

Study Title

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats (Covance Study No.: 6329-183)

Analytical Laboratory Report Title

Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of CrI:CD[®](SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

At signing

Performing Laboratories

Liver and Serum Analyses

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Project Identification

3M Medical Department Study: T-6295.4
Covance In-Life Study: 6329-183
Analytical Report: FACT TOX-002
3M Laboratory Request No. U2121

Total Number of Pages

181

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GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of CrI:CD[®](SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

Study Identification Number: T-6295.4, FACT TOX-002, LRN-U2121

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase study was considered to end at the generation and shipment of specimens. The analytical study was considered to start at the receipt of these specimens for analysis. This resulted in having two separate study directors, one for each phase of the same study. However, since the technical performance of each phase was entirely separate, no effect is expected from this exception.
- Changes in the raw data entries were not all made in accordance with 21 CFR 58.130 (e).
- Specimen stability storage will not be determined.
- One of the control matrices (rabbit liver) was not identified in accordance with 21 CFR 58.120 (a)(2).
- The electronic data systems in use have not been validated and there is not an electronic audit trail of corrections currently available (21 CFR 58.130 (e)). Authenticated hard copies of chromatograms and associated documents will be considered as the original raw data.
- Characterization of the analytical standards is underway, but was not completed at the time this data was assembled (21 CFR 58.105 (a)). When Certificates of Analysis for all remaining lots of PFOS are available, an amendment to this report will be issued. Lot No. 59909 of THPFOS will not be characterized for purity since quantities of this standard are exhausted.

John R. Bortoloff
Study Director

21 FEB 01
Date

Marianne T. Case
Sponsor Representative

21 Feb 2001
Date

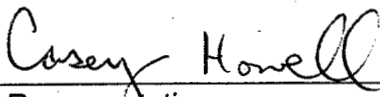
GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of Crl:CD®(SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

Study Identification Number: T-6295.4, FACT TOX-002, LRN-U2121

This study has been inspected by the 3M Lab Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and laboratory management.

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
11/11/99	Extraction	11/30/99	11/30/99
08/28/00 – 09/18/00	Data	09/19/00	09/19/00
10/05/00 – 10/06/00, 10/16/00 – 10/20/00, 10/30/00 – 10/31/00	Data	11/02/00	11/02/00
11/01/00 – 11/03/00	Data	11/06/00	11/06/00
11/02/00 – 11/03/00, 11/06/00 – 11/07/00	Data	11/08/00	11/08/00
01/08/01 – 01/11/01	Draft Report	01/12/01	01/12/01



QAU Representative

Feb 19, 2001

Date

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Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test article and analytical reference standard reserve samples, as well as the specimens pertaining to the analytical phase of this study are archived at the 3M Environmental Laboratory.

Introduction and Purpose

The purpose of the study is to determine the presence and concentration of PFOS in liver and serum specimens collected from Covance Study No.: 6329-183 titled: 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS, T-6295) in Rats, initiated on 22 May 1998.

Test System

360 males and 360 females were used as the test system. Table 1 outlines the rat population demographics and dosage levels for study 6329-183.

The test system species and strain selected was the Crl:CD[®](SD) IGS BR rat received from Charles River Laboratories, Inc., identified using an implanted microchip device. At the initiation of treatment the rats were approximately 6–8 weeks of age and weighed between 100–300 g.

Table 1. Test System Population Demographics for Study (6329-183)

Study Group	Number of Animals		Dietary Levels (ppm)
	Male	Female	
Group 1 Control	70	70	0
Group 2 Low	60	60	0.5
Group 3 Mid	60	60	2.0
Group 4 Mid-High	60	60	5.0
Group 5 High	70	70	20.0
Group 6 High Recovery	40	40	20.0

Specimen Collection and Analysis

Sample specimens were collected by Covance (study 6329-183) and sent to the 3M Environmental Laboratory for analysis. On week 4 and week 14, 50 liver and 50 serum specimens were collected from Groups 1–5 males and females. On week 53, 39 liver and 19 serum specimens were collected from Groups 1 and 5 males and females. On day 719, 9 liver and 9 serum specimens were collected from Group 3 females. At the time of terminal sacrifice week 105), 164 liver and 164 serum specimens were collected from Groups 1–5 males and females. At the time of recovery sacrifice (week 106), 27 liver and 27 serum specimens were collected from Group 6 males and females. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Study Groups 1 through 5 (through 4/21/00):

Serum Specimens—292 specimens

Liver Specimens—312 specimens

Specimens Collected from Study Group 6 (Recovery) on 4/24/00 and 4/27/00:

Serum Specimens—27 specimens

Liver Specimens—27 specimens

Blood specimens were allowed to clot at room temperature and separated by centrifugation. Serum was then harvested and stored in a freezer at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were flash frozen in liquid nitrogen and then stored in a freezer at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver and sera specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice.

Sera and liver specimens were extracted beginning on May 27, 1998 using an ion pairing reagent and either ethyl acetate or methyl-*tert*-butyl ether (MtBE). Liver specimens were homogenized prior to the extraction procedure. Specimen extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple reaction monitoring mode. PFOS levels were quantitated by external calibration of extracted curves. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received liver and serum specimens collected at predetermined time points during and at the end of the *in-life* phase of Covance Study No. 6329-183 from May 1998 through May 2000 from Covance. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-002 were obtained from commercial sources and are presented in Appendix A (see Tables 4 and 5). Specimens analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

Chemical Characterization

Chemical characterization information on the test article used in this study is presented in Appendix A (see Table 6). Chemical characterization information on the analytical reference materials used in this study is presented in tabular form (Table 2) below.

Table 2. Characterization of the Analytical Reference Materials Used in Study FACT TOX-002

Reference Material / Formula	Acronym	Source	Expiration Date	Storage Conditions	Chemical Lot Number	Physical Description	Purity
Potassium Perfluorooctanesulfonate $C_8F_{17}SO_3-K^+$	KPFOS	3M Specialty Chemicals	08/31/00	Ambient temperature	171	White crystalline powder	86.4%
		3M Specialty Chemicals	2010	Ambient temperature	193 ^a	White powder	88.0% ^b
		3M Specialty Chemicals	2010	Ambient temperature (dry)	215 ^a	White powder	TBD
1H, 1H, 2H, 2H-Tetrahydroperfluorooctanesulfonic acid $C_8H_4F_{13}SO_3H$	THPFOS	ICN Biomedics, Inc.	01/01/2010	Ambient temperature	53406	Brown waxy solid	TBD
		ICN	01/01/2010	Ambient temperature	59909	Brown powder	N/A

^a Used for analysis of week 4 and week 14 sera samples.^b Samples were not corrected for purity.

TBD—to be determined

N/A—not applicable. This lot is exhausted and cannot be characterized.

Dose Confirmation Analyses

Dose preparation methods and analysis were performed by Covance, using a validated analytical method provided by the Sponsor (MP-M383-MA), and are reported separately (Reference Covance 6329-183).

Method Summaries

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory. Detailed descriptions of the methods used in this study are located in Appendix C.

As the present study progressed, more advanced methods evolved and earlier methods were used with deviations until amendments to the protocol were written. Protocol and method deviations are located in Appendix B of this report.

3M Environmental Laboratory

PREPARATORY METHODS

- **FACT-M-1.0**, “Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry”. This method was used for week 4 specimens extracted on 6/11/98 and week 14 specimens extracted on 8/6/98.
- **FACT-M-3.0**, “Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry”.

Spectrometry". This method was used for week 4 specimens extracted on 5/27/98 and week 14 specimens extracted on 7/27/98.

- **ETS-8-4.1**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 specimens extracted on 11/10/99, and day 719, week 105, and week 106 specimens extracted on 5/1/00 and 5/2/00.
- **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 specimens extracted on 11/10/99 and 1/25/00, day 719 specimens extracted on 5/16/00 and 5/18/00, week 105 specimens extracted on 5/16-18/00 and 5/22/00, and week 106 specimens extracted on 5/16/00 and 5/18/00.

An ion-pairing reagent was added to the specimen and the analyte ion pair was partitioned into ethyl acetate (FACT-M-1.0 and FACT-M-3.0) or MtBE (ETS-8-4.1 and ETS-8-6.0). The extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, and then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

ANALYTICAL METHODS

- **FACT-M-4.0**, "Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry". With the exception of week 4 sera specimens, data was worked up according to ETS-8-5.1 or ETS-8-7.0 and following control of bias documentation included in study binder.
- **ETS-8-5.1**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry". Sera run 7/15/98 was accepted despite the failure of the continuing calibration checks. Since compliance with method ETS-8-5.1 was in the protocol, deviations from it, including this one are also protocol deviations. A method deviation has been written.
- **ETS-8-7.0**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry".

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of PFOS using HPLC-ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3^-$) analysis, was fragmented further to produce ion 99 (FSO_3^-). The characteristic product ion 99 was monitored for quantitative analysis.

ANALYTICAL EQUIPMENT

The actual analytical equipment settings used in the present analytical phase of this study varied slightly during actual data collection. The following is representative of the analytical equipment settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system

Analytical column: Keystone® Betasil™ C₁₈ 2x50 mm (5 µm)

Column temperature: Ambient

Mobile phase components:

Component A: 2mM ammonium acetate

Component B: methanol

Flow rate: 300 µL/min

Injection volume: 10 µL

Solvent Gradient: 13.5 minutes

<i>Time (minutes)</i>	<i>%B</i>
0.0	40%
8.5	90%
11.0	90%
12.0	40%
13.5	40%

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system

Software: Mass Lynx™ 3.1, 3.3, and 3.4

Cone Voltage: 30–60 V

Collision Gas Energy: 25–45 eV

Mode: Electrospray Negative

Source Block Temperature: 150°C ±10°C

Electrode: Z-spray

Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499.0	80.0, 99.0, 130.0
THPFOS	427.0	80.0

Deviations

As the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Earlier methods were used with deviations until amendments to the protocol were written.

Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated in the method performance section of ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry and ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry:

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980.
- **Limits of Quantitation (LOQ):** The LOQ for PFOS is 5.55 ng/mL for serum and 26.9 ng/g for liver in the respective method performance sections, however, the LOQ for a given run is based upon the concentration of the lowest acceptable standard in the run.
- **Acceptable Matrix Spike Recoveries:** 70–130% for sera / 50–150% for liver.

Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Lab methods ETS-8-5.1 and ETS-8-7.0 (see Appendix C) were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curve was ≥ 0.985 .
- **Calibration Standards:** With the exception of the week 4 sera data, quantitation of the target analytes was based on linear regression analysis of two extracted matrix curves weighted $1/x$ bracketing each group of samples. Calibration standards were not diluted and were spiked to be within the linear range of the instrument. Week 4 sera data was evaluated using an unweighted curve. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of PFOS was based on the response of one or more specific product ions using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within $\pm 30\%$ of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks. For both liver and sera analysis, the LOQ decreased over the course of the study as methods improved. The highest LOQs in both analyses occurred in the week 4 analysis and were 23.2 ng/mL (sera) and 27.8 ng/g (liver).
- **Blanks:** All blanks were below the lower limit of quantitation for PFOS. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed rat sera and liver, rabbit sera and liver were selected as a suitable surrogate matrix for blanks, curves, and CCVs.
- **Precision:** Not determined for this study.

- **Matrix Spike Recoveries:** Rat sera from 18 control animals were spiked prior to extraction. PFOS was spiked at approximately 75 ng/g, 100 ng/g, and 250 ng/g levels. With two exceptions, all Matrix Spike/Matrix Spike Duplicate (MS/MSD) were recovered at $\pm 30\%$ of expected values. One MS, analyzed with the week 4 samples had a recovery of 167%; one MS analyzed with the week 53 samples was recovered at 131%. These high recoveries were confirmed. Because the remaining 16 spikes were acceptable, the high recoveries do not indicate serious analytical deficiencies.

Rat liver from 20 control animals were spiked prior to extraction. PFOS was spiked at approximately 100 ng/g and 250 ng/g levels. Thirteen of the 20 spikes were within 50% of the expected value; seven were outside of this range. Four of the deviant spikes, analyzed with the day 719 samples, were mistakenly prepared from G3 and G6 animals instead of from control animals (G1). The high levels of PFOS in the tissue of these test animals obscured the relatively low level of material spiked (spike was $<3\%$ of endogenous). This mistake in preparation invalidates these results as indicators of method QC. Two of the remaining deviant spikes were analyzed with the week 4 samples and one deviant MS was analyzed with the week 53 samples. Because 13 of 16 matrix spikes that were prepared correctly were acceptable, the deviant recoveries do not indicate serious analytical deficiencies.

All matrix spikes were prepared at levels approximate to G1 samples.

- **Surrogates:** The surrogate (THPFOS) was added to all specimens and standards before extraction, as stated in the methods. THPFOS was spiked into samples at concentrations within the linear range when samples were analyzed without dilution. Prior to May 5, 2000, THPFOS was not used for quantitation, but was used to monitor for gross instrument failure (see Appendix C, Analytical Methods). In analyses performed after May 5, 2000, the surrogate response of each analytical run was verified to determine that it did not vary more than $\pm 50\%$ from the mean within each analytical run. At no time was THPFOS used for quantitation as an internal standard. No problems were observed with these data.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Summary of Specimen Results

- **Specimens from Control Animals:** Low levels of PFOS were often detected in the sera and liver of the control animals.
- **Specimens from Dosed Animals:** Detailed specimen data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. All specimen results were used to calculate the mean values; the LOQ value was used for specimens with $<LOQ$ results. See Appendix F for example calculations used to generate the liver and serum specimen data in FACT TOX-002.

Statement of Conclusion

Under the conditions of the present studies, PFOS was observed in the sera and liver of rats dosed with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) during the in-life phase of the study.

Reference

Covance Study No.: 6329-183, 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats

Appendix A: Identification of Test Article and Control Matrices

Table 4. Characterization of the Control Matrices Used for Sera Analyses in Study FACT TOX-002

Control Matrix	Rabbit Serum	Rabbit Serum	Rat Serum	Rat Serum
Source	Sigma	Sigma	Sigma	Sigma
Expiration Date	2010	2010	2010	2010
Storage Conditions	Frozen	Frozen	Frozen	Frozen
Lot #	47H4641	118H8418	17H9306	19H89292
Physical Description	Rabbit Serum	Rabbit Serum	Rat Serum	Rat Serum

Table 5. Characterization of the Control Matrices Used for Liver Analyses in Study FACT TOX-002

Control Matrix	Rabbit Liver	Rabbit Liver	Rabbit Liver	Rabbit Liver
Source	CHW	CHW	N/R	CHW
Expiration Date	2010	2010	2010	2010
Storage Conditions	Frozen	Frozen	Frozen	Frozen
Lot #	F00014	F00016	N/R	F00007
Physical Description	Rabbit Liver	Rabbit Liver	Rabbit Liver	Rabbit Liver

N/R—not recorded

Table 6. Identification of the Test Article in Study FACT TOX-002

	Test Article
Chemical Name	KPFOS Perfluorooctane sulfonic acid potassium salt
Source	3M ICP/PCP Division
Expiration Date	08/31/01
Storage Conditions	Ambient temperature
Chemical Lot #	217
Physical Description	White crystalline powder
Purity	86.9%

Appendix B: Protocol, Amendments, Deviations

3M Environmental Laboratory

Protocol - Analytical Study Phase

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

In-Vivo Study Reference Number: Covance # 6329-183

Study Number: AMDT - 112296.1

Test Material: T-6295

Name and Address of Sponsor: 3M Toxicology Services
Building 220-2E-02, 3M Center
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Name and Address of Testing Facility: 3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

Proposed Experimental Start Date: May 25, 1998

Proposed Experimental Termination Date: December 31, 2001

Method Numbers and Revisions

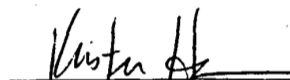
FACT-M-1.0, Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry

FACT-M-2.0, Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

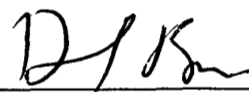
FACT-M-3.0, Extraction of Perfluorooctanesulfonate from Sera for Analysis using HPLC-Electrospray/Mass Spectrometry

FACT-M-4.0, Analysis of Sera Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

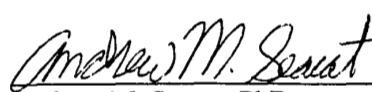
Author: Lisa Clemen


Kris J. Hansen, PhD
Study Director

5/22/98
Date


Dale Bacon
Study Director Management

7/15/99
Date


Andrew M. Seacat, PhD
Sponsor Representative

5/25/99
Date

1.0 PURPOSE

- 1.1** According to this analytical protocol, the 3M Environmental Laboratory will analyze the tissue and fluid samples from the Covance study number 6329-183, "104-Week Dietary Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats." The collected data will be provided to the sponsor for use in the assessment of toxicological effects of the test material when administered in the diets of rats for at least 104 weeks.
- 1.2** Data collected in the Environmental Laboratory will be considered non-quantitative screening data until future studies have been conducted to determine absolute recoveries of specific or general fluorochemical compounds.

2.0 REGULATORY COMPLIANCE

- 2.1** This analytical phase of the study will be conducted in accordance with the FDA Good Laboratory Practices Regulations 21 CFR 58, with the following exceptions:
- 2.1.1** The analytical phase is being conducted as a separate study and therefore has a separate Study Director, protocol, and final report, from those listed in the Covance protocol 6329-183.
- 2.1.2** The characterization of the reference material, including purity, identity, and stability, are the responsibility of the sponsor.
- 2.1.3** Sample storage stability will not be determined.

3.0 TEST MATERIALS

- 3.1 Control, and reference Materials and Matrices**
- 3.1.1 Analytical Reference Material:** T-6295, from 3M ICP/PCP Division
- 3.1.2 Analytical Reference Matrix:** Rat liver, from Covance and rat serum, from Sigma Chemical Company.
- 3.1.3 Analytical Control Material:** None.
- 3.1.4 Analytical Control Matrix:** Rat liver, from Covance and rat serum, from Sigma Chemical Company.
- 3.2 Number of Test and Control Samples:** Throughout the course of the study, liver and serum from 580 test animals and 140 control animals will be made available. Samples will be analyzed as requested by the sponsor or the study director. Other biological tissues (kidney, bile, dermal application site, and cellular fraction) will be available for analysis if deemed appropriate.
- 3.3 Identification of Test and Control Samples:** The samples will be identified using the Covance animal identification number that consists of a letter and five-digit number, plus the tissue identity and day identity (serum).
- 3.4 Purity and Identity of Reference Material:** To be determined by Sponsor.
- 3.5 Stability of Reference Material:** To be determined by Sponsor.

- 3.6 Storage Conditions for Reference Materials:** Reference materials will be stored at room temperature (3.1.1), and samples will be stored at $-20 \pm 10^{\circ}\text{C}$ (3.1.2, 3.1.4). Test and Control samples will be received according to AMDT-S-10-0.
- 3.7 Disposition of Specimens:** Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.
- 3.8 Safety Precautions:** Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting tissue samples.

4.0 EXPERIMENTAL - Overview

- 4.1** The tissues from animals dosed as described (Covance# 6329-183), will be available for analysis for fluorine-containing compounds. At the discretion of the Study Director, a series of analytical tests can be performed. All high dose and control sera and livers will be analyzed initially using HPLC-electrospray mass spectrometry to identify fluorine-containing compounds of interest present in the sera and liver (if any). The screening for organic fluoride in liver via combustion may be performed to present definitive data for fluorine in the liver. Based on the findings from these analyses, additional samples, tissues, or fluids may be analyzed at the discretion of the Study Director to determine the presence of fluorochemicals in these matrices.

