

3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-7132.2; ST-39
PHARMACOKINETIC STUDY OF PERFLUOROOCTANE SULFONAMIDE
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 Sulfonamide (PFOSA) in male Sprague Dawley rats after a single oral dose. Analysis of the serum, liver, urine and feces for potential metabolites of PFOSA will be performed by LCMS and perhaps other methods. Previous studies of the N-ethyl derivative of PFOSA have concluded that PFOSA is the ultimate metabolite in rats (1,2); however, the analytical technique used in these studies was unable to detect all potential metabolites, including perfluorooctanesulfonate (PFOS). No pharmacokinetic studies have yet been done on PFOSA to resolve this issue and search for other possible metabolites in the urine and feces. Recently, validated methods have been developed for the quantitation of PFOSA and its potential metabolite, PFOS, in serum and liver down to the low part per billion level (3). One goal of this study is to determine the potential for, if occurring, the extent of conversion of PFOSA to PFOS. This information will help to explain the pharmacokinetics of NETFOSE and its metabolites and provide data for proper risk characterization.

Research Client: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Sponsor: 3M Specialty Chemicals Division
3M Center, Building 236
Saint Paul, MN 55144

Study Location: 3M Strategic Toxicology Laboratory
3M Center, Building 270-3S-06 room SB314
Saint Paul, MN 55144

Study Director: Andrew M. Seacat, Ph.D.
Toxicology Specialist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144
Ph.: 651-575-3161 FAX: 651-733-1773

Study Toxicologist: Deanna Luebker, MS
Advanced Research Toxicologist
3M Medical Dept. / Corporate Toxicology
3M Center, Building 220-2E-02
Saint Paul, MN 55144

Ph: 651-737-1374 FAX: 651-733-1773

Proposed Study Timeline

In-Life Start Date: October 4th, 1999

In-Life End Date: November 2nd, 1999

Analytical Completion Date: TBA

Final Report Completion Date: TBA

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 PFOSA.

Identification:

Name: Perfluorooctanesulfonamide

Molecular Formula: C₈F₁₇SO₂NH₂

Lot Number: L-10009 (Prepared by George Moore, 3M SMD Lab, Bldg 236, April 1996)

Purity: Analysis by GCMS determined that the starting material was over 99% pure (4). Qualitative and quantitative compositional results that were derived from the single trial ¹H/¹⁹F-NMR cross-integration analysis revealed that the composition was 65.8% CF₃(CF₂)_x-SO₂-NH₂ (Normal chain), 18.7% CF₃(CF₂)_x-CF(CF₃)-(CF₂)_y-SO₂-NH₂ (Internal monomethyl branch), 11.2% (CF₃)₂CF-(CF₂)_x-SO₂-NH₂ (Isopropyl branch), 3.5% C_xF_{2x+1}-CF(CF₃)-SO₂-NH₂ (Alpha branch) and 0.28% (CF₃)₃C-(CF₂)_x-SO₂-NH₂ (t-Butyl branch)(5). HPLC/MS characterization of the PFOSA sample revealed 9,600 ppm of PFOS, 1,100 ppm of C₇F₁₅SO₂NH₂, 510 ppm of C₉F₁₉SO₂NH₂, 6,600 ppm of C₈F₁₆HSO₂NH₂, 24,000 ppm of C₁₈F₃₆HSO₂NH₂, 1,200 ppm of C₈F₁₅H₂SO₂NH₂ and lower concentrations of several other amides. Based on the sum of the impurities, the purity of the PFOSA sample would be approximately 96 % (6 & appendix I).

Stability:

Documentation will be kept on file with the Sponsor.

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 with the Sponsor.

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	15	5 each on days 1, 4 and 29 post dose
2	5 mg/kg	15	5 each on days 1, 4 and 29 post dose

Identification: ear tag with animal number or unique tail mark.
AUA Number: 2246

Husbandry:

Housing:

All rats from groups 1 and 2, which are to be sacrificed on day 29 post dose, 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 PFOSA will be administered via oral gavage to rats in group 2 on day zero of the study. The PFOSA will be prepared from a stock solution of 100mg/ml PFOSA in acetone. A final 0.1% (1mg/ml) uniform suspension (or emulsion) of PFOSA in 2% Tween 80 and 1% acetone will be prepared using a 15 ml dounce 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 vehicle, 2% Tween 80 and 1% acetone, will be administered via oral gavage to rats in group 1 on day zero of the study.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily (excluding weekends and holidays) 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 treatment, weekly thereafter and immediately prior to euthanasia.

