days 1, 4 and 29 post-dose. Liver, kidneys, sera, subcutaneous fat and whole carcass collection will occur at necropsy.

Sample Analysis: 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). 3M Environmental Laboratory or its designee would perform such an analysis if deemed necessary by the Study Director.

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.

Estimated Timeline (Assuming EHS&R approval on Jan. 5th):

In-Life Start: January 11th, 1999

In-Life End: February 9th, 1999

Analytical Completion: March 22nd, 1999

Final Report: April 19th, 1999

Study Director: Andrew M Seacat Ph.D.

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

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
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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

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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

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 $72 \pm 3^{\circ}\text{F}$, 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 CO₂ 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

<u>Specimens</u>	day 1 post dose	day 2 post dose	day 4 post dose	day 15 post dose	day 29 post dose	<u>Total</u>
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:

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna Nabbefeld, MS
Advanced Toxicologist
Study Toxicologist

Date

Sponsor Representative

Date