5.0 EXPERIMENTAL - Methods

- 5.1 Methods (attached):**
- 5.1.1 FACT-M-1.0,** "Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"
 - 5.1.2 FACT-M-2.0,** "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry"
 - 5.1.3 FACT-M-3.0,** "Extraction of Perfluorooctanesulfonate from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry"
 - 5.1.4 FACT-M-4.0,** "Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry"

6.0 DATA ANALYSIS

- 6.1 Quality Control:** Matrix spikes will be extracted and analyzed to determine accuracy of the method. Also, continuing calibration checks will be analyzed to determine response bias.
- 6.2 Transformations:** Any transformations performed on data collected during the analytical phase of the study will be documented in the final report.
- 6.3 Statistics:** At the discretion of the Study Director, statistics used may include regression analysis of serum concentrations with time, averages, and standard deviations of concentrations for the different dose groups. If necessary, simple tests such as the Student's t-test may be applied to determine statistical difference. Any statistical analysis performed will be documented in the final report.
- 6.4 Data Reporting:** A final data package will be submitted to 3M Toxicology Services. The data package will include the following with additional data included as deemed appropriate.

- 6.4.1 A summary of individual sample results, reported as a concentration (weight/weight, weight/volume) of fluoride per tissue or fluid, or as the mass of a specific fluorochemical (HPLC-electrospray mass spectrometry) per unit of tissue or fluid.
- 6.4.2 A summary of quality control results (continuing calibration checks, method blanks, instrument blanks, matrix spikes, and matrix spike duplicates).
- 6.4.3 Certified copies or originals of the written validated methods.
- 6.4.4 Certified copies or originals of sample identification sheets sent from Covance.
- 6.4.5 Certified copies or originals of study specific raw data.
- 6.4.6 A summary of key personnel involved with the analytical phase of the study.
- 6.4.7 A signed QAU statement listing the dates of inspections and reports of findings to management and Study Director.

7.0 MAINTENANCE OF RAW DATA AND RECORDS

- 7.1 The following raw data and records (or certified copies thereof) will be maintained in the study folder in the archives according to appropriate SOPs.
 - 7.1.1 Approved protocol
 - 7.1.2 Approved methods
 - 7.1.3 Data summaries
 - 7.1.4 Study correspondence
 - 7.1.5 Shipping records
 - 7.1.6 Raw data
 - 7.1.7 Electronic copies of data
- 7.2 Supporting records to be retained separately from the study folder in the archives according to 3M ET & SS SOPs, will include, but not necessarily be limited to the following:
 - 7.2.1 Approved validation reports
 - 7.2.2 Training records
 - 7.2.3 Calibration records
 - 7.2.4 Instrument maintenance logs
 - 7.2.5 Standard operating procedures, equipment procedures, and methods
 - 7.2.6 Appropriate specimens

8.0 REFERENCES

- 8.1 Approved AMDT standard operating procedures.
- 8.2 Approved ETS standard operating procedures.

9.0 ATTACHMENTS

- 9.1 **FACT-M-1.0**, Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry

- 9.2 FACT-M-2.0, Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry**
- 9.3 FACT-M-3.0, Extraction of Perfluorooctanesulfonate from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry**
- 9.4 FACT-M-4.0, Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry**

**3M Environmental Technology
and Services**

PO Box 33331
St. Paul, MN 55133-3331
612 778 6442



Study Title

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid
Potassium Salt (PFOS T-6295) in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:

December 13, 1999

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS FACT-TOX-002
LRN U2121

3M Environmental Laboratory

Protocol FACT Tox-002
Amendment Number 1

This amendment modifies the following portion(s) of the protocol:

1. PROTOCOL READS:

The following methods will be used:

FACT-M-1.0, Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry

FACT-M-2.0, Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

FACT-M-3.0, Extraction of Perfluorooctanesulfonate from Sera for Analysis using HPLC-Electrospray/Mass Spectrometry

FACT-M-4.0, Analysis of Sera Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

AMEND TO READ:

The following methods will be used:

ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry

ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

REASON:

The methods originally listed were superseded during the course of the study.

Note: The methods as stated on page 1, in section 5.1, and section 9.0 will be/were amended. UC 12/19/00. (Accepted, Andrew M. Seacat 12/22/00)

Amendment Approval

Andrew M. Seacat
Andrew M. Seacat, Ph.D., Sponsor Representative

12/13/99
Date

Kristen Hansen
Kristen J. Hansen, Ph.D., Study Director

12/20/99
Date

3M Environmental Laboratory

Study Title

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid
Potassium Salt (PFOS T-6295) in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:

20 January 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS LRN-U2121
FACT TOX-002
Covance Study: 6329-183
3M Medical Department Study: T-6295.4

3M Environmental Laboratory

Protocol LRN-U2121
Amendment Number 2

This amendment modifies the following portion(s) of the protocol:

1. PROTOCOL READS:

There is no independent section of the protocol that addresses sample retention.

AMEND TO READ:

Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Any specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. Specimens analyzed at sub-contract laboratories will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:

To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

2. PROTOCOL READS:

Section 7 states that the following raw data and records will be retained in the study folder in the archives according to AMDT-S-8: Approved protocol and amendments; approved methods; data summaries; study correspondence; shipping records; raw data; and electronic copies of data. Additionally, Section 7 states that supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following: Approved validation reports; training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.

AMEND TO READ:

Section 7 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis."

REASON:

To direct subcontract laboratories in the disposition of the items listed above.

3M Environmental Laboratory

Protocol LRN-U2121
Amendment Number 2

3. PROTOCOL READS:

The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.

AMEND TO READ:

The role of study director for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000. The previous study director, Kristen Hansen, has been reassigned to the role of Principle Analytical Investigator.

REASON:

The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58).

4. PROTOCOL READS:

The sponsor for the present study was identified as Andrew M. Seacat, Ph.D.

AMEND TO READ:

The role of sponsor for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of 20 January 2000.

REASON:

The change was made at the request of the sponsor.

Protocol LRN-U2121
Amendment Number 2

Amendment Approval

Andrew M. Seacat 2/10/2000
Andrew M. Seacat, Ph.D., Outgoing Sponsor Representative Date

Kristen Hansen 11-Feb-2000
Kristen J. Hansen, Ph.D., Outgoing Study Director Date

Marvin T. Case 10 February 2000
Marvin T. Case, D.V.M., Ph.D., Incoming Sponsor Representative Date

John L. Butenhoff February 10, 2000
John L. Butenhoff, Ph.D., Incoming Study Director Date

3M Environmental Laboratory

Study Title

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt
(PFOS T-6295) in Rats

PROTOCOL AMENDMENT NO. 3

Amendment Date:

November 21, 2000

Performing Laboratory

3M Environmental Technology & Safety Services
3M Environmental Laboratory
935 Bush Avenue
St. Paul, MN 55106

Laboratory Project Identification

ET&SS LRN-U2121
FACT TOX-002
Covance 6329-183
3M Medical Department Study: T-6295

3M Environmental Laboratory

*Protocol FACT TOX-002
Amendment No. 3*

This amendment modifies the following portion(s) of the protocol:

1. PROTOCOL READS:

1.2 Data Collected in the Environmental Laboratory will be considered non-quantitative screening data until future studies have been conducted to determine absolute recoveries of specific or general fluorochemical compounds.

AMEND TO READ:

1.2 If matrix spike studies provide accurate representation of recovery of endogenous levels of PFOS, the 3M Environmental Laboratory will provide semi-quantitative data for sera and liver samples collected from test animals.

REASON:

Due to improved analytical methods, the decision was made to change the purpose of the study results from non-quantitative to semi-quantitative.

3M Environmental Laboratory

Protocol FACT TOX-002
Amendment No. 3

Amendment Approval

Marvin T Case 27 November 2000
Marvin T. Case, D.V.M., Ph.D., Sponsor Representative Date

John L. Butenhoff 28 NOV 2000
John L. Butenhoff, Ph.D., Study Director Date

3M Environmental Laboratory

Record of Deviation

I. Identification	
Study / Project No. TOX002 (LIMS #U2121)	
Deviation type (Check one)	
☐ SOP	☒ Method
☐ Equipment Procedure	
☐ Protocol ☐ Other:	
Document number ETS-8-7.0	Date(s) of occurrence 7/6/98, 5/25/00, 5/26/00, 6/12/00, 6/14/00, 6/13/00, 5/19/00, 5/22/00
II. Description	
Required procedure/process: Section 10.1.2: Analyze a method blank and a matrix blank prior to each calibration curve.	
Actual procedure/process: On several occasions, the blanks were either a) analyzed after the calibration curves or b) not analyzed at all for a particular MS run	
III. Actions Taken (such as amendment issued, SOP revision, etc.)	
Deviation written.	
Recorded by kh	Date 11/14/00
IV. Impact on Study / Project (completed by Study Director or Project Lead)	
All blanks were analyzed at least once during the course of the study. When blanks were run after the calibration curve, all control-of-bias practices were followed. In the instances where blanks were not run at all, the samples being analyzed were well above the typical run LOQ. In addition, the vast majority of samples run without blanks were high level dilutions. The function of the blank is to determine if contamination was introduced during extraction; any low-level contamination would be inconsequential to extracts requiring dilution. This deviation has no adverse affect on the study data.	
kh 11/14/00	
Authorized by Marvin T. Case	Date 20 Nov 2000 / John Z. Butenhoff 4 Nov 00

Sponsor: Marv Case

Study Director: John Butenhoff

Deviation No. 1

(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. <u>FACT-TOX-002</u>	
Deviation Type (Check one) ☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:	
Document Number	Date(s) of occurrence
<u>Collected FACT-M-2.0 + reduced ETS-8-7.0</u>	<u>07/14/98 original 02/02/00 rework</u>
II. Description: <u>Sample list 071498</u>	
Required Procedure/process:	
<u>Extracts should be diluted into the linear range of the instrument as depicted by the standard curve.</u>	
<u>Two consecutive failed CCVs invalidate the remaining portion of the sample list.</u>	
Actual Procedure/process:	
<u>Despite 1:200 dilutions, some G5/WK4 liver values were above the range of the standard curve. By the time this error was noticed, the sample extracts were over 2 years old. Also, although immediately surrounded by acceptable CCVs, the MS/MSD values were used even though, earlier in the run, 2 consecutive CCVs failed due to extremely high analyte levels in surrounding samples.</u>	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.)	
<u>Results will be noted as "tentative" in the final report.</u>	
Recorded By	Date <u>Kjh 10/09/00</u>
IV. Impact on Study / Project	
<u>G5/WK4 liver values will be considered to be tentative.</u> <u>Kjh 10/03/00</u>	
Authorized By (Study Director / Project Lead)	
<u>Marvin Case 11 Oct 2000</u>	<u>John Z. Bultenhoff 11 OCT 2000</u>

Sponsor Rep - Marvin Case
3M Environmental Laboratory
Form ETS-4-8.0

Study Director - John Bultenhoff

Deviation No. 2

(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. Covance 6329-183	
Deviation Type FACT-TOX-002 (Check one) ☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:	
Document Number FACT-M-4.0	Date(s) of occurrence 07/15/98 (Wk 4 sera)
II. Description:	
Required Procedure/process: The method states a continuing calibration verification will be analyzed after every 10th sample with a minimum of one per batch. Also, continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.	
Actual Procedure/process: None of the continuing calibration verifications were within $\pm 30\%$ recovery. The recoveries ranged from -32 to -38%.	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.) These data were flagged in the Excel spreadsheet and this deviation was written.	
Recorded By <i>Lisa A. Clem</i>	Date 11/27/00
IV. Impact on Study / Project	
Data is noted as non-compliant. Ejh 11/27/00	
Authorized By (Study Director / Project Lead) <i>Mawin Tan 29 Nov 2000</i> <i>John Butenhoff 30 NOV 2000</i>	Date

Sponsor: Mary Cox
3M Environmental Laboratory
Form ETS-4-8.0

Study Director: John Butenhoff

Deviation No. 3

(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. Covance Study Number: 6329-193/ 3M Project Number Tox002 8 (18) IAC 09/03/00	
Deviation Type (Check one)	☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:
Document Number: FACT-M-4.0	Date(s) of occurrence: 07/23/98
II. Description:	
Required Procedure/process: Method FACT-M.4.0, states 10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change ($\pm 30\%$) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.	
Calibration checks in this data packet (collected 071598b, on amelia) are not within the $\pm 30\%$ criteria.	
Actual Procedure/process: Calibration checks within $\pm 40\%$ criteria will be accepted for this data set. The 97 ppb standard points at the beginning and end of the run are excluded from the calibration curve due to the analyst adding the wrong amount of standard. This error appears in both curves and is therefore verified. Deletion of the 97 ppb curve point caused the calibration curve to have a greater r^2 value and an overall better fit to the points.	
III. Actions Taken: (such as amendment issued, SOP revision, etc.)	
This method deviation, increasing the criteria range for this data set is the only action that will need to be taken.	
This new data set will be used for final results.	
Recorded By: Harold Johnson	Date: September 5, 2000
IV. Impact on Study / Project	
<i>affected data will be noted in final results as meeting $\pm 40\%$ criteria.</i> <i>kin 09/05/00</i>	
Authorized By (Study Director / Project Lead)	Date
<i>John Z. Butenhoff 9/13/00</i> Study Director: John Butenhoff 3M Environmental Laboratory Form ETS-4-8.0	<i>Marvin T. Case 15 Sept 2000 kin 09/05/00</i> Sponsor Rep: Marvin Case Deviation No. <u>4</u> (assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. <u>FACT-Tox-002 Covance 6329-183</u>	
Deviation Type (Check one) ☐ SOP ☐ Method ☐ Equipment Procedure ☐ Protocol ☒ Other:	
Document Number <u>21 CFR 58.120 (a) (11)</u>	Date(s) of occurrence <u>05/22/99</u>
II. Description:	
Required Procedure/process: <u>Sponsor Representative approval of analytical protocol prior to Study</u> <u>Director approval</u>	
Actual Procedure/process: <u>The study Director signed/approved the analytical protocol before the</u> <u>Sponsor Representative</u>	
III. Actions Taken: (such as amendment issued, SOP revision, etc.) <u>In the future, analytical protocols will be signed by the Sponsor</u> <u>Representative before the Study Director</u>	
Recorded By <u>Lisa A. Clemen</u>	Date <u>07/27/99</u>
IV. Impact on Study / Project <u>This deviation will not adversely affect the outcome of this study</u>	
Authorized By (<u>Study Director</u> / Project Lead) <u>Karen H</u>	Date <u>7/28/99</u>

Record of Deviation

I. Identification	
Study / Project No. <u>FACT-TDX-002</u> <u>Covance 6329-183</u>	
Deviation Type (Check one) ☐ SOP ☐ Method ☐ Equipment Procedure ☒ Protocol ☐ Other:	
Document Number <u>Protocol FACT-TDX-002</u>	Date(s) of occurrence <u>11/10/99</u>
II. Description:	
Required Procedure/process: <u>Protocol states method FACT-M-3.0 will be used.</u>	
Actual Procedure/process: <u>Method ETS-8-4.1 was used.</u>	
III. Actions Taken: (such as amendment issued, SOP revision, etc.) <u>This Deviation was written/filled out.</u>	
Recorded By <u>Lisa A. Clemens</u>	Date <u>12/01/99</u>
IV. Impact on Study / Project <u>Method is improved; data will not be affected.</u>	
Authorized By (Study Director / Project Lead) <u>kjh</u>	Date <u>06 12/02/99</u> <u>r.e. kjh 12/06/99</u>

3M Environmental Laboratory
Form ETS-4-8.0Deviation No. 6
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. FACT-TOX-002Covance 6329-183	
Deviation Type (Check one) ☐ SOP ☐ Method ☐ Equipment Procedure ☒ Protocol ☐ Other:	
Document Number Protocol FACT-TOX-002	Date(s) of occurrence 11/11/99
II. Description:	
Required Procedure/process: Protocol states method FACT-M-1.D will be used	
Actual Procedure/process: Method ETS-8-6.D was used	
III. Actions Taken: (such as amendment issued, SOP revision, etc.)	
This deviation was written / filled out.	
Recorded By Lisa A. Clemen	Date 12/01/99
IV. Impact on Study / Project	
Data will not be affected.	
Authorized By (Study Director / Project Lead) gh	Date 12/06/99

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 7
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification	
Study / Project No. <i>TOX-002</i>	
Deviation Type (Check one)	☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:
Document Number <i>ETS-8-07.0</i>	Date(s) of occurrence <i>2/00 - 8/00</i>
II. Description:	
Required Procedure/process: <i>14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.</i>	
Actual Procedure/process: <i>Matrix spike recoveries were within $\pm 50\%$ of the spiked concentration.</i>	
III. Actions Taken: (such as amendment issued, SOP revision, etc.) <i>Deviant recoveries were verified, but were not reanalyzed; deviation will be noted in the final report.</i>	
Recorded By <i>ljh 10/05/00</i>	Date
IV. Impact on Study / Project <i>Quality of data for PFOS analysis in liver will be noted in the final report as being accurate to $\pm 50\%$</i> <i>ljh 10/05/00</i>	
Authorized By (Study Director / Project Lead) <i>Marv Case 11/7/00</i> <i>John Rutenhoff 11/7/00</i>	Date

Sponsor Rep - Marv Case
3M Environmental Laboratory
Form ETS-4-8.0

Study Director - John Rutenhoff

Deviation No. 8

(assigned by Study Director or Project Lead at the end of study or project)

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry (8 pages)

FACT-M-3.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry (8 pages)

ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry, (14 pages)

ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry, (14 pages)

FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry (8 pages)

FACT-M-4.0, Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry (8 pages)

ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry, (9 pages)

ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry, (10 pages)

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER ANIONIC FLUORO-CHEMICAL SURFACTANTS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

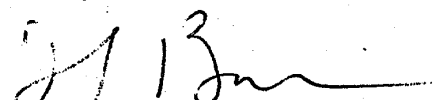
Method Number: FACT-M-1.0

Adoption Date: 5/26/98

Revision Date: N/A

Author: Lisa Clemen

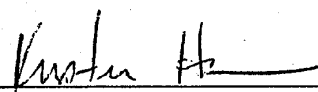
Approved By:



Laboratory Manager

5/26/98

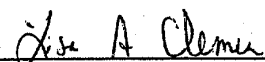
Date



Group Leader

5/26/98

Date



Technical Reviewer

5/27/98

Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of Potassium Perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other livers as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver using ion pairing reagent and 5.0 mLs of ethyl acetate. An ion pairing reagent is added to each sample and partitioned into ethyl acetate. Four mLs of extract is removed to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 µm filter into glass autovials.

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use universal precautions when handling animal livers, they may contain pathogens.

5.0 INTERFERENCES

- 5.1** There are no known interferences at this time.

6.0 EQUIPMENT

- 6.1** The following equipment is used while carrying out this method. Equivalent equipment is acceptable.