Specimen Collection:

Frequency (See table 2):

Urine and feces collections will be made on days 1 - 4 post dose

Necropsies will be performed on days 1, 4 and 29 post dose.

Table 2 - Schedule

Sun	Mon	Tues	Wed	Thurs	Fri	Sat
Oct 3	Oct 4 day 0 DOSING	Oct 5 day 1 PD Collection, Dy 1 PD sac	Oct 6 day 2 PD Collection	Oct 7 day 3 PD Collection	Oct 8 day 4 PD Collection Switch to reg cages. Dy 4 PD sac.	Oct 9 day 5 PD
Oct 10 day 6 PD	Oct 11 day 7 PD	Oct 12 day 8 PD	Oct 13 day 9 PD	Oct 14 day 10 PD	Oct 15 day 11 PD	Oct 16 day 12 PD
Oct 17 day 13 PD	Oct 18 day 14 PD	Oct 19 day 15 PD	Oct 20 day 16 PD	Oct 21 day 17 PD	Oct 22 day 18 PD	Oct 23 day 19 PD
Oct 24 day 20 PD	Oct 25 day 21 PD	Oct 26 day 22 PD	Oct 27 day 23 PD	Oct 28 day 24 PD	Oct 29 day 25 PD	Oct 30 day 26 PD
Oct 31 day 27 PD	Nov 1 day 28 PD	Nov 2 day 29 PD 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 approximately 5-10ml deionized water and the final volume of urine will be brought to 15 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 frozen in dry ice. Livers will be removed, weighed, placed individually into labeled sterile sample bags and flash frozen in liquid nitrogen then maintained on dry ice.

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.

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>Specimen</u>	day 1 post dose	day 2 post dose	Day 3 post dose	day 4 post dose	day 29 post dose	<u>Total</u>
Serum (5/group/day)	10			10	10	30
Liver (5/group/day)	10			10	10	30
Urine (5/group/day)	10	10	10	10		40
Feces (5/group/day)	10	10	10	10		40

Data Analysis:

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

Responsibilities:

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

Signatures:

Andrew M. Seacat 10/27/99
Andrew M. Seacat Ph.D. Date
Toxicology Specialist
Study Director

Deanna Luebker 10/27/99
Deanna Luebker, MS Date
Advanced Research Toxicologist
Study Toxicologist

Daniel Halpern 11/1/99
Sponsor Representative Date

References:

1. Grossman M.R. and Bowen J.M. (1990) Tissue analysis of fluorinated sulfonamide pesticide: an evaluation of distribution, elimination, and potential for bioaccumulation in orally exposed rats. M.S. Thesis, Univ. of Georgia, Athens, GA. (also possibly published as: Grossman Mark R. and Bowen J.M. (1990) Tissue distribution and elimination of a fluorinated sulfonamide pesticide in rats. *Fundam. Appl. Toxicol.*, but not found).
2. Grossman M.R., Mispagel, M.E. and Bowen J.M. (1992) Distribution and tissue in rats during and after prolonged dietary exposure to a highly fluorinated sulfonamide pesticide. *J. Agric. Food Chem.* 40, 2505 – 2509.
3. K.J. Hansen, L.A. Clemen, M.E. Ellefson, H.O. Johnson. (1999). Compound Specific Characterization of Organic Fluorochemicals in General Population Human Sera Samples. 3M Environmental Lab, St. Paul, MN 55133.
4. Payfer R.M. GC/MS analyses of PFOSA (L-10009). SA&C Analytical Request No. 59426. Report 9/24/99. 3M SA&C Lab Building 236-2B-11.
5. Tom Kestner Chemical Characterization of PFOSA, L-10009, by ¹H and ¹⁹F-NMR Spectroscopy Requests # 59426. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, September 25, 1999.
6. DeRoos F.L. Characterization of PFOSA Samples, T-7132-1 (L-10009) and TN-A-1584. Request # A-151254. Report 10/7/99. Corporate Analytical Technology Center, Building 201-1-29, CATC – Chromatography Group.

