3M MEDICAL DEPARTMENT, CORPORATE TOXICOLOGY

Protocol for Study No. T-6295.16 DT-31

Low Level PFOS Dose versus Rat Serum and Liver PFOS

Projected Start Date: October 29th, 1998

Study Objective:

The objective of this study is to obtain data from which to construct standard curves of single oral dose versus serum and liver PFOS concentration in male and female rats. These curves will be used to interpret future oral absorption, distribution, metabolism and excretion (ADME) studies on POSF-based polymeric fluorochemicals (FCs) which may contain low-level contamination with PFOS. These curves will be used to compare serum PFOS concentration after a single oral dose of POSF-based polymeric FCs containing known quantities of PFOS to expected serum level and liver level increases predicted by the standard curve based on an equimolar dose of the residual. These curves will help evaluate whether an increase in serum and liver concentration of PFOS is a result of metabolic degradation of the polymer or absorption of residuals.

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 Alternative Toxicology Laboratory
3M Center, Building 270-SB-181
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:

In-Life Start Date: October 29th, 1998
In-Life End Date: November 19th, 1998

Regulatory Compliance:
This is a rangefinder study and thus non-GLP.

Test Material:
The sponsor will provide a sample of PFOS. Analytical documentation of the starting material will be the responsibility of the sponsor. A chemical composition specification sheet will be kept on file.

Identification:

Name: Perfluorooctane Sulfonic Acid, Potassium Salt; CAS # 2795-39-3
Molecular Formula: $C_8F_{17}OSO_2^-K^+$
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 Laboratories, Inc.
Age at initiation of treatment: 6-8 weeks old
Weight at initiation of treatment: approximately 150-200g
Number and sex: 75 males, 75 females

GROUP 1: control	- 15 male, 15 female
GROUP 2: 3µg/kg	- 15 male, 15 female
GROUP 3: 30µg/kg	- 15 male, 15 female
GROUP 4: 100µg/kg	- 15 male, 15 female
GROUP 5: 300µg/kg	- 15 male, 15 female

Identification: ear tag

Husbandry:

Housing:

All rats will be group housed in standard cages.

Diet/Water:

All rats will be provided tap water and Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet (Harlan Teklad, Madison, WI) *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:

A 1mg/mL stock solution of PFOS in 2% Tween 80 will be prepared immediately prior to dosing. Serial dilutions of the stock solution will be performed to reach the appropriate concentration for each dose level. A single dose of compound, using a dose volume of 5 ml dosing solution / kg body weight, will be administered via oral gavage to rats in groups 2-5 on day zero of the study. The control rats will receive no treatment.

Dose Selection Justification:

The doses selected for this low dose PFOS study are designed to yield serum PFOS levels that are at or near the limit of quantification (15 ppb). Based on previous data, 1 mg of ingested PFOS yields approximately 5 ppm PFOS in the blood. Therefore, using linear extrapolation, 3 µg/kg will theoretically yield about 15 ppb PFOS in the blood. Three µg/kg was thus selected as the low dose.

Mixing procedure:

A 1 mg/ml stock suspension of PFOS will be prepared by homogenizing 10 mg PFOS in 10 ml 2% Tween 80 using a 15 ml glass tissue grinder. A serial dilution will be performed based on the following calculations:

- Assume a dosing volume of (5 ml/kg) (0.3 kg) = 1.5 ml/ rat
- **0.3 mg/ Kg dose group:**
  (0.3 mg/kg dose)(0. 3 kg rat)/ 1.5 ml/ rat = 0.06 mg/ml PFOS solution in 2% Tween 80 needed.
  Dilute 6 ml of the 1 mg/ml solution to 100 ml in 2% Tween 80.
- **0.1 mg/kg dose group:**
  (0.1 mg/kg) (0.3 kg rat)/ 1.5 ml/rat = 0.02 mg/ml PFOS solution in 2% Tween 80 needed.
  Dilute 33.333 ml of 0.06 mg/ml solution up to 100 ml in 2% Tween 80.
- **0.03 mg/kg dose group**
  (0.03mg/kg) (0.3 kg)/ 1.5 ml/rat = 0.006 mg/ml PFOS solution in 2% Tween 80 needed.
  Dilute 30 ml of the 0.02 mg/ml solution up to 100 ml in 2% Tween 80.
- **0.003 mg/kg dose group**
  (0.003) (0.3 kg)/1.5 ml / rat = 0.0006 mg/ml PFOS solution in 2 % Tween 80 needed.
  Dilute 10 ml of the 0.006 mg/ml solution up to 100 ml in 2% Tween 80.