- 6.1.1** Ultra-Turrax T25 Grinder for grinding liver samples
- 6.1.2** Vortex mixer, VWR, Vortex Genie 2
- 6.1.3** Centrifuge, Mistral 1000 or IEC
- 6.1.4** Shaker, Eberbach or VWR
- 6.1.5** Nitrogen Evaporator, Organomation
- 6.1.6** Balance

7.0 SUPPLIES AND MATERIALS

- 7.1** Gloves
- 7.2** Dissecting scalpels
- 7.3** Eppendorf or disposable pipettes
- 7.4** Nalgene bottles, capable of holding 250 mL and 1 L
- 7.5** Glass, type A, volumetric flasks
- 7.6** 40 mL glass I-CHEM vials
- 7.7** Plastic sample vials, Wheaton, 6 mL
- 7.8** Polypropylene centrifuge tubes, 15 mL
- 7.9** Labels

- 7.10 Syringes, capable of measuring 10 μ L to 50 μ L
- 7.11 Glass, type A, volumetric pipettes
- 7.12 Graduated pipettes
- 7.13 Electronic pipettor, Eppendorf or equivalent
- 7.14 Timer
- 7.15 Disposable plastic 3 cc syringes
- 7.16 Filters, nylon syringe filters, 0.2 μ m, 25 mm
- 7.17 Crimp cap autovials

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-QTM water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-QTM water, mix until all solids are dissolved. Store in a 1 L nalgene bottle.
- 8.1.2 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 1:10. Measure 10 mL of the 10N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-QTM water. Store in a 125 mL nalgene bottle.
- 8.1.3 Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-QTM water. Adjust to pH 10 using approximately 64 mL 10N NaOH and dilute to volume with Milli-QTM water. Add NaOH slowly while adding the last 1 mL of NaOH because the pH changes abruptly. Store in a 1 L nalgene bottle.
 - 8.1.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.
- 8.1.4 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker or equivalent), ($\text{Na}_2\text{CO}_3/\text{NaHCO}_3$) 0.25M: Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate (NaHCO_3) into a 1 L volumetric flask and dilute to volume with Milli-QTM water. Store in a 1 L nalgene bottle.
- 8.1.5 PFOS (3M Specialty Chemical Division), molecular weight = 538.
- 8.1.6 Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.
- 8.1.7 Methanol, Omnisolv, glass distilled or HPLC grade.
- 8.1.8 Liver and control liver, received frozen from testing laboratory.
- 8.1.9 Milli-QTM water, all water used in this method should be Milli-QTM water and may be provided by a Milli-Q TOC Plus system.

8.2 Standards

- 8.2.1 Prepare PFOS standards for the standard curve.

- 8.2.2 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.2.3 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu\text{g/mL}$).
- 8.2.4 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.2.5 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.
- 8.2.6 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

9.0 SAMPLE HANDLING

- 9.1 All livers are received frozen and must be kept frozen until the extraction is performed.

10.0 QUALITY CONTROL

10.1 Matrix Spikes

- 10.1.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.
- 10.1.2 Prepare each spike using liver chosen by the analyst, usually a control liver.
- 10.1.3 Expected concentrations will fall in the mid-range of the initial calibration curve.

10.2 Continuing Calibration Checks

- 10.2.1 Prepare and analyze continuing calibration check samples to determine the continued linearity of the initial calibration curve.
- 10.2.2 One check is prepared per group of ten samples. For example, if a sample set = 34, four checks are prepared and extracted.
- 10.2.3 Prepare each continuing calibration check from the same liver homogenate used to prep the initial curve.
- 10.2.4 The expected concentration will fall within the mid-range of the initial calibration curve.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Liver Homogenate to Use for Standards

- 11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-QTM water. Grind to a homogeneous solution.
- 11.1.2 If 40 g is not available, use appropriate amounts of liver and water in keeping with a 1:5 ratio.
- 11.1.3 See section 13.0 to calculate the actual density of liver.

11.1.4 Add 1 mL of homogeneous solution to a 15 mL centrifuge tube. Re-suspend homogeneous solution by shaking between aliquots while preparing a total of sixteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

11.1.5 Two 1 mL aliquots serve as matrix blanks. Use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

Table 1 Approximate Spiking Amounts for Calibration Standards		
Working Standard (Approx. Conc.)	μL	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	4	0.010 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.000 ppm

11.1.1 See section 13.0 to calculate actual concentrations of PFOS in calibration standards.

11.2 Extract spiked liver homogenates following 12.14-12.24 of this method. Use these standards to establish each initial curve on the mass spectrometer.

12.0 PROCEDURES

12.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

12.2 Cut approximately 1 g of liver using a dissecting scalpel.

12.3 Weigh the sample directly into a tared plastic sample vial.

12.4 Record the liver weight in the study notebook.

12.5 Label the sample vial with the study number, weight, liver ID, date and analyst initials.

12.6 Add 2.5 mLs of water to sample vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds.

- 12.11 Pipette 1 mL homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sample vial. (See Worksheet for documenting the remaining steps.)
- 12.12 Spike liver homogenates with the appropriate amount of PFOS standard as described in section 11.1 or Table 1.
- 12.13 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as instrument blanks.
- 12.14 Add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.15 Using a volumetric pipette, add 5 mLs ethyl acetate.
- 12.16 Cap each sample and put on the shaker for 20 minutes.
- 12.17 Centrifuge for 20 to 25 minutes, until layers are well separated. Set power on the centrifuge to approximately 3500 rpm.
- 12.18 Remove 4 mLs of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in 12.5.
- 12.19 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.
- 12.20 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.
- 12.21 Vortex mix for 30 seconds.
- 12.22 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial.
- 12.23 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) who performed the extraction.
- 12.24 Cap and hold for electrospray mass spectrometry analysis.
- 12.25 Complete the worksheet and tape to page of study notebook.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.1 Calculate the density of liver (mg) in 1.0 mL homogenate using the following equation:

$$\frac{\text{g of Liver} \times \text{Average weight of ten 1 mL aliquots (mg)}}{(\text{g of Liver} + \text{g of Water})}$$

- 13.1.2** Calculate actual concentrations of PFOS in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration } (\mu\text{g / mL})}{\text{mg Liver}^* / 1 \text{ mL homogenate}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*Average weight of liver in solution as determined in 13.1.1, by weighing ten 1 mL homogenates of approximately 40 mg liver in 200 mL of Milli-Q water.

14.0 METHOD PERFORMANCE

- 14.1** The method detection limit is equal to half the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1** Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1** Complete the extraction worksheet and tape into the study notebook.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1** The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

18.0 REFERENCES

- 18.1** AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"

19.0 AFFECTED DOCUMENTS

- 19.1** FACT-M-2, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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Extraction Worksheet for FACT-M-1

[illegible]

FACT-M-1.0

Extraction of PFOS from Liver

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3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER ANIONIC FLUORO-CHEMICAL SURFACTANTS FROM SERUM FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

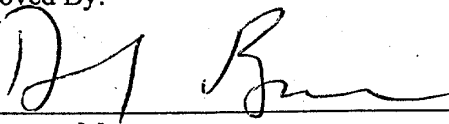
Method Number: FACT-M-3.0

Adoption Date: 4/22/98

Revision Date: N/A

Author: Lisa Clemen

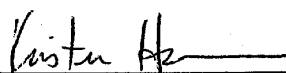
Approved By:



Laboratory Manager

4/22/98

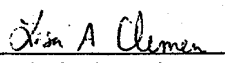
Date



Group Leader

4/22/98

Date



Technical Reviewer

4/22/98

Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, and bovine serum or other sera as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other anionic fluorochemical surfactants from serum using an ion pairing reagent and 5.0 mL of ethyl acetate. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into ethyl acetate. Four mL of extract are removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal serum, it may contain pathogens.

5.0 INTERFERENCES

- 5.1** There are no known interferences at this time.

6.0 EQUIPMENT

- 6.1** The following equipment is used while carrying out this method. Equivalent equipment is acceptable.
- 6.1.1** Vortex mixer, VWR, Vortex Genie 2
 - 6.1.2** Centrifuge, Mistral 1000 or IEC
 - 6.1.3** Shaker, Eberbach or VWR
 - 6.1.4** Nitrogen evaporator, Organomation
 - 6.1.5** Balance, ($\pm$ 0.100 gm)

7.0 SUPPLIES AND MATERIALS

- 7.1** Gloves
- 7.2** Eppendorf or disposable pipettes
- 7.3** Nalgene bottles, capable of holding 250 mL and 1 L
- 7.4** Glass, type A, volumetric flasks
- 7.5** 40 mL glass I-CHEM vials
- 7.6** Polypropylene centrifuge tubes, 15 mL
- 7.7** Labels
- 7.8** Syringes, capable of measuring 10 μ L to 50 μ L
- 7.9** Glass, type A, volumetric pipettes
- 7.10** Graduated pipettes

7.11 Electronic pipettor, Eppendorf or equivalent

7.12 Timer

7.13 Disposable plastic 3 cc syringes

7.14 Filters, nylon syringe filters, 0.2 μ m, 25 mm

7.15 Crimp cap autovials

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Sodium hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.1.2 Sodium hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 1:10. Measure 10 mL of 10N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.1.3 Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-Q™ water. Adjust to pH 10 using approximately 64 mL of 10N NaOH and dilute to volume with Milli-Q™ water. Add NaOH slowly while adding the last mL of NaOH because the pH changes abruptly. Store in a 1 L Nalgene bottle.

8.1.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.

8.1.4 Sodium carbonate/sodium bicarbonate buffer (J.T. Baker or equivalent), ($\text{Na}_2\text{CO}_3/\text{NaHCO}_3$) 0.25M: Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate (NaHCO_3) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

8.1.5 PFOS (3M Specialty Chemical Division), molecular weight = 538.

8.1.6 Other fluorochemicals, as appropriate.

8.1.7 Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.

8.1.8 Methanol, Omnisolv, glass distilled or HPLC grade.

8.1.9 Serum, frozen liquid from Sigma.

8.1.10 Control serum received with each sample set.

8.1.11 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.2 Standards

- 8.2.1 Prepare PFOS standards for the standard curve.
- 8.2.2 Prepare other fluorochemical standards, as appropriate.
- 8.2.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.2.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu\text{g/mL}$).
- 8.2.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.2.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.
- 8.2.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

9.0 SAMPLE HANDLING

- 9.1 All sera are received frozen and must be kept frozen until the extraction is performed.

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

- 10.1.1 Two 1.0 mL aliquots of the serum are extracted following this procedure and used as matrix blanks. See section 11.1.2.
- 10.1.2 Two 1.0 mL aliquots of Milli-Q™ water are extracted following this procedure and used as method blanks.

10.2 Matrix Spikes

- 10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.
- 10.2.2 Prepare each spike using serum chosen by the analyst, usually control serum received with each sample set.
- 10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

- 10.3.1 Prepare and analyze continuing calibration check samples to determine the continued linearity of the initial calibration curve.
- 10.3.2 One check is prepared per group of ten samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same serum used to prep the initial curve.

10.3.4 The expected concentration will fall within the mid-range of the initial calibration curve.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Serum Standards

11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.

11.1.2 If the majority of serum sample volumes are less than 1.0 mL, extract standards using serum volumes in the standards equal to the serum volumes in samples. Do not extract below 0.50 mL of serum. Record the serum volume on the extraction sheet.

11.1.3 Mix or shake between aliquots while preparing a total of sixteen aliquots of serum in 15 mL centrifuge tubes.

11.1.4 Two 1 mL or appropriate aliquots serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

11.1.5 Refer to the validation report FACT-M-3.0-V-1 and FACT-M-4.0-V-1 which lists the working ranges for calibration curves.

Table 1 Approximate Spiking Amounts for Standards and Spikes Using 1.0 mL of Serum		
Working Standard (Approx. Conc.)	µL	Approx. final conc. of PFOS in serum
-	-	Blank
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm

11.1.4 See section 13.0 to calculate actual concentrations of PFOS in calibration standards.

11.2 Extract spiked serum standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

12.0 PROCEDURES

- 12.1 Obtain frozen serum samples and allow to thaw.
- 12.2 Vortex mix for 15 seconds then remove 1.0 mL or appropriate volume to a 15 mL polypropylene centrifuge tube.
- 12.3 Return serum samples to freezer after extraction amount has been removed.
- 12.4 Record the serum volume on the extraction worksheet. The final methanol volume will equal the initial serum volume.
- 12.5 Label the tube with the study number, serum ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike serum with the appropriate amount of PFOS standard as described in section 11.1 or Table I for the calibration curve standards. Also spike matrix spikes and continuing calibration standards.
- 12.7 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.8 Add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.9 Using a volumetric pipette, add 5 mL ethyl acetate.
- 12.10 Cap each sample and put on the shaker for 20 minutes.
- 12.11 Centrifuge for 20 to 25 minutes, until layers are well separated. Set power on the centrifuge to approximately 3500 rpm.
- 12.12 Transfer 4 mL of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in 12.5.
- 12.13 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.
- 12.14 Add 1.0 mL or appropriate volume of methanol to each centrifuge tube using a graduated pipette. (This volume equals the initial volume of serum used for the extraction.)
- 12.15 Vortex mix for 30 seconds.
- 12.16 Attach a 0.2 μ m nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial.
- 12.17 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) who performed the extraction.
- 12.18 Cap and hold for HPLC-electrospray/mass spectrometry analysis. Extracts may be stored at 4° C until analysis.
- 12.19 Complete the extraction worksheet, attached to this document, and tape to page of study notebook.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.1 Calculate actual concentrations of PFOS, or other appropriate fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of Standard} \times \text{Concentration } (\mu\text{g/mL})}{\text{mL of Standard} + \text{Initial Serum Volume (mL)}} = \text{Final Concentration } (\mu\text{g/mL}) \text{ of PFOS in Serum}$$

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit is equal to half the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Complete the extraction worksheet attached to this method, and tape into the study notebook.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 The validation report associated with this method is FACT-M-3.0 & 4.0-V-1.

18.0 REFERENCES

- 18.1 None

19.0 AFFECTED DOCUMENTS

- 19.1 FACT-M-4, "Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision</u> <u>Number.</u>	<u>Reason For Revision</u>	<u>Revision</u> <u>Date</u>
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Extraction Worksheet for FACT-M-3

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FACT-M-3.0

Extraction of PFOS from Serum

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3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

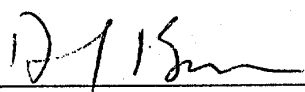
Method Number: ETS-8-4.1

Adoption Date: 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

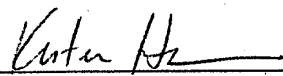
Approved By:



Laboratory Manager

4/27/99

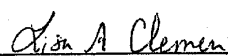
Date



Group Leader

4/26/99

Date



Technical Reviewer

04/26/99

Date

1.0 SCOPE AND APPLICATION

- 1.1 **Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.
- 1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.
- 1.3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see 3.0 *Definitions*). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 DEFINITIONS

- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and safety warnings

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Vortex mixer, VWR, Vortex Genie 2
- 6.1.2 Centrifuge, Mistral 1000 or IEC
- 6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

- 7.1 Gloves
- 7.2 Eppendorf or disposable pipettes
- 7.3 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.4 Volumetric flasks, glass, type A
- 7.5 I-CHEM vials, glass, 40 mL glass
- 7.6 Centrifuge tubes, polypropylene, 15 mL
- 7.7 Labels
- 7.8 Oxford Dispenser – 3.0 to 10.0 mL
- 7.9 Syringes, capable of measuring 5 μ L to 50 μ L
- 7.10 Graduated pipettes
- 7.11 Syringes, disposable plastic, 3 cc
- 7.12 Syringe filters, nylon, 0.2 μ m, 25 mm
- 7.13 Timer
- 7.14 Crimp cap autovials and caps
- 7.15 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T. Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO_3), J.T. Baker or equivalent
- 8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Serum or blood, frozen from supplier
- 8.9 **Fluorochemical standards**
 - 8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
 - 8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

- 8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H, 1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428
- 8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-QTM water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-QTM water. Store in a 125 mL Nalgene bottle.
- 8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric flask containing 500 mL Milli-QTM water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-QTM water. Store in a 1 L Nalgene bottle.
 - 8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-QTM water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

- 8.11.1 Prepare PFOS standards for the standard curve.
- 8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu g/mL$).
- 8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method blanks and matrix blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See 11.1.4.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

- 10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

- 11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.
- 11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.
- 11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.
- 11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.
- 11.1.5 Refer to validation report ETS-8-4.0 & ETS-8-5.0-V-1, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.
- 11.3 Extract spiked matrix standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	μL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

- 12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.
- 12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.
- 12.3 Return unused samples to freezer after extraction amounts have been removed.
- 12.4 Record the initial volume on the extraction worksheet.
- 12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in 11.2.
- 12.7 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.
- 12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

- 12.14 Label a fresh 15 mL centrifuge tube with the same information as in 12.5.
- 12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.
- 12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.
- 12.18 Vortex mix for 30 seconds.
- 12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations

- 13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard} \times \text{concentration of standard } (\mu\text{g / mL})}{\text{mL of standard} + \text{mL of surrogate standard} + \text{initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
 - 14.2.1 Method blanks and matrix blanks.
 - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
 - 14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 ATTACHMENTS

- 17.1 Attachment A, Extraction worksheet
17.2 Attachment B, MDL/LOQ values and summary
17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

- 18.1 The validation report associated with this method is ETS-8-4.0 & 5.0-V-1.
18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL.	04/02/99

Extraction Worksheet ETS-8-4.1

[illegible]

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

MDL/LOQ values in rat, bovine, monkey, and human serum, and monkey plasma were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit serum curves to determine equivalence. Responses in the rat, bovine, monkey, and human were equivalent to the rabbit responses, therefore, their MDL and LOQ will be the same values as determined in rabbit serum.

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

Compound: PFOS

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.995 - 978	24.8 - 978	83-108	4.67-11.0
Low Curve	4.94 - 248	4.94 - 248	85-104	5.34-12.0
High curve	97.8 - 978	97.8 - 978	85-106	4.84-9.80
1/X	0.995 - 978	4.94 - 978	94-111	4.60-10.5

Compound: PFOSA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	4.93 - 976	88-103	5.10-14.7
Low Curve	4.93 - 97.6	4.93 - 97.6	87-105	9.85-14.7
High curve	24.8 - 976	24.8 - 978	93-102	5.08-13.9
1/X	0.993 - 976	4.93 - 976	94-103	5.10-14.5

Compound: PFOSAA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.991 - 974	24.7 - 974	81-111	4.18-10.6
Low Curve	4.92 - 247	9.74 - 247	97-107	6.38-21.8
High curve	49.2 - 974	97.4 - 974	85-108	4.33-12.5
1/X	0.991 - 974	9.74 - 974	95-115	4.11-23.2

Compound: EtFOSE-OH

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	49.3 - 976	77-110	11.2-25.5
Low Curve	4.93 - 97.6	9.76 - 97.6	97-107	14.1-21.3
High curve	49.3 - 976	97.6 - 976	90-109	11.5-19.6
1/X	0.993 - 493	9.76 - 976	86-111	11.1-21.2

Compound: PFOSEA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	96-106	10.1-16.2
Low Curve	4.93 - 248	9.76 - 248	91-110	11.8-19.5
High curve	49.3 - 976	49.3 - 976	86-106	10.2-18.2
1/X	0.993 - 976	9.76 - 976	95-117	10.1-19.1

Compound: M556

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	24.8 - 976	88-106	4.82-17.9
Low Curve	4.93 - 97.6	9.76 - 97.6	100-105	5.95-18.2
High curve	97.6 - 976	97.6 - 976	81-111	5.11-9.74
1/X	0.993 - 976	9.76 - 976	97-110	4.77-19.5

Ion Pair Standard Curves – Fluids

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Blank fluid/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Am't spiked mL	All Final vol mL
0.500	0.507	0.532	0.501	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked mL
4.93	5.00	5.24	4.94	5.01	5.13	100	0.005
9.76	9.89	10.4	9.78	9.93	10.2	Surrogate Final conc ng/mL 500	
24.8	25.1	26.3	24.8	25.2	25.8		
49.3	50.0	52.4	49.4	50.1	51.3		
97.6	98.9	104	97.8	99.3	102		
248	251	263	248	252	258		
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges -- approximate concentrations

Serum	PFOS	PFOSA	PFOSAA	EtFOSE-OH	PFOSEA	M556
Rabbit	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000	5.00-1000
Bovine	Estimates only. Use values for rabbit.					
Rat	Estimates only. Use values for rabbit.					
Monkey & Plasma	Estimates only. Use values for rabbit.					
Human	Estimates only. Use values for rabbit.					

