3M ENVIRONMENTAL LABORATORY

Protocol - Analytical Study

Quantitative Analysis of Perfluorooctane Sulfonic Acid Potassium Salt (PFOS, T-6295) in Cynomolgus Monkeys Following Administration of a 4-Week Capsule Toxicity Study

3M ET & SS Study Number: AMDT-041598.1

In-Vivo Study Reference Number: Covance 6329-222
Test Substance: Perfluorooctane Sulfonic Acid Potassium Salt (Potassium Perfluorooctane Sulfonate) $C_8F_{17}SO_3^-K^+$ (PFOS, T-6295)

Name and Address of Sponsor:

3M Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

Name and Address of Biological-Phase Facility:

Covance Laboratories Inc.
P.O. Box 7545
Madison, WI 53707-7545

Name and Address of Analytical Testing Facility:

3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Date of Approval by Sponsor: 4/21/98
Proposed Analytical Initiation Date: 7/15/98
Proposed Analytical Completion Date: 7/15/99

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8/6/98
Study Director ① Date

7/18/98
Principal Analytical Investigator Date

① Biological Phase Study Director 8/6/98

1.0 PURPOSE

This study is designed to quantitatively determine the extent of absorption of PFOS and various metabolites in cynomolgus monkeys orally administered PFOS.

2.0 REGULATORY COMPLIANCE

This study will be conducted in accordance with the EPA Good Laboratory Practice regulations 40 CFR part 792 and applicable current Environmental Testing & Safety Services (ET&SS) SOP's and methods (*see sections **8.0 and 9.0** for specific SOP's and methods related to this protocol*).

3.0 TEST MATERIALS

3.1 Test, Control, and Reference Substances and Matrices

3.1.1 Analytical Reference Substance: Perfluorooctane Sulfonic Acid Potassium Salt ($C_8F_{17}SO_3^-K^+$ (PFOS, T-6295))

3.1.2 Analytical Reference Substance Matrix: Cynomolgus monkey serum and liver tissue

3.1.3 Analytical Control Substance Matrix: Cynomolgus monkey serum and liver tissue (Control tissue, I05346).

3.2 Source of Materials

3.2.1 Analytical Reference Substance: 3M ICP/PCP Division

3.2.2 Analytical Reference Substance Matrix: Covance Research Products (The source of the monkeys will be documented in the raw data)

3.2.3 Analytical Control Substance Matrix: Covance Research Products. The tissue was from a control animal, I05346.

3.3 Number of Test and Control Samples

3.3.1 Test samples

Group	Number & Sex	Route	Dose Level PFOS (mg/kg/day)	Collections[1]
1 (Control)	2 Male 2 Female	Orally by gel capsules	0	• Blood predose (-6 days from dosing), and on Days 2, 7, 14,and 29. • Liver tissue on Day 29.
2 (low-dose)	3 Male 3 Female	Orally by gel capsules	0.02	• Blood predose (-6 days from dosing), and on Days 2, 7, 14,and 29. • Liver tissue on Day 29.
3 (high-dose)	1 Male 1 Female	Orally by gel capsules	2.0	• Blood predose (-6 days from dosing), and on Days 2, 7, 14,and 29 • Liver tissue on Day 29.

1-Blood collected from animals will be centrifuged by Covance, serum harvested, and serum shipped to 3M ET&SS for analysis

3.4 Identification of Test and Control Samples. Test samples will be identified by Covance at the time of collection.

3.5 **Purity and Identity of Materials.** Characterization of the test materials is the responsibility of the Sponsor.

3.6 **Stability of Reference Material.** Testing for the stability of the test material under the conditions of administration is the responsibility of the Sponsor.

3.7 **Storage Conditions for Test Materials.** Analytical reference substance will be stored at ambient temperature. Analytical reference matrices will be stored at or below approximately -20 °C, until extraction. All extracts will be stored nominally at 4 °C until analyzed. Test and Control samples will be received according to AMDT-S-10.

3.8 **Disposition of Specimens.** Biological tissues and fluids will be retained for the required time period as per GLP regulation 40 CFR part 792.195 for studies longer than 28 days.