Dose Verification:

Samples of the concentrated 1 mg/ ml PFOS stock, the 0.06 mg/ml stock (for the 0.3 mg/kg dose group) and the 0.02 mg/ml stock (for the 0.1 mg/kg dose group) will be analyzed by LCMS for dose verification by Kris Hansen, 3M Environmental Lab. It will not be possible to sufficiently dilute the 0.006 and 0.0006 mg/ml dose solutions and still quantify the PFOS. Extrapolation to the lower dose levels will

therefore, be performed based on the quantification of the more concentrated dose solutions. All doses will also be confirmed by total organic fluorine analysis performed by Dr. Ventakaswarlu Pothapragada (Dr. V.), 3M SCD Lab.

Observation of Animals:

Clinical Observations:

Each animal will be observed daily for mortality and morbidity and notable findings will be recorded. Weekly, each animal will be removed from its cage and a record of abnormal findings or normality will be made. 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.

Sample Collection:

Frequency and Number of Animals:

Scheduled sacrifices:

Five males and five females from each group will be euthanized on days 1, 4 and 14 post dose. See Table 1 - schedule.

TABLE 1 - SCHEDULE

				Oct 29 day 0 DOSE grps 2 & 3 (60)	**Oct 30 day 1 post dose NECROPSY** 5 M, 5 F each, grps 2 & 3 (20)	**Oct 31**
Nov 1	**Nov 2 day 4 post dose NECROPSY** 5 M, 5 F each, grps 2 & 3 (20)	**Nov 3**	**Nov 4**	**Nov 5 day 0 DOSE** grps 1, 4 & 5 (90)	**Nov 6 day 1 post dose NECROPSY** 5 M, 5 F each, grps 1, 4 & 5 (30)	**Nov 7**
Nov 8	**Nov 9 day 4 post dose NECROPSY** 5 M, 5 F each, grps 1, 4 & 5 (30)	**Nov 10**	**Nov 11**	**Nov 12 day 14 post dose NECROPSY** 5 M, 5 F each, grps 2 & 3 (20)	**Nov 13**	**Nov 14**
Nov 15	**Nov 16**	**Nov 17**	**Nov 18**	**Nov 19 day 14 post dose NECROPSY** 5 M, 5 F each, grps 1, 4 & 5 (30)	**Nov 12**	**Nov 21**

Method of Sample Collection:
At all designated times, animals will be euthanized by CO_2 and gross necropsy performed. During necropsy, systemic blood (≈ 5 ml) will be collected via the abdominal aorta and transferred to blood collection tubes without anticoagulant. All 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. The serum will then be transferred to labeled polypropylene microfuge tubes and flash-frozen in liquid nitrogen. Livers will be removed, weighed, placed individually into labeled tubes and flash frozen in liquid nitrogen.

Sample Handling:
Samples will temporarily be stored in a freezer set to maintain -60 to -80°C. 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.

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

TABLE 2 - SAMPLES

Sample	Oct 30	Nov 2	Nov 6	Nov 9	Nov 12	Nov 19	Total
Serum	20	20	30	30	20	30	150
Liver	20	20	30	30	20	30	150

Analysis:
Samples will be analyzed by 3M Environmental for PFOS concentration. Results will be provided for inclusion in the final report.

Report:
Best-fit curves will be obtained by linear regression based on mean serum and liver PFOS concentrations for each group.

Responsibilities:

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

Signatures:

Andrew M. Seacat 10/29/98

Dr. Andrew Seacat — Date
Senior Research Toxicologist
Study Director

Deanna Nabbefeld 10/29/98

Deanna Nabbefeld, MS — Date
Advanced Toxicologist
Study Toxicologist

Protocol Amendment # 1
Study No. T-6295.16, DT-31
Low Level PFOS Dose versus Rat Serum and Liver PFOS

Sponsor:	3M Specialty Chemicals Division
Study Location:	3M Strategic Alternative Toxicology Laboratory
Study Director:	Andrew M. Seacat Ph.D.

This amendment modifies the following portions of the protocol:
Effective November 5, 1998

1. **Page 2. Animals, Number and Sex:**
   To strengthen the low-dose portion of the dose response curve, Dose Group 5 was replaced with a 10 ug/kg dose group. The dose groups will now be as follows:

   GROUP 1: control - 15 male, 15 female
   GROUP 2: 3µg/kg - 15 male, 15 female
   GROUP 3: 30µg/kg - 15 male, 15 female
   GROUP 4: 100µg/kg - 15 male, 15 female
   GROUP 5: 10 µg/kg - 15 male, 15 female

2. **Page 3. Mixing Procedure:**
   The 0.010 mg/kg dosing solution will be made by making a 10 fold dilution of the 0.1 mg/kg dosing solution. Therefore, 10 ml of the 0.02 mg/ml PFOS solution in 2% Tween 80 will be diluted to 100 ml in 2% Tween 80 to yield the 0.002 mg/ ml PFOS solution in 2 % Tween 80 needed.