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemical COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

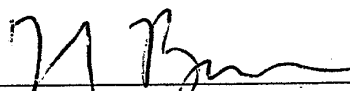
Method Number: ETS-8-6.0

Adoption Date: 07/22/99

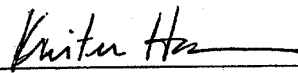
Revision Date: NR

Author: Lisa Clemen, Robert Wynne

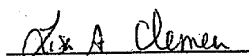
Approved By:



Laboratory Manager7/22/99

Date

Group Leader7/14/99

Date

Technical Reviewer07/14/99

Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 DEFINITIONS

- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3$
- 3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples
- 6.1.2 Vortex mixer, VWR, Vortex Genie 2
- 6.1.3 Centrifuge, Mistral 1000 or IEC
- 6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

- 7.1 Gloves
- 7.2 Dissecting scalpels
- 7.3 Eppendorf or disposable pipettes
- 7.4 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.5 Volumetric flasks, glass, type A
- 7.6 I-CHEM vials, 40 mL glass
- 7.7 Plastic sample vials, Wheaton, 6 mL (or appropriate size)
- 7.8 Centrifuge tubes, polypropylene, 15 mL
- 7.9 Labels
- 7.10 Oxford Dispensor – 3.0 to 10.0 ml
- 7.11 Syringes, capable of measuring 5 µL to 50 µL
- 7.12 Graduated pipettes
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2 µm, 25 mm
- 7.15 Timer
- 7.16 Crimp cap autovials and caps
- 7.17 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T. Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na₂CO₃), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO₃), J.T. Baker or equivalent
- 8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Liver, frozen from supplier
- 8.9 Dry ice from supplier
- 8.10 Fluorochemical standards
 - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

- 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
- 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428
- 8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.
 - 8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ($Na_2CO_3/NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

- 8.12.1 Prepare PFOS standards for the standard curve.
- 8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ($\mu g/mL$).
- 8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

10.0 QUALITY CONTROL

10.1 Matrix blanks and method blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of liver homogenate following this procedure and use as matrix blanks. Refer to 11.1.6.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually a control liver received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration verifications

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification sample per group of 10 samples. For example, if a sample set = 34, four verifications are prepared and extracted.

- 10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.
- 10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

- 11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.
- 11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.
- 11.1.3 Refer to 13.0 to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.
- 11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.
- 11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.
- 11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.
- 11.1.8 Refer to validation reports ETS-8-6.0 and ETS-8-7.0-V-1 or Attachment B, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.9 Use Attachment C as an aid in calculating the concentrations of the working standards. Refer to 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

- 11.3 Extract spiked liver homogenates following 12.14-12.25 of this method. Use these standards to establish each initial curve on the mass spectrometer.

Table 1 Approximate Spiking Amounts for Calibration Standards		
Working Standard (Approx. Conc.)	μ l	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

12.3 Weigh the sample directly into a tared plastic sample vial.

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sample vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sample vial with the study number, weight, liver ID, date and analyst initials.

- 12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sample vial. Refer to attached worksheet for documenting the remaining steps.
- 12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.
- 12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section 11.2.
- 12.14 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.
- 12.21 Label a fresh 15 mL centrifuge tube with the same information as in 12.10.
- 12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.
- 12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.
- 12.25 Vortex mix for 30 seconds.
- 12.26 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{1.0 \text{ mL aliquot}}$$

- 13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver} \times \text{Average density* of homogenate (mg/mL)}}{(\text{g of Liver} + \text{g of Water})}$$

* refer to 13.1.1 for details.

- 13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration } (\mu\text{g / mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*refer to 13.1.2 for details.

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration verification samples to determine the continued accuracy of the initial calibration curve.

- 14.3 Refer to section 14 of ETS-8-7.0 for method performance criteria.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A, Extraction worksheet
- 17.2 Attachment B, MDL/LOQ values and summary
- 17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

- 18.1 The validation report associated with this method is ETS-8-6.0 & 7.0-V-1.
- 18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"
- 18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-7.0, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision
Number

Reason For Revision

Revision
Date

[illegible]

MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	8.45	26.9	30 ppb – 1200 ppb
PFOSA	3.50	11.1	12 ppb – 1200 ppb
PFOSAA	24.6	78.3	30 ppb – 1200 ppb
EtFOSE-OH	108	345	60 ppb – 900 ppb*
M556	82.3	262	60 ppb – 1200 ppb
PFOSEA	33.9	108	30 ppb- 1200 ppb

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information

* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	6 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	12 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSAA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.16 - 1232	12 - 1200	30 - 1200	30 - 900	60 - 900	N/A	N/A

Compound: EtFOSE-OH

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 900	31 - 900	N/A	N/A	N/A	N/A

Compound: PFOSEA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	31 - 1200	N/A	N/A	N/A	N/A

Compound: M556

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	60 - 1200	N/A	N/A	N/A	N/A

Ion Pair Standard Curves – Tissue

Prep date(s):

Analyte(s):

Sample matrix:

Standard number:

Equipment number:

Final solvent and TN:

Blank liver/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density g
0.500	0.500	0.500	0.500	0.500	0.500		0.002	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.004	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.010	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.020	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.040	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.010	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.020	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.030	0.167
50.0	50.0	50.0	50.0	50.0	50.0		0.004	0.167

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked mL
5.99	5.99	5.99	5.99	5.99	5.99		100	0.005
12.0	12.0	12.0	12.0	12.0	12.0		Surrogate Final conc ng/mL 0.500	
29.9	29.9	29.9	29.9	29.9	29.9			
59.9	59.9	59.9	59.9	59.9	59.9			
120	120	120	120	120	120			
299	299	299	299	299	299			
599	599	599	599	599	599			
898	898	898	898	898	898			
1198	1198	1198	1198	1198	1198			

Validated ranges – approximate concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Bovine	Estimates only, use rabbit values.					
Rat	Estimates only, use rabbit values.					
Monkey	Estimates only, use rabbit values.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF FLUOROchemicals IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

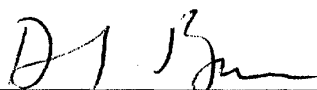
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Adoption Date: 5/26/98

Revision Date: N/A

Author: Lisa Clemen

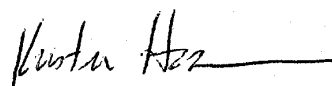
Approved By:



Laboratory Manager

5/26/98

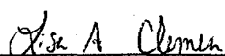
Date



Group Leader

5/26/98

Date



Technical Reviewer

5/27/98

Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of extracts of liver or other tissues for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Potassium perfluorooctanesulfonate, anionic fluorochemical surfactants, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other livers as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, $M/Z = 499$. Samples may also be screened to verify compound identification.

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

4.2 Cautions:

- 4.2.1** Do not run solvent pumps above capacity of 400 bar (5800 psi). If pressure goes over 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2** Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1** Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1** Equipment listed below may be changed in order to optimize the system.
- 6.1.1** Micromass Electrospray Mass Spectrometer
- 6.1.2** HP1100 low pulse solvent pumping system and autosampler.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1** Nitrogen gas, refrigerated liquid, regulated to approximately 100 psi.
- 7.1.2** HPLC column, specifics to be determined by the analyst.
- 7.1.3** Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1** Methanol, HPLC grade or equivalent.

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

8.2.1 Typically one H₂O blank, one liver blank, and seven liver standards are prepared during the extraction procedure. See FACT-M-1.

9.0 SAMPLE HANDLING

9.1 Fresh liver standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be refrigerated until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

10.1.1 Analyze a method blank and matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate with each analysis.

10.2.2 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change ($\pm 30\%$) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

10.4.1 System suitability (e.g. peak area, retention time and peak shape, etc.) will be assessed for each run.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted liver standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression for the calibration curve using MassLynx or other suitable software.

- 11.2 The r^2 value for the data should be 0.98 or greater. Lower values may be acceptable at the discretion of the analyst.
- 11.3 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

12.0 PROCEDURES

12.1 Acquisition Set up

- 12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using letter-MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.
- 12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A scan is usually collected along with the SIRs. Save method.
- 12.1.3 Typically the sample list begins with the first set of liver standards and ends with the second set of standards.
- 12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

- 12.2.1 Set up sample tray according to the sample list prepared in section 12.1.1.
- 12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:
- 12.2.2.1 Sample size = 10 μ L injection with a sample wash
- 12.2.2.2 Inject/sample = 1
- 12.2.2.3 Cycle time = 15 minutes
- 12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	45%	55%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 min.	45%	55%

Note: In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

- 12.2.2.5 Press the "Start" button.

12.3 Instrument Sep-up

- 12.3.1 Refer to AMDT-EP-31 for more details.
- 12.3.2 Check the solvent level in reservoirs and refill if necessary.
- 12.3.3 Check the stainless steel capillary at the end of the probe. Use an eye piece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.
- 12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
 - 12.3.6.1 Drying gas 250-400 liters/hour
 - 12.3.6.2 ESI nebulizing gas 10-15 liters/hour
 - 12.3.6.3 LC constant flow mode flow rate 10 - 500 uL/min
 - 12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the instrument is operating correctly.)
- 12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8 Record tune parameters in the instrument log.
- 12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10 Click on start button in the Acquisition Control Panel. Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.1 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.2 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.3 Calculate actual concentration of PFOS anion in total liver (mg):

$$\frac{\left(\frac{\text{ug PFOS anion calc. from std curve}}{\text{g of liver used for analysis}} \right)}{1000 \text{ ug / 1 mg}} \times \text{Total mass of liver (g)}$$

14.0 METHOD PERFORMANCE

- 14.1 The method detection limit is equal to at least three times the baseline noise in the matrix blank.
- 14.2 The practical quantitation limit is equal to the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers. All containers are located in the laboratory.

16.0 RECORDS

- 16.1 Store chromatograms in the study folder. Each chromatogram should have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).
- 16.2 Plot calibration curve by linear regression and store in the study folder.
- 16.3 Print sample list from MassLynx and tape into the instrument runlog.
- 16.4 Print data integration summary from MassLynx and tape into the instrument runlog.
- 16.5 Copy instrument runlog pages, including instrument parameters and sample results, and tape into appropriate study notebook.
- 16.6 Summarize data using suitable software and store in the study folder.
- 16.7 Back up electronic data to appropriate media. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A: FACT-M-2 Data reporting spreadsheet
- 17.2 The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

18.0 REFERENCES

- 18.1 AMDT-EP-31, "Operation of VG Platform Electrospray Mass Spectrometer"

19.0 AFFECTED DOCUMENTS

19.1 FACT-M-1.0, "Extraction of Potassium Perfluorooctanesulfonate from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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Laboratory Study #

Study:

Test Material:

Matrix/Final Solvent:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Filename:

R-Squared Value:

Slope:

Y Intercept:

Date of Extraction/Analyst:

Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.**Group/Dose:** Taken from the study folder.**Sample#:** Taken from the study folder.**Concentration (ug/mL):** Taken from the MassLynx integration summary.**Initial Volume (mL):** Taken from the study folder.**Dilution Factor:** Taken from the study folder.**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF FLUOROchemicals IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

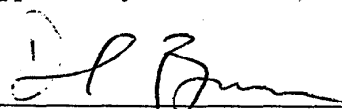
Method Number: FACT-M-4.0

Adoption Date: 4/22/98

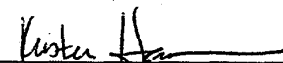
Revision Date: N/A

Author: Lisa Clemen

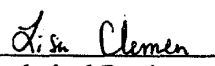
Approved By:


Laboratory Manager

4/22/98
Date


Group Leader

4/22/98
Date


Technical Reviewer

4/14/98
Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of extracts of serum or tissue for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Potassium perfluorooctanesulfonate, anionic fluorochemical surfactants, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, and bovine serum or other sera as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from serum using HPLC-electrospray/mass spectrometry. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, $M/Z = 499$. Samples may also be screened to verify compound identification.

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

4.2 Cautions:

- 4.2.1** Do not run solvent pumps above capacity of 400 bar (5800 psi). If pressure goes over 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2** Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1** Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1** Equipment listed below may be changed in order to optimize the system.

- 6.1.1** Micromass Electrospray Mass Spectrometer
- 6.1.2** HP1100 low pulse solvent pumping system and autosampler.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1** Nitrogen gas, refrigerated liquid, regulated to approximately 100 psi.
- 7.1.2** HPLC column, specifics to be determined by the analyst.
- 7.1.3** Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1** Methanol, HPLC grade or equivalent.

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

8.2.1 Typically one H₂O blank, one serum blank, and seven serum standards are prepared during the extraction procedure. See FACT-M-3.

9.0 SAMPLE HANDLING

9.1 Fresh serum standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be refrigerated at 4° C until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

10.1.1 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate with each analysis.

10.2.2 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change ($\pm 30\%$) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

10.4.1 System suitability (e.g., peak area, retention time, peak shape, etc.) will be assessed for each run.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted serum standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression for the calibration curve using MassLynx or other suitable software.

11.2 The r^2 value for the data should be 0.98 or greater. Lower values may be acceptable at the discretion of the analyst.

11.3 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using letter-MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A scan is usually collected along with the SIRs. Save method.

12.1.3 Typically the sample list begins with the first set of serum standards and ends with the second set of standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in section 12.1.1.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 μ L injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 15 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	45%	55%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 min.	45%	55%

Note: In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

- 12.3.1 Refer to AMDT-EP-31 for more details.
- 12.3.2 Check the solvent level in reservoirs and refill if necessary.
- 12.3.3 Check the stainless steel capillary at the end of the probe. Use an eye piece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.
- 12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
 - 12.3.6.1 Drying gas 250-400 liters/hour
 - 12.3.6.2 ESI nebulizing gas 10-15 liters/hour
 - 12.3.6.3 HPLC constant flow mode flow rate 10 – 500 µL/min
 - 12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8 Record tune parameters in the instrument log.
- 12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10 Click on start button in the Acquisition Control Panel. Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6** Calculate actual concentration of PFOS, or other fluorochemical, anion in serum (µg/mL):

$$\frac{\mu\text{g of PFO calc. from std. Curve}}{\text{Initial Volume of serum (mL)}} \times \text{Dilution Factor} \times \text{Final Volume (mL)}$$

14.0 METHOD PERFORMANCE

- 14.1** The method detection limit is equal to half the lowest standard in the calibration curve.
- 14.2** The practical quantitation limit is equal to the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1** Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1** Store chromatograms in the study folder. Each chromatogram must have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).
- 16.2** Plot calibration curve by linear regression and store in the study folder.
- 16.3** Print sample list from MassLynx and tape into the instrument runlog.
- 16.4** Print data integration summary from MassLynx and tape into the instrument runlog.
- 16.5** Copy instrument runlog pages, including instrument parameters and sample results, and tape into appropriate study notebook.
- 16.6** Summarize data using suitable software and store in the study folder.
- 16.7** Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1** Attachment A: FACT-M-4 Data reporting spreadsheet
- 17.2** The validation report associated with this method is FACT-M-3.0 & 4.0-V-1.

18.0 REFERENCES

- 18.1** AMDT-EP-31, "Operation of VG Platform Electrospray Mass Spectrometer"

19.0 AFFECTED DOCUMENTS

- 19.1** FACT-M-3.0, "Extraction of Fluorochemical Anions from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision
Number.Reason For RevisionRevision
Date

Laboratory Study #

Study:

Test Material:

Matrix/Final Solvent:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Filename:

R-Squared Value:

Slope:

Y Intercept:

Date of Extraction/Analyst:

Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.**Group/Dose:** Taken from the study folder.**Sample#:** Taken from the study folder.**Concentration (ug/mL):** Taken from the MassLynx integration summary.**Initial Volume (mL):** Taken from the study folder.**Dilution Factor:** Taken from the study folder.**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

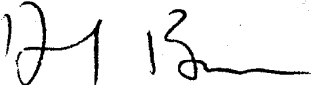
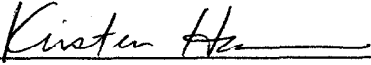
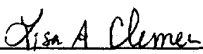
Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

	4/26/99
Laboratory Manager	Date
	4/26/99
Group Leader	Date
	04/26/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, $m/z = 499$. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

3.0 DEFINITIONS

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.
- 4.1.2** When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

- 4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
 - 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source
 - 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.
- 7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

- 8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See ETS-8-4.1.

9.0 SAMPLE HANDLING

- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

- 9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted $1/x$, not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

- 12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.
- 12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).
- 12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.
- 12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

- 12.2.1 Set up sample tray according to the sample list prepared in Section 12.1.1.
- 12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:
- 12.2.2.1 Sample size = 10 μ L injection
- 12.2.2.2 Inject/sample = 1
- 12.2.2.3 Cycle time = 13.5 minutes
- 12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.50 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

- 12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

- 12.3.1 Refer to ETS-9-24.0 for more details.
- 12.3.2 Check the solvent level in reservoirs and refill if necessary.

- 12.3.3** Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4** Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5** Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.
- 12.3.6** The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
- 12.3.6.1** Drying gas 250-400 liters/hour
 - 12.3.6.2** ESI nebulizing gas 10-15 liters/hour
 - 12.3.6.3** HPLC constant flow mode, flow rate 10 – 500 µL/min
 - 12.3.6.4** Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7** Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8** Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9** Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10** Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

- 13.1.4** Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5** Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6** Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{(\text{Initial Volume of matrix (mL)} + \text{mL of Surrogate Standard})} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \text{Final Volume (mL)}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-4.1, Attachment B, for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration curve must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries are must be within $\pm 30\%$ of the spiked concentration.

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be $\pm 30\%$ of the spiked concentration.

14.6 If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

16.3 Plot the calibration curve by linear regression, weighted $1/x$, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

- 16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 REFERENCES

- 18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

Laboratory Study #

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.

Group/Dose: Taken from the study folder.

Sample#: Taken from the study folder.

Concentration (ug/mL): Taken from the MassLynx integration summary.

Initial Volume (mL): Taken from the study folder.

Dilution Factor: Taken from the study folder.

Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

Study #: FACT-TOX-002

3M Environmental Lab -- Method Modification

Method:

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Sera Extracts Using HPLC-Electrospray/Mass Spectrometry"

Section modified: 10.3.2, 14.5.1, add sections 14.3.2-14.3.6

Effective date of modifications: April 26, 1999

Section 10.3.2

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

Section 14.5.1

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within $\pm 30\%$ of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

Study #: FACT-TOX-002

Section 14.3.3

Method reads:

NA

Modify method to read:

- 14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

Section 14.3.4

Method reads:

NA

Modify method to read:

- 14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

Section 14.3.5

Method reads:

NA

Modify method to read:

- 14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

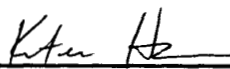
Section 14.3.6

Method reads:

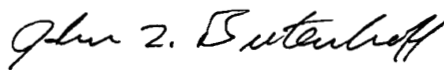
NA

Modify method to read:

- 14.3.6 A valid calibration curve must contain at least 5 active points.

 11/29/00
Signature of PAI and date

 12 Dec 2000
Signature of Sponsor and date

 13 DEC 2000
Signature of Study Director and date

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

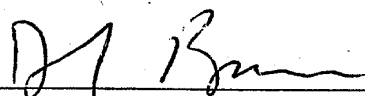
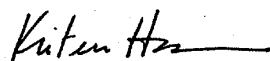
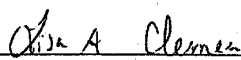
Method Number: ETS-8-7.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:


Laboratory Manager7/22/99
Date
Group Leader7/14/99
Date
Technical Reviewer07/14/99
Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of liver extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey liver, or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, $m/z = 499$. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

3.0 DEFINITIONS

- 3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).
- 3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.
- 3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)
- 3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

- 4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

- 4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

- 4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

- 5.1 To minimize interferences when analyzing samples, Teflon shall not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
- 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.
- 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

- 7.1.1 High purity grade air regulated to approximately 100 psi (house air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data
- 7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent
- 8.1.3.1 When preparing different amounts than those listed, adjust accordingly.
- 8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

- 8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to ETS-8-6.0.