3.9 **Safety Precautions.** Refer to the Material Safety Data Sheets for the substances used for associated safety information. Exercise caution when handling knives for cutting the sample matrices. Safety glasses, protective clothing and appropriate hand protection should always be worn when working in the Environmental Laboratory.

4.0 EXPERIMENTAL - Overview

Animals were administered gel capsules orally, daily (7 days/week) for 28 days. Dose levels of PFOS in the capsule varied (0, 0.02, and 2.0 mg/kg) between groups (control, low-dose, and high-dose respectively). Blood samples were collected at regular intervals (see 3.3.1). Liver tissue samples were collected at the time of sacrifice (Day 29). Sera and liver tissue were shipped to 3M ET&SS for the analytical portion of this study. See Covance Study #CHW 6329-222 for information regarding the in-life portion of the study.
The sera and liver tissue will be extracted via an ion pairing method, or other method as appropriate, and then analyzed via Electrospray Ionization/Mass Spectrometry (Tandem Mass Spectrometry) ESI/MS(MS), or other appropriate technique, for PFOS and other fluorochemical metabolites.

5.0 EXPERIMENTAL- Analytical Methods

Liver tissue and sera will be targeted for analysis. Methods are available for extraction and analysis of fluorochemicals from sera and liver tissue various animal matrices (see attachments). The methods will be validated for cynomolgus monkey matrices prior to or concurrently with sample analysis.

6.0 DATA ANALYSIS

Data will be reported as the milligrams (mg) of PFOS or metabolite per unit of tissue, or as a concentration of fluoride per tissue, or in other clearly defined units, as appropriate. Quantitation

will be performed using calibration curves. These curves will be determined using linear regression analysis. Statistics used, at the discretion of the Principal Investigator, may include averages and standard deviations of concentrations for different dose groups. If necessary, simple statistical tests such as Student's t-test may be applied to determine statistical difference.

No final report will be prepared for this study. A data package will be provided to Covance for inclusion in their final report for CHW 6329-222.

7.0 Maintenance of Raw Data and Records

7.1 The following raw data and records will be retained in the study folder in the archives according to AMDT-S-8.

- **7.1.1** Approved protocol and amendments
- **7.1.2** Study correspondence
- **7.1.3** Shipping records
- **7.1.4** Raw data
- **7.1.5** Approved final report (original signed copy)
- **7.1.6** Electronic copies of data

7.2 Supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include, but not necessarily be limited to, the following:

- **7.2.1** Training records
- **7.2.2** Calibration records
- **7.2.3** Instrument maintenance logs
- **7.2.4** Standard Operating Procedures, Equipment Procedures, and Methods
- **7.2.5** Appropriate specimens

8.0 References

8.1 AMDT-S-1, Chemical Tracking
8.2 AMDT-S-2, Solutions and Standards Making
8.3 AMDT-S-3, Personnel Training
8.4 AMDT-S-4, General Lab Documentation Systems
8.5 AMDT-S-5, GLP-related Documentation Systems
8.6 AMDT-S-6, General Lab Records
8.7 AMDT-S-7, GLP-related Records
8.8 AMDT-S-8, Archives
8.9 AMDT-S-9, GLP Program and Responsibilities
8.10 AMDT-S-10, Sample Tracking System
8.11 AMDT-S-12, Analytical Method Validation
8.12 AMDT-S-15, Laboratory Practices and Data Management
8.13 AMDT-S-16, Quality Assurance Unit
8.14 AMDT-S-17, Analytical Equipment Systems Documentation

8.15 AMDT-S-18, Routine Calibration Checks of Balances
8.16 AMDT-S-20, Daily Calibration Checks of Automatic Pipettors
8.17 AMDT-S-21, Annual Calibration Checks of Automatic Pipettors

9.0 ATTACHMENTS

9.1 FACT-M-1.0 Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver Using Analysis Using HPLC Electrospray Mass Spectrometry
9.2 FACT-M-2.0 Analysis of Fluorochemicals in Liver Extracts using HPLC Electrospray/Mass Spectrometry
9.3 FACT-M-3.0 Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Serum Using Analysis Using HPLC Electrospray Mass Spectrometry
9.4 FACT-M-4.0 Analysis of Fluorochemicals in Serum Extracts using HPLC Electrospray/Mass Spectrometry