3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY
Protocol for Study No. T-6316.17, T-6295.21, T-7132.3; ST-41
Feces Method Development Metabolism Study for Perfluorooctanesulfonate Derivatives.

Study Objective:
This study is designed to generate specimens for the 3M Environmental Laboratory Fluorine Analytical Chemistry Team to use for development and validation a method of feces metabolite analysis.

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: Monday November 22, 1999
In-Life End Date: Wednesday November 24, 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, has previously furnished high-purity N-EtFOSE, PFOS and PFOSA to the Strategic Toxicology Lab.

Identification:

Name:

- N-EtFOSE: Narrow Range N-Ethyl perfluorooctanesulfonamido ethanol, FM-3923.
- PFOS: Perfluorooctane Sulfonic Acid, Potassium Salt; CAS # 2795-39-3
- PFOSA: Perfluorooctanesulfonamide

Molecular Formula:

- N-EtFOSE: C8F17SO2N(CH2CH3)CH2CH2OH
- PFOS: $C_8F_{17}OSO_2^-K^+$
- PFOSA: $C_8F_{17}SO_2NH_2$

Lot Number:

- N-EtFOSE: Lots 30035, 30037, 30039 mixed and analyzed as one sample.
- PFOS: Lot # 217
- PFOSA: L-10009

Purity:

- **N EtFOSE:** 98 % as determined by GC, GC/MS, ^{19}F-NMR, ^{1}H-NMR and DSC techniques (1).
- **PFOS:** >99% as determined by ^{19}F-NMR (2).
- **PFOSA:**
  - Analysis by GCMS determined that the staring material was over 99% pure (3).
  - Qualitative and quantitative compositional results that were derived from the single trial ^{1}H/^{19}F-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% CxF2x+1-CF(CF3)-SO2-NH2 (Alpha branch) and 0.28% $(CF_3)_3C$-$(CF_2)_x$-SO_2-NH_2 (t-Butyl branch)(4).
  - HPLC/MS characterization of the PFOSA sample revealed 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 % (5).

Stability: Documentation will be kept on file.
Storage Conditions:
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: 9-11 weeks
Weight at initiation of treatment: approximately 200-250g
Number and sex: 12 males
GROUP 1: **control**, n=3
GROUP 2: **N-Et FOSE**, n=3
GROUP 3: **PFOS**, n=3
GROUP 4: **PFOSA**, n=3
Identification: unique tail mark

Husbandry:

Housing:
All rats will be individually housed in wire bottom metabolism cages to allow for collection of urine and feces.

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:
All rats will be dosed via oral gavage on day zero of the study using a volume of 5 ml dosing suspension / kg body weight. Rats in group 2 will receive a single 400 mg/kg dose of N-Et FOSE, rats in group 3 will receive a single 100 mg/kg dose of PFOS and rats in group 4 will receive a single 100 mg/kg dose of PFOSA. Uniform suspensions of N-Et FOSE (8%; 80 mg/mL) and PFOS (2%; 20 mg/mL) will be prepared in 2% Tween 80 using a 15-ml tissue grinder. Re-suspension of PFOS and N-EtFOSE solids will be performed with 5 strokes of the tissue grinder pestel before each sample is drawn-up in the syringe for dosing.

PFOSA (2%; 20 mg/mL) will be prepared by dissolving in acetone, then forming an emulsion in 2% Tween 80 to a final concentration of 1% acetone, 2% Tween 80. Re-suspension of PFOSA solids will be performed by pumping the emulsion through the syringe immediately before dosing. A single 5 ml / kg body weight dose of vehicle will be administered via oral gavage to rats in group 1 on day zero of the study. Two of the rats will receive 2% Tween 80 and 1 will receive a mixture of 1% acetone/2% Tween 80.

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 treatment and immediately prior to euthanasia.

Frequency and Number of Animals:

One control and one PFOSA rat will be sacrificed on day 1 post dose. All remaining animals will be sacrificed on day 2-post dose.

Specimen Collection & Analysis:

Method of Specimen Collection:

Urine and feces will be collected from each metabolism cage on days one and two post dose. The initial volume of urine will be recorded, the sides of the urine collection apparatus will be washed with 5-10 ml deionized water and the final volume of urine will be recorded. Daily feces weight will be recorded for each animal.

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 divided into 2 aliquots, transferred to a separate labeled polypropylene microfuge tube and frozen in dry ice. One aliquot will be used for metabolite analysis and the other for biochemical analysis. Each liver will be excised and weighed. The liver will be flash frozen in liquid nitrogen. A small section (approximately 3 grams) will be removed and stored at –70°C for metabolite analysis. The remainder will be stored at -70°C for biochemical analysis. Kidneys from animals sacrificed on day 2-post dose will be excised, weighed and flash frozen in liquid nitrogen for future biochemical analysis.

Specimen Handling:

All urine and feces and the serum and liver for metabolite analysis will be temporarily stored at -70°C in the Strategic Toxicology Laboratory. For analysis, these samples 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
Telephone No.: 651-778-6081, Facsimile No.: 651-778-6176.

All kidney specimens and the remainder of serum and liver will be kept in the Strategic Toxicology lab for biochemical analysis. These specimens will be stored at -70°C.

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

TABLE 1 –Specimens for Environmental Lab

	Specimen	Collection date		Total
		Day 1 post dose	Day 2 post dose	
Environmental Lab Specimens	Urine	12	10	22
	Feces	12	10	22
	Sera	2	10	12
	Liver	2	10	12
Strategic Tox. Lab Specimens	Sera	2	10	12
	Liver	2	10	12
	Kidneys		10	10

Data Analysis:

All results will be provided for inclusion in the final report.

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.
- 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 11/30/99

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna J. Luebker 11/24/99

Deanna J. Luebker, MS
Advanced Toxicologist
Study Toxicologist

Date

References:

1. Payfer, R. Characterization of FM-3923, Mixture of Lots 30035, 30037 & 30039 for Two Year Feeding Study. SMD Analytical Request #52489. Analytical Report # 816, 6/23/97. 3M SMD Lab Building 236-2B-11.

2. Kestner, T. Fluorochemical Isomer Distribution by ^{19}F-NMR Spectroscopy. Spectroscopy Request # 53030. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, December 1, 1997.

3. Payfer, R. GC/MS analysis of PFOSA (L-10009). SA&C Analytical Request No. 59426. Report 9/24/99. 3M SA&C Lab Building 236-2B-11.

4. Kestner, T. Chemical Characterization of PFOSA, L-10009, by 1H and 19F-NMR. Spectroscopy Request # 59426. 3M Specialty Adhesives & Chemicals Analytical Laboratory / SMMD-236-2B-11, September 25, 1999.

5. DeRoos F. Characterization of PFOSA Samples, T-7132-1 (L-10009) and TN-A-1584. Request # A-151254. Report 10/7/99, and Letter addendum to report 10/14/99. Corporate Analytical Technology Center, Building 201-1-29, CATC – Chromatography Group.