9.0 SAMPLE HANDLING

- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.
- 9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Method Blanks and Matrix Blanks

- 10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.
- 10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

- 10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.
- 10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.
- 10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.
- 10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

- 10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.
- 10.3.2 Analyze a mid-range calibration standard every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

- 11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted $1/x$, not forced through the origin, using MassLynx or other suitable software.
- 11.2 If the curve does not meet requirements perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

- 11.3** For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration verification injected standard after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section 12.1.1.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 μ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 mi.	40%	60%

12.2.2.5 Press the "Start" button.**12.3 Instrument Set-up**

12.3.1 Refer to ETS-9-24.0, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

12.3.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 – 500 µL/min

12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentrations in matrix (µg/g):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\frac{(\text{Initial Weight of Liver (g)})}{\text{Final Volume (mL)}}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to ETS-8-6.0, Attachment B for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

- 14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

- 15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

16.0 RECORDS

- 16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.
- 16.4 Print data integration summary, integration method, and chromatograms from MassLynx and store in the study folder.
- 16.5 Summarize data using suitable software (Excel 5.0+) and store in the study folder, refer to **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A: ETS-8-7.0 Data summary spreadsheet

18.0 REFERENCES

- 18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
----------------------------	----------------------------	--------------------------

Laboratory Study #

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

Slope: Taken from linear regression equation.

Group/Dose: Taken from the study folder.

Sample#: Taken from the study folder.

Concentration (ng/g): Taken from the MassLynx integration summary.

Initial Wt. (g): Taken from the study folder.

Dilution Factor: Taken from the study folder.

Final Conc. (ug/g): Calculated by dividing the initial volume from the concentration

Study #: FACT-TOX-002

3M Environmental Lab -- Method Modification

Method:

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

Section modified: 10.3.2, 14.5.1, add sections 14.3.2-14.3.6

Effective date of modifications: July 22, 1999

Section 10.3.2

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

Section 14.5.1

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within $\pm 30\%$ of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

Study #: FACT-TOX-002

Section 14.3.3

Method reads:

NA

Modify method to read:

14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

Section 14.3.4

Method reads:

NA

Modify method to read:

14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

Section 14.3.5

Method reads:

NA

Modify method to read:

14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

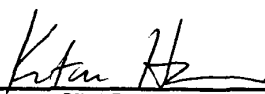
Section 14.3.6

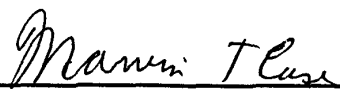
Method reads:

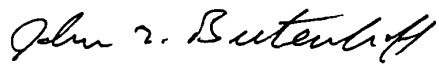
NA

Modify method to read:

14.3.6 A valid calibration curve must contain at least 5 active points.

 11/29/02
Signature of PAI and date

 13 Dec 2000
Signature of Sponsor and date

 13 DEC 2000
Signature of Study Director and date

Appendix D: Data Summary Tables

Table 7. FACT TOX-002 Data Summary of PFOS Concentration—Serum (µg/mL)

Timepoint	Sex	Group 1 Control Average ±SD	Group 2 Low Average ±SD	Group 3 Mid Average ±SD	Group 4 Mid-High Average ±SD	Group 5 High Average ±SD	Group 6 High Recovery Average ±SD
Week 4 ^a	Male	<LOQ ^b (n = 5)	0.907 ±0.0619 (n = 5)	4.33 ±1.16 (n = 5)	7.57 ±2.17 (n = 5)	41.8 ±7.92 (n = 5)	
	Female	0.0259 ±0.00663 (n = 5)	1.61 ±0.207 (n = 5)	6.62 ±0.499 (n = 5)	12.6 ±1.73 (n = 5)	54.0 ±7.34 (n = 5)	
Week 14 ^a	Male	<LOQ ^c (n = 5)	4.04 ±0.801 (n = 5)	17.1 ±1.22 (n = 5)	43.9 ±4.90 (n = 5)	148 ±13.8 (n = 5)	
	Female	2.67 ±4.58 (n = 5)	6.96 ±0.993 (n = 4 ^d)	27.3 ±2.34 (n = 5)	64.4 ±5.48 (n = 5)	223 ±22.4 (n = 5)	
Week 53	Male	0.0249 ±0.0182 (n = 5)				146 ±33.5 (n = 4)	
	Female	0.395 ±0.777 (n = 5)				220 ±44.0 (n = 5)	
Day 719	Male						
	Female			20.2 ±13.3 (n = 9)			
Week 105	Male	0.0118 ±0.0104 (n = 11)	1.31 ±1.30 (n = 10)	7.60 ±8.60 (n = 17)	22.5 ±23.5 (n = 25)	69.3 ±57.9 (n = 22)	
	Female	0.0836 ±0.134 (n = 24)	4.35 ±2.78 (n = 15)		75.0 ±45.7 (n = 15)	233 ±124 (n = 25)	
Week 106	Male						2.42 ±5.09 (n = 10)
	Female						9.51 ±8.70 (n = 17)

^a Not corrected for purity of the standard material.^b LOQ—Limit of Quantitation = 0.00910 µg/mL^c LOQ—Limit of Quantitation = 0.0457 µg/mL^d C92987F sample spilled during extraction, no sample remaining for analysis.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 8. FACT TOX-002 Data Summary of PFOS Concentration—Liver (µg/g)

Timepoint	Sex	Group 1 Control Average ±SD	Group 2 Low Average ±SD	Group 3 Mid Average ±SD	Group 4 Mid-High Average ±SD	Group 5 High Average ±SD	Group 6 High Recovery Average ±SD
Week 4 ^a	Male	0.104 ±0.0673 (n = 5)	11.0 ±2.31 (n = 5)	31.3 ±5.84 (n = 5)	47.6 ±12.5 (n = 5)	282 ±45.3 ^b (n = 5)	
	Female	0.107 ±0.0486 (n = 5)	8.71 ±0.552 (n = 5)	25.0 ±6.11 (n = 5)	83.0 ±14.1 (n = 5)	373 ±44.1 ^b (n = 5)	
Week 14 ^a	Male	0.459 ±0.0573 (n = 5)	23.8 ±3.45 (n = 5)	74.0 ±6.16 (n = 5)	358 ±28.8 (n = 5)	568 ±107 (n = 5)	
	Female	12.0 ±22.4 (n = 5)	19.2 ±3.77 (n = 5)	69.2 ±3.46 (n = 5)	370 ±22.3 (n = 5)	635 ±49.0 (n = 5)	
Week 53	Male	0.635 ±1.04 (n = 10)				435 ±96.9 (n = 9)	
	Female	0.923 ±1.77 (n = 10)				560 ±180 (n = 10)	
Day 719	Male						
	Female			55.1 ±31.5 (n = 9)			
Week 105	Male	0.114 ±0.148 (n = 11)	7.83 ±7.34 (n = 10)	26.4 ±20.4 (n = 17)	70.5 ±63.1 (n = 25)	189 ±141 (n = 22)	
	Female	0.185 ±0.184 (n = 24)	12.9 ±6.81 (n = 15)		131 ±61.4 (n = 15)	381 ±176 (n = 25)	
Week 106	Male						3.12 ±5.97 (n = 10)
	Female						12.9 ±10.4 (n = 17)

^a Not corrected for purity of the standard material.^b The values in this mean were tentative

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 9. Week 4 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92506M	<LOQ
	C92524M	<LOQ
	C92526M	<LOQ
	C92529M	<LOQ
	C92546M	<LOQ
Group 2 Low	C92574M	0.910*
	C92575M	0.989*
	C92610M	0.944*
	C92613M	0.852*
	C92618M	0.842*
Group 3 Mid	C92646M	3.97*
	C92650M	2.85*
	C92652M	4.80*
	C92668M	5.98*
	C92678M	4.04*
Group 4 Mid-High	C92715M	8.46*
	C92718M	9.89*
	C92731M	4.03*
	C92734M	7.69*
	C92744M	7.81*
Group 5 High	C92752M	29.0*
	C92759M	40.7*
	C92779M	49.5*
	C92793M	42.7*
	C92798M	47.0*

Note: Not corrected for purity of the standard material.

LOQ—Limit of Quantitation = 0.00910 µg/mL

* Continuing calibration verifications did not meet ±30% criteria; data may be biased low.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 10. Week 4 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92861F	0.0227
	C92864F	0.0309
	C92914F	0.0347
	C92919F	0.0193
	C92926F	0.0216
Group 2 Low	C92982F	1.69*
	C92941F	1.67*
	C92945F	1.89*
	C92950F	1.38*
	C92969F	1.44*
Group 3 Mid	C92996F	6.84*
	C93006F	5.75*
	C93008F	6.85*
	C93031F	6.65*
	C93045F	6.99*
Group 4 Mid-High	C93058F	15.3*
	C93067F	13.2*
	C93070F	11.6*
	C93075F	10.8*
	C93084F	12.1*
Group 5 High	C93114F	62.8*
	C93123F	52.4*
	C93142F	56.4*
	C93144F	42.7*
	C93159F	55.8*

Note: Not corrected for purity of the standard material.

* Continuing calibration verifications did not meet $\pm 30\%$ criteria; data may be biased low.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Table 11. Week 14 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92509M	<LOQ
	C92511M	<LOQ
	C92521M	<LOQ
	C92528M	<LOQ
	C92532M	<LOQ
Group 2 Low	C92593M	4.70
	C92600M	4.58
	C92616M	4.09
	C92621M	4.13
	C92627M	2.69
Group 3 Mid	C92640M	15.9
	C92645M	16.9
	C92662M	16.1
	C92676M	17.9
	C92684M	18.8
Group 4 Mid-High	C92713M	49.1
	C92714M	37.2
	C92719M	41.6
	C92722M	43.3
	C92728M	48.2
Group 5 High	C92765M	139
	C92777M	145
	C92789M	149
	C92799M	136
	C92812M	171

Note: Not corrected for purity of the standard material.

LOQ—Limit of Quantitation = 0.0457 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 12. Week 14 Reported PFOS Levels in Sera Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92880F	0.332
	C92887F	10.8
	C92898F	0.252
	C92903F	1.62
	C92905F	0.333
Group 2 Low	C92944F	5.90
	C92962F	8.21
	C92967F	7.23
	C92978F	6.50
	C92987F	*
Group 3 Mid	C92993F	28.7
	C93000F	26.7
	C93018F	25.1
	C93023F	25.4
	C93035F	30.6
Group 4 Mid-High	C93051F	56.7
	C93064F	64.5
	C93071F	71.3
	C93085F	62.3
	C93109F	67.3
Group 5 High	C93111F	229
	C93127F	210
	C93143F	258
	C93155F	220
	C93169F	199

Note: Not corrected for purity of the standard material.

* Sample spilled during extraction, no sample remaining for analysis.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Table 13. Week 53 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92504M	0.0278
	C92512M	0.0149
	C92534M	0.0251
	C92562M	0.0529
	C92570M	<LOQ
Group 5 High	C92778M	96.6
	C92795M	162
	C92796M	171
	C92817M	154

LOQ—Limit of Quantitation = 0.00395 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 14. Week 53 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92876F	0.0310
	C92889F	1.78
	C92899F	0.0656
	C92923F	0.0891
	C92930F	<LOQ
Group 5 High	C93112F	240
	C93120F	144
	C93154F	223
	C93163F	245
	C93171F	250

LOQ—Limit of Quantitation = 0.00395 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 15. Day 719 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 3 Mid	C92991F G3 Day 719	15.1
	C93003F G3 Day 719	13.0
	C93007F G3 Day 719	30.9
	C93011F G3 Day 719	3.57
	C93022F G3 Day 719	2.02
	C93039F G3 Day 719	27.6
	C93040F G3 Day 719	43.8
	C93047F G3 Day 719	21.9
	C93048F G3 Day 719	24.3

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92503M	0.0148
	C92505M	0.0161
	C92510M	0.0285
	C92513M	0.0127
	C92514M	0.0325
	C92518M	<LOQ
	C92541M	<LOQ
	C92542M	<LOQ
	C92544M	<LOQ
	C92551M	<LOQ
	C92569M	<LOQ
Group 2 Low	C92577M	0.309
	C92583M	0.212
	C92590M	2.55
	C92597M	1.95
	C92598M	0.230
	C92601M	3.94
	C92602M	1.39
	C92619M	0.461
	C92623M	2.05
	C92626M	1.48
Group 3 Mid	C92631M	8.98
	C92633M	14.3
	C92637M	1.81
	C92641M	15.1
	C92642M	6.05
	C92643M	2.75
	C92644M	1.21
	C92649M	8.63
	C92653M	0.487
	C92657M	5.20
	C92659M	14.4
	C92660M	1.66
	C92669M	3.13
	C92674M	35.3
	C92682M	4.77
	C92683M	2.72
	C92690M	2.83

LOQ—Limit of Quantitation = 0.00428 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/mL
Group 4 Mid-High	C92691M	4.48
	C92693M	7.71
	C92694M	74.2
	C92695M	75.6
	C92696M	38.7
	C92698M	12.1
	C92699M	73.3
	C92700M	28.8
	C92702M	3.14
	C92704M	10.9
	C92705M	23.0
	C92708M	9.72
	C92716M	13.5
	C92717M	3.01
	C92724M	12.8
	C92726M	38.8
	C92730M	6.72
	C92735M	24.4
	C92736M	4.10
	C92739M	5.72
	C92740M	35.7
	C92742M	2.28
	C92747M	45.7
	C92748M	3.66
	C92750M	3.40

LOQ—Limit of Quantitation = 0.00428 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/mL
Group 5 High	C92753M	35.8
	C92757M	196
	C92761M	27.3
	C92762M	130
	C92774M	10.4
	C92775M	11.1
	C92776M	108
	C92780M	67.4
	C92782M	16.7
	C92786M	27.5
	C92788M	118
	C92791M	21.4
	C92792M	18.0
	C92800M	64.2
	C92802M	169
	C92803M	141
	C92804M	127
	C92808M	37.2
	C92809M	6.02
	C92814M	89.2
	C92815M	11.9
	C92818M	90.1

LOQ—Limit of Quantitation = 0.00428 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 1 Control	C92866F	0.624
	C92867F	0.225
	C92868F	0.0467
	C92869F	0.306
	C92871F	0.00650
	C92873F	0.0602
	C92874F	0.0427
	C92877F	0.100
	C92881F	0.0258
	C92882F	0.0483
	C92885F	<LOQ (1)
	C92890F	0.0675
	C92892F	0.0140
	C92895F	0.0233
	C92896F	0.0407
	C92897F	0.0443
	C92906F	0.0795
	C92907F	0.0176
	C92909F	0.0402
	C92911F	0.0687
	C92912F	0.0311
	C92913F	0.0575
	C92925F	<LOQ (2)
	C92927F	0.0229
Group 2 Low	C92936F	8.72
	C92938F	0.349
	C92946F	9.08
	C92953F	6.64
	C92957F	5.67
	C92960F	1.70
	C92964F	5.97
	C92970F	1.73
	C92973F	1.34
	C92974F	4.06
	C92975F	3.72
	C92983F	6.81
	C92984F	2.78
	C92986F	5.24
	C92988F	5.76

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/mL
Group 4 Mid-High	C93059F	103
	C93060F	89.5
	C93062F	102
	C93063F	171
	C93072F	114
	C93077F	38.0
	C93078F	25.9
	C93079F	72.8
	C93080F	73.6
	C93081F	70.5
	C93090F	99.1
	C93098F	5.17
	C93101F	117
	C93102F	11.4
	C93107F	33.2

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/mL
Group 5 High	C93113F	403
	C93115F	286
	C93124F	160
	C93125F	353
	C93133F	242
	C93135F	274
	C93137F	143
	C93139F	375
	C93140F	3.85
	C93146F	51.5
	C93149F	432
	C93151F	139
	C93153F	121
	C93157F	28.6
	C93158F	287
	C93161F	103
	C93162F	446
	C93164F	321
	C93165F	165
	C93166F	189
	C93168F	339
	C93174F	304
	C93177F	248
	C93178F	216
	C93179F	190

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 18. Week 106 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 6 High Recovery	C92821M	8.32
	C92823M	0.533
	C92827M	0.0303
	C92829M	0.0243
	C92833M	0.0795
	C92834M	0.0138
	C92848M	14.9
	C92851M	0.0331
	C92853M	0.210
	C92856M	<LOQ

LOQ—Limit of Quantitation = 0.00482 µg/mL

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 19. Week 106 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/mL
Group 6 High Recovery	C93181F	5.76
	C93182F	1.69
	C93183F	5.68
	C93184F	1.64
	C93185F	11.8
	C93190F	31.6
	C93192F	16.8
	C93197F	9.35
	C93198F	8.63
	C93201F	27.0
	C93202F	10.1
	C93205F	0.0594
	C93209F	6.57
	C93211F	12.3
	C93216F	2.85
	C93219F	2.39
	C93220F	7.44

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 20. Week 4 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92506M	<LOQ
	C92524M	0.169
	C92526M	0.186
	C92529M	<LOQ
	C92546M	<LOQ
Group 2 Low	C92574M	12.4
	C92575M	9.67
	C92610M	8.54
	C92613M	14.2
	C92618M	9.94
Group 3 Mid	C92646M	40.7
	C92650M	26.1
	C92652M	31.2
	C92668M	32.0
	C92678M	26.7
Group 4 Mid-High	C92715M	53.5
	C92718M	59.6
	C92731M	26.7
	C92734M	49.8
	C92744M	48.3
Group 5 High	C92752M	219*
	C92759M	264*
	C92779M	342*
	C92793M	286*
	C92798M	298*

Note: Not corrected for purity of the standard material.

LOQ—Limit of Quantitation = 0.0552µg/g

* Results were outside the range of the curve and should be considered estimates.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 21. Week 4 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92861F	0.0817
	C92864F	0.0738
	C92914F	0.184
	C92919F	0.0700
	C92926F	0.126
Group 2 Low	C92982F	9.09
	C92941F	7.99
	C92945F	9.40
	C92950F	8.43
	C92969F	8.67
Group 3 Mid	C92996F	28.9
	C93006F	14.6
	C93008F	29.7
	C93031F	27.1
	C93045F	24.8
Group 4 Mid-High	C93058F	107
	C93067F	83.9
	C93070F	77.0
	C93075F	70.7
	C93084F	76.7
Group 5 High	C93114F	435*
	C93123F	360*
	C93142F	395*
	C93144F	317*
	C93159F	359*

Note: Not corrected for purity of the standard material.

* Results were outside the range of the curve and should be considered estimates.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 22. Week 14 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92509M	0.440
	C92511M	0.542
	C92521M	0.492
	C92528M	0.403
	C92532M	0.418
Group 2 Low	C92593M	24.6
	C92600M	19.3
	C92616M	27.2
	C92621M	21.2
	C92627M	26.6
Group 3 Mid	C92640M	82.7
	C92645M	73.5
	C92662M	68.3
	C92676M	68.3
	C92684M	77.4
Group 4 Mid-High	C92713M	375
	C92714M	372
	C92719M	377
	C92722M	308
	C92728M	356
Group 5 High	C92765M	722
	C92777M	618
	C92789M	559
	C92799M	456
	C92812M	485

Note: Not corrected for purity of the standard material.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 23. Week 14 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92880F	1.02
	C92887F	52.0
	C92898F	0.697
	C92903F	5.26
	C92905F	1.18
Group 2 Low	C92944F	18.9
	C92962F	17.7
	C92967F	24.8
	C92978F	14.5
	C92987F	19.8
Group 3 Mid	C92993F	69.0
	C93000F	71.1
	C93018F	67.4
	C93023F	64.9
	C93035F	73.9
Group 4 Mid-High	C93051F	355
	C93064F	361
	C93071F	355
	C93085F	373
	C93109F	408
Group 5 High	C93111F	692
	C93127F	619
	C93143F	621
	C93155F	675
	C93169F	569

Note: Not corrected for purity of the standard material.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 24. Week 53 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92501M	0.0326
	C92504M	0.358
	C92507M	0.301
	C92512M	0.283
	C92517M	0.761
	C92523M	0.529
	C92534M	0.359
	C92540M	0.106
	C92562M	3.52
	C92570M	0.103
Group 5 High	C92751M	559
	C92754M	486
	C92755M	486
	C92778M	279
	C92790M	405
	C92795M	554
	C92796M	427
	C92817M	403
	C92820M	316

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 25. Week 53 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92862F	5.66
	C92876F	0.131
	C92889F	2.08
	C92894F	0.276
	C92899F	0.217
	C92910F	0.198
	C92917F	0.246
	C92922F	0.0580
	C92923F	0.154
	C92930F	0.215
Group 5 High	C93112F	296
	C93116F	534
	C93117F	514
	C93120F	430
	C93130F	504
	C93138F	565
	C93147F	619
	C93154F	424
	C93163F	883
	C93171F	830

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Table 26. Day 719 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS $\mu\text{g/g}$
Group 3 Mid	C92991F	43.6
	C93003F	51.7
	C93007F	97.2
	C93011F	13.2
	C93022F	9.02
	C93039F	79.7
	C93040F	94.1
	C93047F	53.3
	C93048F	53.8

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to $\pm 30\%$; liver data are accurate to $\pm 50\%$.

Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92503M	0.152
	C92505M	0.106
	C92510M	0.511
	C92513M	0.0663
	C92514M	0.233
	C92518M	<LOQ
	C92541M	0.0283
	C92542M	0.0342
	C92544M	0.0291
	C92551M	<LOQ
	C92569M	0.0690
Group 2 Low	C92577M	1.60
	C92583M	1.64
	C92590M	3.89
	C92597M	17.2
	C92598M	2.02
	C92601M	21.6
	C92602M	10.0
	C92619M	1.39
	C92623M	13.4
	C92626M	5.61
Group 3 Mid	C92631M	41.5
	C92633M	51.2
	C92637M	3.82
	C92641M	3.35
	C92642M	23.9
	C92643M	18.5
	C92644M	4.32
	C92649M	54.0
	C92653M	2.85
	C92657M	19.8
	C92659M	61.4
	C92660M	10.6
	C92669M	17.4
	C92674M	59.0
	C92682M	38.2
	C92683M	16.9
	C92690M	21.5

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/g
Group 4 Mid-High	C92691M	27.7
	C92693M	30.5
	C92694M	208
	C92695M	195
	C92696M	120
	C92698M	18.9
	C92699M	164
	C92700M	69.1
	C92702M	13.1
	C92704M	65.3
	C92705M	67.7
	C92708M	51.6
	C92716M	85.8
	C92717M	13.7
	C92724M	46.1
	C92726M	95.0
	C92730M	18.0
	C92735M	73.7
	C92736M	15.1
	C92739M	35.6
	C92740M	155
	C92742M	7.16
	C92747M	170
	C92748M	11.5
	C92750M	6.80

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/g
Group 5 High	C92753M	113
	C92757M	329
	C92761M	80.2
	C92762M	175
	C92774M	39.4
	C92775M	35.0
	C92776M	320
	C92780M	163
	C92782M	53.1
	C92786M	98.6
	C92788M	385
	C92791M	69.0
	C92792M	54.8
	C92800M	159
	C92802M	409
	C92803M	411
	C92804M	330
	C92808M	119
	C92809M	23.3
	C92814M	358
	C92815M	72.0
	C92818M	352

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 1 Control	C92866F	0.907
	C92867F	0.367
	C92868F	0.108
	C92869F	0.250
	C92871F	<LOQ
	C92873F	0.254
	C92874F	0.151
	C92877F	0.271
	C92881F	0.0904
	C92882F	0.228
	C92885F	<LOQ
	C92890F	0.248
	C92892F	0.0490
	C92895F	0.113
	C92896F	0.116
	C92897F	0.158
	C92906F	0.328
	C92907F	0.0346
	C92909F	0.113
	C92911F	0.206
	C92912F	0.0667
	C92913F	0.183
	C92925F	<LOQ
	C92927F	0.161

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/g
Group 2 Low	C92936F	10.5
	C92938F	1.72
	C92946F	15.3
	C92953F	20.1
	C92957F	14.5
	C92960F	4.94
	C92964F	20.1
	C92970F	6.69
	C92973F	5.08
	C92974F	15.8
	C92975F	13.5
	C92983F	27.0
	C92984F	8.58
	C92986F	13.2
	C92988F	17.2
Group 4 Mid-High	C93059F	183
	C93060F	133
	C93062F	242
	C93063F	133
	C93072F	167
	C93077F	152
	C93078F	61.7
	C93079F	113
	C93080F	149
	C93081F	118
	C93090F	186
	C93098F	19.2
	C93101F	193
	C93102F	40.0
	C93107F	79.8

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002 (continued)

Dosage Group	Specimen ID	PFOS µg/g
Group 5 High	C93113F	373
	C93115F	378
	C93124F	174
	C93125F	444
	C93133F	403
	C93135F	582
	C93137F	281
	C93139F	446
	C93140F	9.20
	C93146F	131
	C93149F	575
	C93151F	292
	C93153F	234
	C93157F	76.8
	C93158F	617
	C93161F	212
	C93162F	695
	C93164F	510
	C93165F	411
	C93166F	394
	C93168F	462
	C93174F	493
	C93177F	623
	C93178F	396
	C93179F	328

LOQ—Limit of Quantitation = 0.0107 µg/g

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 29. Week 106 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 6 High Recovery	C92821M	9.68
	C92823M	1.91
	C92827M	0.144
	C92829M	0.117
	C92833M	0.382
	C92834M	0.203
	C92848M	17.9
	C92851M	0.363
	C92853M	0.431
	C92856M	0.0332

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Table 30. Week 106 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

Dosage Group	Specimen ID	PFOS µg/g
Group 6 High Recovery	C93181F	4.32
	C93182F	2.10
	C93183F	9.08
	C93184F	1.69
	C93185F	15.9
	C93190F	22.8
	C93192F	15.0
	C93197F	8.65
	C93198F	6.18
	C93201F	19.2
	C93202F	17.0
	C93205F	0.132
	C93209F	14.9
	C93211F	42.6
	C93216F	14.7
	C93219F	4.96
	C93220F	19.5

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

Appendix E: Data Spreadsheets

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number/Test Substance: T-6295 (PFOS)
Matrix: Rat Serum - Curve linear regression, no weighting
Method/Revision: FACT-M-3.0 & FACT-M-4.0 - Reworked x dropping 97 ppb std
Analytical Equipment System Number: Madeline 041098
Instrument Software/Version: MassLynx 3.1
Filename: See Attachments
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Date of Extraction/Analyst: 05/27/98 OK
Date of Analysis/Analyst: 06/26/98, 07/15/98 HOI/MEE/KJH
Date of Data Reduction/Analyst: 07/02/98, 07/23/98 HOI

Blks
Grp 1
Grp 2
Grp 3
Grp 4
Grp 5
MS, MSD

Sample Data

WEEK 4 RAT SERA

Lot 215

Group Dose	Sample #	Initial Vol. mL	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1	0.9275	ND	1	0.00	<LOQ (0.00453 ug/mL) *		
	H2O Blk-2	1	0.9275	ND	1	0.00	<LOQ (0.00453 ug/mL) *	<LOQ (0.00453 ug/mL)	NA
Matrix Blk	Rat Serum Blk-1	1	0.9275	ND	1	1.75	<LOQ (0.00453 ug/mL) *		
	Rat Serum Blk-2	1	0.9275	ND	1	1.73	<LOQ (0.00453 ug/mL) *	<LOQ (0.00453 ug/mL)	NA
QC-75 ppb	MS	1	NA	NA	1	122	167% *		
	MSD	1	NA	NA	1	92.6	127% *	147%	27%
Group 1 Control 0.0 mg/kg	C92506M	1	0.9275	ND	1	1.57	<LOQ (0.00910 ug/mL)		
	C92524M	1	0.9275	ND	1	0.00	<LOQ (0.00910 ug/mL)		
	C92526M	1	0.9275	ND	1	4.31	<LOQ (0.00910 ug/mL)		
	C92529M	1	0.9275	ND	1	4.54	<LOQ (0.00910 ug/mL)		NA
	C92546M	1	0.9275	ND	1	0.00	<LOQ (0.00910 ug/mL)	<LOQ (0.00910 ug/mL)	NA
	C92861F	1	0.9275	ND	1	24.5	0.0227		
	C92864F	1	0.9275	ND	1	33.4	0.0309		
	C92914F	1	0.9275	ND	1	37.5	0.0347		
	C92919F	1	0.9275	ND	1	20.8	0.0193		25.7
	C92926F	1	0.9275	ND	1	23.3	0.0216	0.0259	0.00663
Group 2 Low Dose 0.5 mg/kg	C92574M	1	0.9275	ND	20	49.0	0.910 *		
	C92575M	1	0.9275	ND	20	53.3	0.989 *		
	C92610M	1	0.9275	ND	20	50.9	0.944 *		
	C92613M	1	0.9275	ND	20	46.0	0.852 *		6.83
	C92618M	1	0.9275	ND	20	45.4	0.842 *	0.907	0.0619
	C92982F	1	0.9275	ND	20	91.1	1.69 *		
	C92941F	1	0.9275	ND	20	89.8	1.67 *		
	C92945F	1	0.9275	ND	20	102	1.89 *		
	C92950F	1	0.9275	ND	20	74.6	1.38 *		12.8
	C92969F	1	0.9275	ND	20	77.5	1.44 *	1.61	0.207
Group 3 Mid Dose 2.0 mg/kg	C92646M	1	0.9275	ND	50	85.5	3.97 *		
	C92650M	1	0.9275	ND	50	61.4	2.85 *		
	C92652M	1	0.9275	ND	50	103	4.80 *		
	C92668M	1	0.9275	ND	50	129	5.98 *		26.7
	C92678M	1	0.9275	ND	50	87.2	4.04 *	4.33	1.16
	C92996F	1	0.9275	ND	50	147	6.84 *		
	C93006F	1	0.9275	ND	50	124	5.75 *		
	C93008F	1	0.9275	ND	50	148	6.85 *		
	C93031F	1	0.9275	ND	50	143	6.65 *		7.54
	C93045F	1	0.9275	ND	50	151	6.99 *	6.62	0.499
Group 4 Mid-High Dose 5.0 mg/kg	C92715M	1	0.9275	ND	100	91.2	8.46 *		
	C92718M	1	0.9275	ND	100	107	9.89 *		
	C92731M	1	0.9275	ND	100	43.4	4.03 *		
	C92734M	1	0.9275	ND	100	82.9	7.69 *		28.6
	C92744M	1	0.9275	ND	100	84.2	7.81 *	7.57	2.17
	C93058F	1	0.9275	ND	100	165	15.3 *		
	C93067F	1	0.9275	ND	100	142	13.2 *		
	C93070F	1	0.9275	ND	100	125	11.6 *		
	C93075F	1	0.9275	ND	100	116	10.8 *		13.8
	C93084F	1	0.9275	ND	100	131	12.1 *	12.6	1.73
Group 5 High Dose 20 mg/kg	C92752M	1	0.9275	ND	200	157	29.0 *		
	C92759M	1	0.9275	ND	200	219	40.7 *		
	C92779M	1	0.9275	ND	200	267	49.5 *		
	C92793M	1	0.9275	ND	200	230	42.7 *		19.0
	C92798M	1	0.9275	ND	200	253	47.0 *	41.8	7.92
	C93114F	1	0.9275	ND	200	338	62.8 *		
	C93123F	1	0.9275	ND	200	282	52.4 *		
	C93142F	1	0.9275	ND	200	304	56.4 *		
	C93144F	1	0.9275	ND	200	230	42.7 *		13.6
	C93159F	1	0.9275	ND	200	301	55.8 *	54.0	7.34

Original PFOS LOQ (4.88 ng/mL, 9.81 ng/mL) updated to reflect K+ information on 10/04/00. LAC 10/04/00

Correction factors not applicable for MS/MSD QC data

ND = Not Determined

NA = Components were not integrated because they were not detected in the group 5 samples.

Date Entered/By: 07/30/98, 09/05/00 LAC

Date Verified/By: 08/24/00 KJH

Purity Entered/Verified: 09/12/00 LAC

* Data entered. Continuing calibration verifications did not meet +/- 30% criteria. LAC 09/05/00

PFOS = Perfluorooctanesulfonate

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295 (PFOS)
 Matrix: Rat Serum - Curve linear regression, 1/X weighted
 Method/Revision: FACT-M-3.0 & ETS-8-5.1
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: MassLynx 3.3
 Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Date of Extraction/Analyst: 07/28/98 IAS
 Date of Analysis/Analyst: 07/29/98, 08/14/98, 08/17/98 HOJ/MEE
 Date of Data Reduction/Analyst: 02/01/00, 02/02/00 IAS

Files	PFOS	Dilutions
Blks	A072998002-3 & 71-72	1/1
Grp 1 M	072998013-17	1/1
Grp 1 F	072998021, 24, 25	1/1
Grp 1 F	81498012-13	1/25
Grp 2	81498016-26	1/25
Grp 3	81498029-40	1/50
Grp 4	81498043-54	1/100
Grp 5 M	81498064-68	1/250
Grp 5 F	08179825-29	1/500
MS, MSD	81498071-72	1/1

Sample Data**WEEK 14 RAT SERA REWORK**

Lot 193

Group Dose	Sample #	Initial Vol. mL	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1	0.9275	ND	1	0.00	<LOQ (0.0457 ug/mL)		
	H2O Blk-2	1	0.9275	ND	1	7.82	<LOQ (0.0457 ug/mL)	<LOQ (0.0457 ug/mL)	NA
Matrix Blk	Rabbit Serum Blk-1	1	0.9275	ND	1	0.00	<LOQ (0.0457 ug/mL)		
	Rabbit Serum Blk-2	1	0.9275	ND	1	6.79	<LOQ (0.0457 ug/mL)	<LOQ (0.0457 ug/mL)	NA
QC-100 ppb Rat Serum	TN-A-1902-MS	1	NA	NA	1	105	107%		
	TN-A-1902-MSD	1	NA	NA	1	105	108%	108%	1%
Group 1 Control 0.0 ppm in Diet	C92509M	1	0.9275	ND	1	18.0	<LOQ (0.0457 ug/mL)		
	C92511M	1	0.9275	ND	1	17.4	<LOQ (0.0457 ug/mL)		
	C92521M	1	0.9275	ND	1	17.2	<LOQ (0.0457 ug/mL)		
	C92528M	1	0.9275	ND	1	18.9	<LOQ (0.0457 ug/mL)		NA
	C92532M	1	0.9275	ND	1	17.5	<LOQ (0.0457 ug/mL)	<LOQ (0.0457 ug/mL)	NA
	C92880F	1	0.9275	ND	1	360	0.332		
	C92887F	1	0.9275	ND	25	468	10.8		
	C92898F	1	0.9275	ND	1	273	0.252		
	C92903F	1	0.9275	ND	25	70.0	1.62		172
	C92905F	1	0.9275	ND	1	361	0.333	2.67	4.58
Group 2 Low Dose 0.5 ppm in Diet	C92593M	1	0.9275	ND	25	204	4.70		
	C92600M	1	0.9275	ND	25	198	4.58		
	C92616M	1	0.9275	ND	25	177	4.09		
	C92621M	1	0.9275	ND	25	179	4.13		19.8
	C92627M	1	0.9275	ND	25	117	2.69	4.04	0.801
	C92944F	1	0.9275	ND	25	256	5.90		
	C92962F	1	0.9275	ND	25	356	8.21		
	C92967F	1	0.9275	ND	25	314	7.23		
	C92978F	1	0.9275	ND	25	282	6.50		14.3
	C92987F	1	0.9275	ND	25	**	**	6.96	0.993
Group 3 Mid Dose 2.0 ppm in Diet	C92640M	1	0.9275	ND	50	344	15.9		
	C92645M	1	0.9275	ND	50	367	16.9		
	C92662M	1	0.9275	ND	50	350	16.1		
	C92676M	1	0.9275	ND	50	387	17.9		7.13
	C92684M	1	0.9275	ND	50	408	18.8	17.1	1.22
	C92993F	1	0.9275	ND	50	621	28.7		
	C93000F	1	0.9275	ND	50	579	26.7		
	C93018F	1	0.9275	ND	50	543	25.1		
	C93023F	1	0.9275	ND	50	549	25.4		8.57
	C93035F	1	0.9275	ND	50	663	30.6	27.3	2.34
Group 4 Mid-High Dose 5.0 ppm in Diet	C92713M	1	0.9275	ND	100	532	49.1		
	C92714M	1	0.9275	ND	100	403	37.2		
	C92719M	1	0.9275	ND	100	451	41.6		
	C92722M	1	0.9275	ND	100	469	43.3		11.2
	C92728M	1	0.9275	ND	100	522	48.2	43.9	4.90
	C93051F	1	0.9275	ND	100	614	56.7		
	C93064F	1	0.9275	ND	100	699	64.5		
	C93071F	1	0.9275	ND	100	773	71.3		
	C93085F	1	0.9275	ND	100	675	62.3		8.51
	C93109F	1	0.9275	ND	100	729	67.3	64.4	5.48
Group 5 High Dose 20 ppm in Diet	C92765M	1	0.9275	ND	250	602	139		
	C92777M	1	0.9275	ND	250	629	145		
	C92789M	1	0.9275	ND	250	645	149		
	C92799M	1	0.9275	ND	250	588	136		9.35
	C92812M	1	0.9275	ND	250	740	171	148	13.8
	C93111F	1	0.9275	ND	500	496	229		
	C93127F	1	0.9275	ND	500	456	210		
	C93143F	1	0.9275	ND	500	559	258		
	C93155F	1	0.9275	ND	500	477	220		10.0
	C93169F	1	0.9275	ND	500	432	199	223	22.4

Original PFOS LOQ (49.3 ng/mL) updated to reflect K+ information on 10/04/00. LAC 10/04/00

Correction factors not applicable for MS/MSD QC data

ND = Not Determined

PFOS = Perfluorooctanesulfonate

** Sample spilled during extraction, no sample remaining for analysis.

Date Entered/By: 02/09/00, 09/07/00 LAC

Date Verified/By: 08/24/00 KJH

Purity Entered/Verified: 09/12/00 LAC, 9/12/00 MMH

Original PFOS LOQ = 25 ng/mL updated to reflect purity information on 10/04/00. LAC 10/04/00

AMDT# 112296.1

LRN-U2121

Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295 (PFOS)
 Matrix: Rat Serum
 Method/Revision: ETS-8-4.1 and ETS-8-5.1
 Analytical Equipment System Number: Soup 020199
 Instrument Software/Version: MassLynx 3.3
 Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Date of Extraction/Analyst: 11/10/99 SEE
 Date of Analysis/Analyst: 11/15/99, 11/19/99, 11/22/99, 11/24/99 IAS, 12/15/99 IAS
 Date of Data Reduction/Analyst: 11/16/99, 11/22/99, 11/23/99, 11/29/99 IAS, 12/13/99 MMH

FileNames
Blanks S121499043-45.63-65
Grp 1 M 111599019-25
Grp 1 F 111599026,28-32
Grp 1 F 121499057
Grp 5 M 112299017-20
Grp 5 F 112499016-19
MS, MSD 121499058-59
Box 99-220 1/1
 Dilutions
 1/1
 1/1
 1/2
 1/200&2000
 1/1
 1/1