Appendix 1:

Tel: 736-0665
201-1W-29

Corporate Analytical Technology Center

To: Larry A. Wendling/US-Corporate/3M/US
cc: Andrew Seacat/US-Corporate/3M/US
Subject: Characterization of PFOSA Sample for Toxicology

Larry,

I have completed the HPLC/MS characterization of the PFOSA sample (T-7132-, L-10009 prepared by G. Moore 4/96) that is proposed to be used for the animal feeding study. I found 9,600 ppm of PFOS, 1,100 ppm of $C_7F_{15}SO_2NH_2$, 510 ppm of $C_9F_{19}SO_2NH_2$, 6,600 ppm of $C_8F_{16}HSO_2NH_2$, 24,000 ppm of $C_{18}F_{36}HSO_2NH_2$, 1,200 ppm of $C_8F_{15}H_2SO_2NH_2$ and lower concentrations of several other amides. Based on the sum of the impurities, the purity of the PFOSA sample would be approximately 96 %.

I've thought a little more about apparent presence of $C_{18}F_{36}HSO_2NH_2$ in the sample. The identification of this compound was based primarily on the observed molecular weight and the relative elution order in the chromatogram. Rather than $C_{18}F_{36}HSO_2NH_2$, it seems more probable that this compound is actually $(C_8F_{17}SO_2)_2NH$. Confirmation of this identification will require additional analyses. I also need to think more about whether the other partially hydrogenated perfluoroamides may actually be of this

The PFOS was quantified using a standard curve prepared by analyzing PFOS standards so its concentration should be accurate. The amide impurities, however, including the partially hydrogenated amides, were all quantified using a PFOSA standard curve assuming that they had the same mass spectral response factor as did PFOSA. While this assumption will introduce some error to the quantitative data, it is the best we can do since we do not have standards for each of these amides. Also, this calculated purity assumes that all of the impurities are amenable to HPLC and are detected by the analysis. While not always true, this sample was analyzed by Rick Payfer using GC/MS and by CATC using our GC method. Neither of the analyses found volatile or semivolatile impurities, e.g., N-methyl FOSE, N-ethyl FOSE, etc. at concentrations greater than 50 ppm.

I also carried out a semiquantitative assessment of the purity of the PFOSA by comparing the PFOSA response to a PFOSA standard curve. Using this technique, the purity of the PFOSA sample was found to be 118 %. These analyses were carried out in duplicate, with triplicate injections of each solution, so they should be relatively accurate as analytical variation would be averaged. In general, however, it is not highly accurate to use a chromatographic method to quantify a relatively pure material due to the extremely large dilution factor, in this case >100,000, that must be applied. It is not expected that instrumental variation would bias the purity high. It is possible that the purity of the sample is actually higher than the purity of the PFOSA that we are using as a standard! If this were true, the purity would be determined to be > 100%.

Andrew Seacat has reviewed the concentrations of the impurities that were determined and calculated that the concentration of PFOS will not adversely affect the study. At 9,600 ppm, he calculates that the rats receiving 3 mg/kg/day would ingest app. 0.20 mg PFOS total in

Appendix I Continued:

28 days assuming a 0.25 kg rat. If we further assume that 30 % of that is deposited in the liver (based on previous feeding studies) and liver is approximately 12 grams, then the predicted concentration in the liver from the residual PFOS would be $200 \text{ ug PFOS} \times 0.3 / 12 \text{ g liver weight} = 5 \text{ ug/gram}$, or 5 ppm, which in itself should not impose any toxicological consequences. Any PFOS measured significantly over that could be attributed to metabolism of PFOSA to PFOS.

He believes that the 1-H and 2-H perfluoro amides are interesting as they may be subject to metabolic attack, however these amides should be analytically distinct from the Perfluorinated species on down the line, and would not amount to a foreseeable toxic concentration and therefore would be acceptable if no other substitute can be prepared. Andrew is still thinking about whether the $(\text{C}_8\text{F}_{17}\text{SO}_2)_2\text{NH}$ will have a negative impact on the study.

Please give me a call at 6-0665 if you have any questions concerning our analyses or if i can provide additional information.

Fred L. DeRoos
CATC