3. **Page 4. Dose Verification:**
   The 0.002 mg/ ml PFOS solution in 2 % Tween 80 will be confirmed by total organic fluorine analysis performed by Dr. Ventakaswarlu Pothapragada (Dr. V.), 3M SCD Lab.

4. **Page 6. Analysis:**
   Serum and liver samples will be analyzed by Battelle Laboratories for PFOS concentration. Results will be provided for inclusion in the final report. Shipping Address:

   Battelle Laboratories
   Attention: Angela Capers
   505 King Avenue
   Columbus, Ohio 43201
   Phone: (614) 424-5588, Fax: (614) 424 -3268

Amendment Approval:

Signatures:

Andrew Seacat 11/5/98

Dr. Andrew Seacat
Senior Research Toxicologist
Study Director

Date

Deanna J Nabbefeld 11/5/98

Deanna Nabbefeld, MS
Advanced Toxicologist
Study Toxicologist

Date

Protocol Amendment # 2
Study No. T-6295.16, DT-31
Low Level PFOS Dose versus Rat Serum and Liver PFOS

Sponsor:	3M Specialty Chemicals Division
Study Location:	3M Strategic Alternative Toxicology Laboratory
Study Director:	Andrew M. Seacat Ph.D.

Effective April 26th, 1999
The purpose of this amendment is to reflect the decision of the Sponsor to add a dose group of 5mg/kg body weight and a necropsy date of 28 days post dose to the overall study design. All modifications are **additions** to the original protocol and Amendment #1. The modifications are as follows:

1. **Page 1 Protocol, Proposed Study Timeline:**
   Additional Proposed Timeline specific to Amendment #2:
   In-Life Start Date: 3/2/99
   In-Life End Date: 5/24/99

2. **Page 2 Protocol / Page 1 Amendment #1, Animals, Number and Sex:**
   The following 2 dose groups will be added to the protocol:
   GROUP 6: vehicle control - 12 male, 12 female
   GROUP 7: 5mg/kg - 20 male, 20 female

3. **Page 3 Protocol / Page 1 Amendment #1, Mixing Procedure:**
   A single 5mg/kg dose of compound will be administered via oral gavage to rats in groups 7 on day zero of the study. The compound 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. Re-suspension of solids will be performed with 5 strokes of the tissue grinder pestle before each sample is drawn-up in the syringe for dosing. The 10 control rats (group 6) will be dosed with 5 ml/kg 2% Tween 80 (vehicle control).

4. **Page 4 Protocol / Page 1 Amendment #1, Dose Verification:**
   The vehicle control sample (2% Tween 80) and the 1 mg/ ml PFOS solution in 2 % Tween 80 will be confirmed by total organic fluorine analysis performed by Dr. Ventakaswarlu Pothapragada (Dr. V.), 3M SCD Lab.

5. **Page 4 Protocol. Sample Collection, Frequency and Number of Animals:**
   Frequency and Number of Animals:
   Scheduled sacrifices:
   Five males and five females from group 7 and 3 males and 3 females from group 6 will be euthanized on days 1, 4, 14 and 28 post dose. See Table 1 - schedule.

TABLE 1 - SCHEDULE

	April 26 day 0 DOSE grps 6 & 7 (64)	**April 27** day 1 post dose NECROPSY (16)	**April 28**	**April 29**	**April 30** day 4 post dose NECROPSY (16)	**May 1**
May 2	**May 3**	**May 4**	**May 5**	**May 6**	**May 7**	**May 8**
May 9	**May 10** day 14 post dose NECROPSY (16)	**May 11**	**May 12**	**May 13**	**May 14**	**May 15**
May 16	**May 17**	**May 18**	**May 19**	**May 20**	**May 21**	**May 22**
May 23	**May 24** Day 28 post dose NECROPSY (16)					

Amendment Approval:

Signatures:

Andrew M. Seacat 4/23/99

Andrew M. Seacat, Ph.D. Date
Senior Research Toxicologist
Study Director

Deanna Nabbefeld 4/23/99

Deanna Nabbefeld, MS Date
Advanced Toxicologist
Study Toxicologist