Sample Data

WEEK 53 RAT SERA

Lot 171

Group Dose	Sample #	Initial Vol. mL	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-11	1.0	0.9275	0.8640	1	9.75	<LOQ (0.00782 ug/mL)		
	H2O Blk-12	1.0	0.9275	0.8640	1	5.11	<LOQ (0.00782 ug/mL)	<LOQ (0.00782 ug/mL)	NA
Matrix Blk	Rabbit Serum Blk-11	1.0	0.9275	0.8640	1	6.61	<LOQ (0.00782 ug/mL)		
	Rabbit Serum Blk-12	1.0	0.9275	0.8640	1	3.62	<LOQ (0.00782 ug/mL)	<LOQ (0.00782 ug/mL)	NA
Matrix Blk	Rat Serum Blk-11	1.0	0.9275	0.8640	1	46.6	0.0373		
	Rat Serum Blk-12	1.0	0.9275	0.8640	1	476	0.3814 *	0.209	0.243
QC-250 ppb	RTS11109-250ppb-MS-11	1.0	NA	NA	1	189	76%		
	RTS11109-250ppb-MSD-11	1.0	NA	NA	1	224	90%	83%	17%
Group 1 Control 0.0 ppm in Diet	C92504M	0.600	0.9275	0.8640	1	20.8	0.0278		
	C92512M	0.700	0.9275	0.8640	1	13.1	0.0149		
	C92534M	0.700	0.9275	0.8640	1	21.9	0.0251		
	C92562M	0.700	0.9275	0.8640	1	46.2	0.0529		73.2
	C92570M	0.700	0.9275	0.8640	1	4.71	<LOQ (0.00395 ug/mL)	0.0249	0.0182
	C92876F	0.800	0.9275	0.8640	1	31.0	0.0310		
	C92889F	0.700	0.9275	0.8640	2	779	1.78		
	C92899F	0.800	0.9275	0.8640	1	65.5	0.0656		
	C92923F	0.700	0.9275	0.8640	1	77.9	0.0891		197
	C92930F	0.700	0.9275	0.8640	1	3.93	<LOQ (0.00395 ug/mL)	0.395	0.777
Group 5 High Dose 20 ppm in Diet	C92778M	0.800	0.9275	0.8640	200	482	96.6		
	C92795M	0.800	0.9275	0.8640	200	806	162		
	C92796M	0.600	0.9275	0.8640	200	640	171		23.0
	C92817M	0.800	0.9275	0.8640	200	767	154	146	33.5
	C93112F	1.000	0.9275	0.8640	2000	150	240		
	C93120F	1.000	0.9275	0.8640	200	897	144		
	C93154F	0.900	0.9275	0.8640	2000	125	223		
	C93163F	0.900	0.9275	0.8640	2000	138	245		20.0
	C93171F	0.900	0.9275	0.8640	2000	140	250	220	44.0

Original PFOS LOQ (4.93 ng/mL, 9.76 ng/mL) updated to reflect K+ and purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

PFOS = Perfluorooctanesulfonate

* Second analysis confirmed high result. LAC 08/24/00

Date Entered/By: 11/22/99, 11/24/99 GML/LAC, 12/1/99 GML, 12/30/99 MMH

Date Verified/ By: 08/31/00 KJH

Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1

Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number(Test Substance): T-6295 (PFOS)
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
ANALytical Equipment System Number: Ruby 100699
Instrument Software/Version: MassLynx 3.3, 3.4
FileName: See listing to the right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/ANALyst: 05/01/00, 05/02/00 SAL/KJK/RWW
Dates of ANALysis/ANALyst: 05/02/00 05/04/00, 05/10/00 05/26/00, 05/30/00 MMH/IAS
Date of Data Reduction/ANALyst: 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH

Sample Data**DAY 719 RAT SERA**

Group Dose	Sample #	Initial Vol. mL	Surrogate Verified	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	FileName (optional)	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Group 3 mg/kg/day	C92991F G3 Day719	1.00	NA	0.9949	0.8640	50	348	D051000054	15.1		
	C93003F G3 Day719	1.00	NA	0.9949	0.8640	50	299	D051000055	13.0		
	C93007F G3 Day719	0.80	NA	0.9949	0.8640	50	569	D051000058	30.9		
	C93011F G3 Day719	0.75	NA	0.9949	0.8640	50	61.7	D051000059	3.57		
	C93022F G3 Day719	0.85	NA	0.9949	0.8640	50	39.5	D051000060	2.02		
	C93039F G3 Day719	1.00	NA	0.9949	0.8640	50	637	D051000061	27.6		
	C93040F G3 Day719	0.80	NA	0.9949	0.8640	200	201	R053100030	43.6		
	C93047F G3 Day719	0.85	NA	0.9949	0.8640	50	429	D051000065	21.9		65.9
	C93048F G3 Day719	0.75	NA	0.9949	0.8640	50	420	D051000066	24.3	20.2	13.3

Original PFOS LOQ (24.8 ng/mL, 4.93 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

Date Entered/By: 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/17/00 MMH/CSH/LAC

Date Verified/ By: 08/31/00 KJH, 11/21/00 MMH

Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

LRN-U2121

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number/Test Substance: T-6295 (PFOS)
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
ANALYTICAL Equipment System Number: Davey 070799, Ruby 100699, Amelia 062498
Instrument Software/Version: MassLynx 3.3, 3.4
FileName: See listing to the right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 05/01/00, 05/02/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00 MMH/IAS
Date of Data Reduction/Analyst: 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH

Sample Data

WEEK 105 RAT SERA

Lot 171

Group Dose	Sample #	Initial Vol. mL	Surrogate Verified	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (optional)	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1 mg/kg/day	C92503M	0.64	NA	0.9949	0.8640	1	10.9	A051000043	< LOQ (0.00428 ug/mL)	0.0148	
	C92505M	0.85	NA	0.9949	0.8640	1	15.8	D050200028	0.0161		
	C92510M	0.84	NA	0.9949	0.8640	1	27.6	A051000060	0.0285		
	C92513M	0.82	NA	0.9949	0.8640	1	12.0	A051000061	0.0127		
	C92514M	0.85	NA	0.9949	0.8640	1	31.8	A051000046	0.0325		
	C92518M	0.70	NA	0.9949	0.8640	1	0.00	D050200029	< LOQ (0.00428 ug/mL)		
	C92541M	0.74	NA	0.9949	0.8640	1	3.28	R053100019	< LOQ (0.00428 ug/mL)		
	C92542M	0.84	NA	0.9949	0.8640	1	3.10	R053100028	< LOQ (0.00428 ug/mL)		
	C92544M	0.78	NA	0.9949	0.8640	1	0.360	R053100020	< LOQ (0.00428 ug/mL)		
	C92551M	0.70	NA	0.9949	0.8640	1	0.820	D050200030	< LOQ (0.00428 ug/mL)		
	C92569M	0.90	NA	0.9949	0.8640	1	3.06	D050200033	< LOQ (0.00428 ug/mL)	0.0118	87.6 0.0104
	C92866F	0.98	NA	0.9949	0.8640	1	705	A051000048	0.624		
	C92867F	0.76	NA	0.9949	0.8640	1	197	A051000064	0.225		
	C92868F	0.90	NA	0.9949	0.8640	1	48.4	D050200034	0.0467		
	C92869F	0.87	NA	0.9949	0.8640	1	307	A051000067	0.306		
	C92871F	0.90	NA	0.9949	0.8640	1	6.74	D050200035	0.00650		
	C92873F	0.98	NA	0.9949	0.8640	1	68.0	A051000049	0.0602		
	C92874F	0.85	NA	0.9949	0.8640	1	41.8	D050200036	0.0427		
	C92877F	0.85	NA	0.9949	0.8640	1	97.9	A051000068	0.100		
	C92881F	0.87	NA	0.9949	0.8640	1	25.9	A051000050	0.0258		
	C92882F	0.95	NA	0.9949	0.8640	1	52.8	A051000053	0.0483		
	C92885F	0.76	NA	0.9949	0.8640	1	0.00	R053100029	< LOQ (0.00428 ug/mL)		
	C92890F	0.89	NA	0.9949	0.8640	1	69.2	A051000055	0.0675		
	C92892F	0.96	NA	0.9949	0.8640	1	15.5	A051000069	0.0140		
	C92895F	1.00	NA	0.9949	0.8640	1	26.9	A051000070	0.0233		
	C92896F	1.00	NA	0.9949	0.8640	1	46.8	D050200037	0.0407		
	C92897F	0.85	NA	0.9949	0.8640	1	43.3	D050200040	0.0443		
	C92906F	0.85	NA	0.9949	0.8640	1	77.9	D050200041	0.0795		
	C92907F	0.73	NA	0.9949	0.8640	1	14.8	A051000056	0.0176		
	C92909F	1.00	NA	0.9949	0.8640	1	46.3	A051000071	0.0402		
	C92911F	0.81	NA	0.9949	0.8640	1	64.1	A051000074	0.0687		
	C92912F	1.00	NA	0.9949	0.8640	1	35.8	A051000075	0.0311		
	C92913F	0.90	NA	0.9949	0.8640	1	59.6	D050200042	0.0575		
	C92925F	1.00	NA	0.9949	0.8640	1	3.62	A051000057	< LOQ (0.00428 ug/mL)		161
	C92927F	1.00	NA	0.9949	0.8640	1	26.4	D050200043	0.0229	0.0836	0.134
Group 2 mg/kg/day	C92577M	0.90	NA	0.9949	0.8640	1	320	R052600022	0.309		
	C92583M	1.00	NA	0.9949	0.8640	1	244	D050200044	0.212		
	C92590M	0.43	NA	0.9949	0.8640	10	127	D051000017	2.55		
	C92597M	1.00	NA	0.9949	0.8640	10	225	R050400017	1.95		
	C92598M	0.85	NA	0.9949	0.8640	1	226	D050200048	0.230		
	C92601M	0.81	NA	0.9949	0.8640	10	367	D051000018	3.94		
	C92602M	0.40	NA	0.9949	0.8640	10	64.0	D051000019	1.39		
	C92619M	0.81	NA	0.9949	0.8640	10	43.0	D051000020	0.461		
	C92623M	0.75	NA	0.9949	0.8640	10	177	R051000023	2.05		99.0
	C92626M	1.00	NA	0.9949	0.8640	10	171	R051000024	1.48	1.31	1.30
	C92936F	1.00	NA	0.9949	0.8640	50	201	R052500106	8.72		
	C92938F	0.92	NA	0.9949	0.8640	50	37.0	D051000026	0.349		
	C92946F	0.83	NA	0.9949	0.8640	50	174	R052500107	9.08		
	C92953F	0.70	NA	0.9949	0.8640	10	535	R051000030	6.64		
	C92957F	1.00	NA	0.9949	0.8640	10	653	R051000031	5.67		
	C92960F	1.00	NA	0.9949	0.8640	10	196	R051000032	1.70		
	C92964F	0.75	NA	0.9949	0.8640	10	515	R050400018	5.97		
	C92970F	0.85	NA	0.9949	0.8640	10	169	R050400019	1.73		
	C92973F	0.95	NA	0.9949	0.8640	10	147	R050400020	1.34		
	C92974F	0.90	NA	0.9949	0.8640	10	421	R051000033	4.06		
	C92975F	0.95	NA	0.9949	0.8640	10	407	R051000034	3.72		
	C92983F	1.00	NA	0.9949	0.8640	10	784	R050400023	6.81		
	C92984F	0.98	NA	0.9949	0.8640	10	313	R051000037	2.78		
	C92986F	1.00	NA	0.9949	0.8640	10	603	R051000038	5.24		63.8
	C92988F	0.85	NA	0.9949	0.8640	10	563	R050400024	5.76	4.35	2.78
Group 3 mg/kg/day	C92631M	0.75	NA	0.9949	0.8640	10	776	R050400025	8.98		
	C92633M	0.33	NA	0.9949	0.8640	50	109	R051000039	14.3		
	C92637M	0.85	NA	0.9949	0.8640	10	178	R050400026	1.81		
	C92641M	0.74	NA	0.9949	0.8640	50	257	R052500108	15.1		
	C92642M	0.90	NA	0.9949	0.8640	10	627	R050400027	6.05		
	C92643M	0.75	NA	0.9949	0.8640	50	47.5	R051000041	2.75		
	C92644M	1.00	NA	0.9949	0.8640	10	139	R050400030	1.21		
	C92649M	0.82	NA	0.9949	0.8640	50	163	R051000044	8.63		
	C92653M	1.00	NA	0.9949	0.8640	1	561	R052600026	0.487		
	C92657M	0.90	NA	0.9949	0.8640	50	108	R051000046	5.20		
	C92659M	0.78	NA	0.9949	0.8640	50	258	R051000047	14.4		
	C92660M	0.96	NA	0.9949	0.8640	50	36.7	R051000048	1.66		
	C92669M	0.35	NA	0.9949	0.8640	50	25.2	R051000051	3.13		
	C92674M	1.00	NA	0.9949	0.8640	100	406	R050400031	35.3		
	C92682M	0.90	NA	0.9949	0.8640	10	495	R050400032	4.77		
	C92683M	0.85	NA	0.9949	0.8640	50	53.3	R051000052	2.72		113
	C92690M	0.80	NA	0.9949	0.8640	50	52.1	R051000053	2.83	7.60	8.60

Original PFOS LOQ (4.93 ng/mL, 9.76 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

Date Entered/By: 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/14/00, 11/17/00 MMH/CSH/LAC
Date Verified/By: 08/31/00 KJH, 11/21/00 MMH
Purity Entered/Verified: 10/04/00 LAC / 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number(Test Substance): T-6295 (PFOS)
Matrix: Rat Serum
Method/Revision: ETS-8.4.1 & ETS-8.5.1
Analytical Equipment System Number: Ruby 100699, Davey 070799
Instrument Software/Version: MassLynx 3.3, 3.4
Filename: See listing to the right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 05/01/00, 05/02/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00 MMH/LAS
Date of Data Reduction/Analyst: 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH

Sample Data

WEEK 105 RAT SERA

Group Dose		Sample #	Initial Vol. mL	Surrogate Verified	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (optional)	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Group 4 mg/kg/day		C92691M	0.90	NA	0.9949	0.8640	20	232	R050400033	4.48		
		C92693M	0.85	NA	0.9949	0.8640	100	75.5	D051100016	7.71		
		C92694M	0.75	NA	0.9949	0.8640	100	640	R050400034	74.2		
		C92695M	0.85	NA	0.9949	0.8640	100	740	R050400037	75.6		
		C92696M	0.95	NA	0.9949	0.8640	100	423	R050400038	38.7		
		C92698M	1.00	NA	0.9949	0.8640	100	140	D051100017	12.1		
		C92699M	0.70	NA	0.9949	0.8640	100	591	R050400039	73.3		
		C92700M	0.75	NA	0.9949	0.8640	100	248	D051100018	28.8		
		C92702M	0.90	NA	0.9949	0.8640	100	32.6	D051100019	3.14		
		C92704M	0.79	NA	0.9949	0.8640	100	98.9	D051100020	10.9		
		C92705M	0.73	NA	0.9949	0.8640	200	96.8	R053100024	23.0		
		C92708M	0.67	NA	0.9949	0.8640	20	375	R053100025	9.72		
		C92716M	0.75	NA	0.9949	0.8640	20	584	R053100018	13.5		
		C92717M	1.00	NA	0.9949	0.8640	100	34.7	D051100024	3.01		
		C92724M	0.85	NA	0.9949	0.8640	20	626	R050400044	12.8		
		C92726M	0.75	NA	0.9949	0.8640	100	335	D051100025	38.8		
		C92730M	0.70	NA	0.9949	0.8640	100	54.1	D051100026	6.72		
		C92735M	0.70	NA	0.9949	0.8640	200	98.5	R050900029	24.4		
		C92736M	0.75	NA	0.9949	0.8640	100	35.5	D051100027	4.10		
		C92739M	1.00	NA	0.9949	0.8640	100	65.8	D051100030	5.72		
		C92740M	0.85	NA	0.9949	0.8640	100	349	D051100031	35.7		
		C92742M	1.00	NA	0.9949	0.8640	100	26.3	D051100032	2.28		
		C92747M	0.57	NA	0.9949	0.8640	100	300	D051100033	45.7		
		C92748M	1.00	NA	0.9949	0.8640	100	42.1	D051100034	3.66		105
		C92750M	0.85	NA	0.9949	0.8640	100	33.3	D051100037	3.40	22.5	23.5
		C93059F	0.80	NA	0.9949	0.8640	500	189	R052500109	103		
		C93060F	0.85	NA	0.9949	0.8640	500	175	R052600028	89.5		
		C93062F	1.00	NA	0.9949	0.8640	1000	117	R050900030	102		
		C93063F	1.00	NA	0.9949	0.8640	1000	197	R050900031	171		
		C93072F	0.85	NA	0.9949	0.8640	500	223	R052500109	114		
		C93077F	1.00	NA	0.9949	0.8640	100	438	D051100041	38.0		
		C93078F	0.94	NA	0.9949	0.8640	50	562	R050400048	25.9		
		C93079F	1.00	NA	0.9949	0.8640	500	168	R052500113	72.8		
		C93080F	0.90	NA	0.9949	0.8640	500	153	R052600029	73.6		
		C93081F	0.78	NA	0.9949	0.8640	500	127	R052500114	70.5		
		C93090F	1.00	NA	0.9949	0.8640	200	570	R050400051	99.1		
		C93098F	1.00	NA	0.9949	0.8640	100	59.5	D051100047	5.17		
		C93101F	0.75	NA	0.9949	0.8640	500	202	R052500115	117		
		C93102F	0.80	NA	0.9949	0.8640	100	105	R051100051	11.4		60.9
		C93107F	0.83	NA	0.9949	0.8640	50	635	R050400052	33.2	75.0	45.7
Group 5 mg/kg/day		C92753M	0.80	NA	0.9949	0.8640	2000	16.5	D051100052	35.8		
		C92757M	0.87	NA	0.9949	0.8640	1000	196	R050900032	196		
		C92761M	0.65	NA	0.9949	0.8640	2000	10.2	D051100053	27.3		
		C92762M	1.00	NA	0.9949	0.8640	200	751	R050400054	130		
		C92774M	1.00	NA	0.9949	0.8640	50	239	R052600033	10.4		
		C92775M	0.85	NA	0.9949	0.8640	50	217	R052500117	11.1		
		C92776M	0.96	NA	0.9949	0.8640	2000	59.9	D051100058	108		
		C92780M	1.00	NA	0.9949	0.8640	2000	38.8	D051100059	67.4		
		C92782M	0.57	NA	0.9949	0.8640	50	220	R052500120	16.7		
		C92786M	0.91	NA	0.9949	0.8640	50	577	R050400055	27.5		
		C92788M	0.80	NA	0.9949	0.8640	2000	54.4	D051100061	118		
		C92791M	0.93	NA	0.9949	0.8640	50	459	R050400058	21.4		
		C92792M	0.64	NA	0.9949	0.8640	2000	6.62	D051100062	18.0		
		C92800M	0.91	NA	0.9949	0.8640	200	336	R050400059	64.2		
		C92802M	0.92	NA	0.9949	0.8640	1000	179	R050900033	169		
		C92803M	0.75	NA	0.9949	0.8640	2000	60.7	D051100065	141		
		C92804M	0.88	NA	0.9949	0.8640	200	645	R050400061	127		
		C92808M	0.86	NA	0.9949	0.8640	50	736	R050400062	37.2		
		C92809M	0.65	NA	0.9949	0.8640	50	90.1	R052600034	6.02		
		C92814M	0.85	NA	0.9949	0.8640	2000	43.7	D051100067	89.2		
		C92815M	0.70	NA	0.9949	0.8640	2000	4.79	D051100068	11.9		83.6
		C92818M	0.85	NA	0.9949	0.8640	2000	44.1	D051100069	90.1	69.3	57.9
		C93113F	0.94	NA	0.9949	0.8640	2000	218	R050900036	403		
		C93115F	0.90	NA	0.9949	0.8640	2000	148	D051100072	286		
		C93124F	1.00	NA	0.9949	0.8640	2000	91.9	D051100073	160		
		C93125F	1.00	NA	0.9949	0.8640	2000	203	R050900037	353		
		C93133F	1.00	NA	0.9949	0.8640	2000	139	D051100074	242		
		C93135F	1.00	NA	0.9949	0.8640	2000	158	R050900038	274		
		C93137F	0.95	NA	0.9949	0.8640	2000	78.0	D051100075	143		
		C93139F	0.75	NA	0.9949	0.8640	2000	162	D051100076	375		
		C93140F	0.67	NA	0.9949	0.8640	50	59.5	R052500123	3.85		
		C93146F	1.00	NA	0.9949	0.8640	2000	29.6	D051100080	51.5		
		C93149F	0.80	NA	0.9949	0.8640	2000	199	R050900039	432		
		C93151F	1.00	NA	0.9949	0.8640	2000	80.1	D051100081	139		
		C93153F	1.00	NA	0.9949	0.8640	2000	69.7	D051100082	121		
		C93157F	0.85	NA	0.9949	0.8640	2000	14.0	D051100083	28.6		
		C93158F	1.00	NA	0.9949	0.8640	2000	165	R050900040	287		
		C93161F	0.84	NA	0.9949	0.8640	200	499	R050400072	103		
		C93162F	1.00	NA	0.9949	0.8640	2000	257	R050900043	446		
		C93164F	1.00	NA	0.9949	0.8640	2000	185	R050900044	321		
		C93165F	1.00	NA	0.9949	0.8640	2000	94.7	D051100086	165		
		C93166F	0.70	NA	0.9949	0.8640	2000	76.0	D051100087	189		
		C93168F	0.92	NA	0.9949	0.8640	2000	180	D051100088	339		
		C93174F	1.00	NA	0.9949	0.8640	2000	175	R050900045	304		
		C93177F	0.90	NA	0.9949	0.8640	2000	128	D051100089	248		
		C93178F	1.00	NA	0.9949	0.8640	2000	124	D051100090	216		53.2
		C93179F	0.99	NA	0.9949	0.8640	2000	109	D051100093	190	233	124

Original PFOS LOQ updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

Date Entered/By: 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/14/00, 11/17/00 MMH/CSH/LAC
Date Verified/By: 08/31/00 kjh, 11/21/00 MMH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

LRN-U2121

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number/Test Substance: T-6295 (PFOS)
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
ANALtical Equipment System Number: Ruby 100699, Davey 070799
Instrument Software/Version: MassLynx 3.3, 3.4
Filename: See listing to the right
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 05/01/00, 05/02/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00, 07/28/00 MMH/LAS
Date of Data Reduction/Analyst: 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/1/00, 07/31/00, 11/16/00 LAS/MMH

Sample Data

WEEK 106 RAT SERA

Lot 171

Group Dose	Sample #	Initial Vol. mL	Surrogate Verified	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	FileName (optional)	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Group 6 mg/kg/day	C92821M	0.82	NA	0.9949	0.8640	20	393	R052600036	8.32		
	C92823M	0.84	NA	0.9949	0.8640	20	25.8	R052600037	0.533		
	C92827M	0.96	NA	0.9949	0.8640	1	33.6	R053100031	0.0303		
	C92829M	0.93	NA	0.9949	0.8640	1	26.0	R053100032	0.0243		
	C92833M	0.66	NA	0.9949	0.8640	1	60.4	R053100035	0.0795		
	C92834M	0.69	NA	0.9949	0.8640	1	11.0	R053100036	0.0138		
	C92848M	0.80	NA	0.9949	0.8640	20	686	R052600044	14.9		
	C92851M	0.84	NA	0.9949	0.8640	1	32.0	R053100037	0.0331		
	C92853M	0.84	NA	0.9949	0.8640	1	203	D072800017	0.210		211
	C92856M	0.90	NA	0.9949	0.8640	1	3.65	R053100038	< LOQ (0.00428 ug/mL)	2.42	5.09
	C93181F	0.86	NA	0.9949	0.8640	20	285	R052600050	5.76		
	C93182F	0.86	NA	0.9949	0.8640	20	83.5	R052600051	1.69		
	C93183F	1.00	NA	0.9949	0.8640	20	327	R052600054	5.68		
	C93184F	0.82	NA	0.9949	0.8640	20	77.6	R052600055	1.64		
	C93185F	0.95	NA	0.9949	0.8640	20	647	R052600056	11.8		
	C93190F	0.76	NA	0.9949	0.8640	50	553	D072800018	31.6		
	C93192F	0.95	NA	0.9949	0.8640	20	918	R052600058	16.8		
	C93197F	1.00	NA	0.9949	0.8640	20	538	R052600061	9.35		
	C93198F	0.81	NA	0.9949	0.8640	20	403	R052600062	8.63		
	C93201F	0.79	NA	0.9949	0.8640	50	492	D072800019	27.0		
	C93202F	0.77	NA	0.9949	0.8640	20	447	R052600064	10.1		
	C93205F	0.78	NA	0.9949	0.8640	1	53.3	R053100039	0.0594		
	C93209F	0.87	NA	0.9949	0.8640	20	329	R052600068	6.57		
	C93211F	0.88	NA	0.9949	0.8640	20	623	R052600069	12.3		
	C93216F	1.00	NA	0.9949	0.8640	20	164	R052600070	2.85		
	C93219F	0.85	NA	0.9949	0.8640	20	117	R052600071	2.39		91.5
	C93220F	1.00	NA	0.9949	0.8640	20	428	R052600072	7.44	9.51	8.70

Original PFOS LOQ (4.93 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

Date Entered/By: 06/08/00, 06/19/00, 08/14/00, 11/14/00, 11/17/00 CSH/LAC

Date Verified/ By: 08/31/00 KJH, 11/21/00 MMH

Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1

Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295 (PFOS)
 Matrix: Rat Liver
 Method/Revision: FACT-M-1.0 & ETS-8-7.0
 Analytical Equipment System Number: Madeline 041098, Amelia 062498
 Instrument Software/Version: MassLynx 3.3
 Date of Extraction/Analyst: 6/11/98 IAS/RWW
 Date of Analysis/Analyst: 07/06/98, 07/14/98 KJH/MEE
 Date of Data Reduction/Analyst: 02/02/00 IAS

Filename: See List to Right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Sample Data

WEEK 4 RAT LIVER REWORK

Lot 193

Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1.0000	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)		
	H2O Blk-2	1.0000	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)	<LOQ (0.0552 ug/g)	NA
Matrix Blk	Rabbit Liver Blk-1	1.0000	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)		
	Rabbit Liver Blk-2	1.0000	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)	<LOQ (0.0552 ug/g)	NA
QC - 100 ppb	C92506M-MS	1.0108	NA	NA	231	1	207	174%		
	C92506M-MSD	1.0108	NA	NA	311	1	286	241%	207%	32.15%
Group 1 Control 0.0 ppm in Diet	C92506M	1.0108	NA	0.9275	21.8	1	20.0	<LOQ (0.0552 ug/g)		
	C92524M	0.9998	NA	0.9275	182	1	169	0.169		
	C92526M	1.0287	NA	0.9275	207	1	186	0.186		
	C92529M	1.0117	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)		64.6
	C92546M	1.0034	NA	0.9275	0.00	1	0.00	<LOQ (0.0552 ug/g)	0.104	0.0673
	C92861F	1.0140	NA	0.9275	89.4	1	81.7	0.0817		
	C92864F	1.0172	NA	0.9275	80.9	1	73.8	0.0738		
	C92914F	1.0186	NA	0.9275	202	1	184	0.184		
	C92919F	1.0145	NA	0.9275	76.6	1	70.0	0.0700		45.4
	C92926F	1.0092	NA	0.9275	137	1	126	0.126	0.107	0.0486
Group 2 Low Dose 0.5 ppm in Diet	C92574M	0.9928	NA	0.9275	398	33.3	12376	12.4		
	C92575M	1.0187	NA	0.9275	319	33.3	9670	9.67		
	C92610M	1.0060	NA	0.9275	278	33.3	8539	8.54		
	C92613M	1.0075	NA	0.9275	465	33.3	14248	14.2		21.1
	C92618M	1.0133	NA	0.9275	326	33.3	9937	9.94	11.0	2.31
	C92982F	0.9971	NA	0.9275	293	33.3	9089	9.09		
	C92941F	1.0104	NA	0.9275	261	33.3	7988	7.99		
	C92945F	1.0161	NA	0.9275	309	33.3	9398	9.40		
	C92950F	1.0132	NA	0.9275	277	33.3	8429	8.43		6.33
	C92969F	1.0038	NA	0.9275	282	33.3	8669	8.67	8.71	0.552
Group 3 Mid Dose 2.0 ppm in Diet	C92646M	1.0116	NA	0.9275	444	100	40692	40.7		
	C92650M	1.0072	NA	0.9275	283	100	26094	26.1		
	C92652M	1.0030	NA	0.9275	337	100	31167	31.2		
	C92668M	1.0036	NA	0.9275	346	100	31975	32.0		18.7
	C92678M	1.0010	NA	0.9275	289	100	26736	26.7	31.3	5.84
	C92996F	1.0047	NA	0.9275	313	100	28866	28.9		
	C93006F	0.9943	NA	0.9275	157	100	14601	14.6		
	C93008F	1.0158	NA	0.9275	325	100	29691	29.7		
	C93031F	1.0166	NA	0.9275	297	100	27099	27.1		24.4
	C93045F	1.0076	NA	0.9275	270	100	24847	24.8	25.0	6.11
Group 4 Mid-High Dose 5.0 ppm in Diet	C92715M	1.0003	NA	0.9275	577	100	53503	53.5		
	C92718M	1.0026	NA	0.9275	645	100	59632	59.6		
	C92731M	1.0139	NA	0.9275	292	100	26740	26.7		
	C92734M	0.9930	NA	0.9275	533	100	49826	49.8		26.2
	C92744M	1.0150	NA	0.9275	528	100	48285	48.3	47.6	12.5
	C93058F	1.0083	NA	0.9275	1161	100	106760	107		
	C93067F	1.0038	NA	0.9275	908	100	83858	83.9		
	C93070F	0.9957	NA	0.9275	826	100	76985	77.0		
	C93075F	1.0068	NA	0.9275	767	100	70660	70.7		17.0
	C93084F	1.0133	NA	0.9275	838	100	76723	76.7	83.0	14.1
Group 5 High Dose 20 ppm in Diet	C92752M	1.0100	NA	0.9275	1190	200	218558	219	*	
	C92759M	1.0122	NA	0.9275	1439	200	263670	264	*	
	C92779M	1.0082	NA	0.9275	1858	200	341904	342	*	
	C92793M	0.9949	NA	0.9275	1533	200	285921	286	*	16.1
	C92798M	1.0158	NA	0.9275	1630	200	297613	298	*	45.3
	C93114F	1.0043	NA	0.9275	2355	200	434951	435	*	
	C93123F	1.0010	NA	0.9275	1943	200	360089	360	*	
	C93142F	1.0076	NA	0.9275	2144	200	394644	395	*	
	C93144F	1.0102	NA	0.9275	1728	200	317269	317	*	11.8
	C93159F	1.0130	NA	0.9275	1959	200	358782	359	*	44.1

Original PFOS LOQ (59.5 ng/g) updated to reflect k+ information on 10/04/00. LAC 10/04/00

NA=Not Applicable

PFOS = Perfluorooctanesulfonate

Correction Factors not applicable to MS/MSD QC data

* Results were outside the range of the curve and should be considered to be estimates. LAC 10/04/00

Date Entered/Analyst: 02/08/00 LAC
 Date Verified/Analyst: 10/03/00 KJH
 Purity Entered/Verified: 10/04/00 LAC

AMDT# 112296.1

Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295
 Matrix: Rat Liver
 Method/Revision: FACT-M-1.0 & ETS-8-7.0
 Analytical Equipment System Number: Madeline 041098, Amelia 062498
 Instrument Software/Version: MassLynx 3.3
 Date of Extraction/Analyst: 08/06/98 SAH
 Date of Analysis/Analyst: 08/10/98, 08/14/98, 08/17/98 MEE/HOJ
 Date of Data Reduction/Analyst: 02/03/00 IAS

Filename: See List to Right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Sample Data

WEEK 14 RAT LIVER REWORK

Lot 193

Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1.0000	NA	0.9275	0.00	1	0.00	<LOQ(0.0569 ug/g)		
	H2O Blk-2	1.0000	NA	0.9275	60.9	1	56.5	<LOQ(0.0569 ug/g)	<LOQ(0.0569 ug/g)	NA
Matrix Blk	Rabbit Liver Blk-1	1.0000	NA	0.9275	0.00	1	0.00	<LOQ(0.0569 ug/g)		
	Rabbit Liver Blk-2	1.0000	NA	0.9275	12.1	1	11.2	<LOQ(0.0569 ug/g)	<LOQ(0.0569 ug/g)	NA
QC - 100 ppb	C92509M-MS	0.9979	NA	NA	533	1	59.6	50%		
	C92509M-MSD	0.9979	NA	NA	658	1	184	154%	102%	102%
Group 1 Control 0.0 ppm in Diet	C92509M	0.9979	NA	0.9275	473	1	440	0.440		
	C92511M	1.0044	NA	0.9275	587	1	542	0.542		
	C92521M	0.9960	NA	0.9275	529	1	492	0.492		
	C92528M	1.0131	NA	0.9275	440	1	403	0.403		
	C92532M	1.0140	NA	0.9275	457	1	418	0.418	0.459	12.5
	C92800F	1.0008	NA	0.9275	110	10	1017	1.02		0.0573
	C92807F	1.0047	NA	0.9275	563	100	51996	52.0		
	C92809F	1.0165	NA	0.9275	76.4	10	697	0.697		186
	C92903F	1.0078	NA	0.9275	572	10	5263	5.26		22.4
	C92905F	0.9945	NA	0.9275	126	10	1175	1.18	12.0	
Group 2 Low Dose 0.5 ppm in Diet	C92593M	0.9975	NA	0.9275	265	100	24625	24.6		
	C92600M	1.0058	NA	0.9275	209	100	19274	19.3		
	C92616M	1.0112	NA	0.9275	296	100	27191	27.2		
	C92621M	1.0082	NA	0.9275	230	100	21166	21.2		14.5
	C92627M	0.9940	NA	0.9275	285	100	26634	26.6	23.8	3.45
	C92944F	1.0005	NA	0.9275	204	100	18933	18.9		
	C92962F	0.9994	NA	0.9275	191	100	17718	17.7		
	C92967F	1.0132	NA	0.9275	271	100	24839	24.8		
	C92978F	1.0013	NA	0.9275	156	100	14461	14.5		19.7
	C92987F	1.0095	NA	0.9275	216	100	19842	19.8	19.2	3.77
Group 3 Mid Dose 2.0 ppm in Diet	C92640M	1.0095	NA	0.9275	180	500	82666	82.7		
	C92645M	0.9974	NA	0.9275	158	500	73468	73.5		
	C92662M	0.9977	NA	0.9275	147	500	68263	68.3		
	C92676M	0.9926	NA	0.9275	146	500	68329	68.3		8.32
	C92684M	0.9987	NA	0.9275	167	500	77352	77.4	74.0	6.16
	C92993F	1.0006	NA	0.9275	149	500	69020	69.0		
	C93000F	0.9994	NA	0.9275	153	500	71061	71.1		
	C93018F	1.0081	NA	0.9275	146	500	67380	67.4		
	C93023F	0.9965	NA	0.9275	139	500	64869	64.9		4.99
	C93035F	1.0019	NA	0.9275	160	500	73916	73.9	69.2	3.46
Group 4 Mid-High Dose 5.0 ppm in Diet	C92713M	1.0048	NA	0.9275	406	1000	374738	375		
	C92714M	1.0044	NA	0.9275	403	1000	372357	372		
	C92719M	1.0050	NA	0.9275	409	1000	377202	377		
	C92722M	1.0095	NA	0.9275	336	1000	308422	308		8.05
	C92728M	1.0011	NA	0.9275	384	1000	356019	356	358	28.8
	C93051F	0.9944	NA	0.9275	381	1000	355218	355		
	C93064F	1.0103	NA	0.9275	393	1000	360819	361		
	C93071F	1.0001	NA	0.9275	383	1000	354845	355		
	C93085F	1.0038	NA	0.9275	404	1000	373393	373		6.03
	C93109F	1.0022	NA	0.9275	441	1000	408111	408	370	22.3
Group 5 High Dose 20 ppm in Diet	C92765M	0.9983	NA	0.9275	777	1000	722350	722		
	C92777M	1.0067	NA	0.9275	670	1000	617695	618		
	C92789M	0.9920	NA	0.9275	598	1000	559277	559		
	C92799M	1.0046	NA	0.9275	494	1000	456225	456		18.8
	C92812M	1.0064	NA	0.9275	526	1000	485214	485	568	107
	C93111F	0.9982	NA	0.9275	745	1000	692038	692		
	C93127F	1.0056	NA	0.9275	672	1000	619486	619		
	C93143F	1.0136	NA	0.9275	679	1000	621011	621		
	C93155F	1.0125	NA	0.9275	737	1000	674725	675		7.72
	C93169F	0.9981	NA	0.9275	612	1000	569017	569	635	49.0

Original PFOS LOQ (61.3 ng/mL) updated to reflect k+ information on 10/04/00. LAC 10/04/00

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctanesulfonamide

PFOSAA = Perfluorooctanesulfonamidoacetate

EAFOS = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

Correction Factors not applicable to MS/MSD QC data

NA = Not Applicable

Date Entered/Analyst: 02/09/00 LAC
 Date Verified/Analyst: 10/03/00 K/JH
 Purity Entered/Verified: 10/04/00 LAC

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295
 Matrix: Rat Liver
 Method/Revision: ETS-8-6.0 and ETS-8-7.0
 Analytical Equipment System Number: Soup 020199
 Instrument Software/Version: MassLynx 3.3
 Date of Extraction/Analyst: 11/11/99, 01/25/00 MCH/SAL
 Date of Analysis/Analyst: 11/15/99, 11/19/99 11/22/99 IAS/MMH, 11/24/99 GML, 12/16/99 IAS
 Date of Data Reduction/Analyst: 11/16/99, 11/22/99, 11/23/99 IAS, 12/1/99 MMH, 12/17/99, 11/20/00 IAS/KJH

FileNames

	PFOS	PFOSA
Blanks	121699046-47,92-93	NA
Grp 1 M	111999056-58, 121699067-76	NA
Grp 1 F	111999059-, 121699063,77-87	NA
Grp 5 M	112499088-99	NA
Grp 5 F	112499100-115	NA
MS, MSD	121699060	NA
Box 99-222		

Sample Data

WEEK 53 RAT LIVER

Lot 171												
Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	
Method Blk	H2O Blk-9	1.0000	NA	0.9275	0.8640	24.4	1	19.6	<LOQ (0.0491 ug/g)			
	H2O Blk-10	1.0000	NA	0.9275	0.8640	21.4	1	17.1	<LOQ (0.0491 ug/g)	<LOQ (0.0491 ug/g)	NA	
Matrix Blk	Rabbit Liver Blk-9	1.0000	NA	0.9275	0.8640	29.0	1	23.2	<LOQ (0.0491 ug/g)			
	Rabbit Liver Blk-10	1.0000	NA	0.9275	0.8640	13.7	1	11.0	<LOQ (0.0491 ug/g)	<LOQ (0.0491 ug/g)	NA	
QC	C92517M-250 ppb-MS9	0.9979	NA	NA	NA	109	10	119	40%			
	C92876F-250 ppb-MSD10	0.9937	NA	NA	NA	338	1	176	58%	49%	39%	
Group 1 Control 0.0 ppm in Diet	C92501M	1.0185	NA	0.9275	0.8640	41.5	1	32.6	0.0326			
	C92504M	1.0095	NA	0.9275	0.8640	451	1	358	0.358			
	C92507M	0.9916	NA	0.9275	0.8640	372	1	301	0.301			
	C92512M	0.9982	NA	0.9275	0.8640	352	1	283	0.283			
	C92517M	1.0192	NA	0.9275	0.8640	96.8	10	761	0.761			
	C92523M	0.9909	NA	0.9275	0.8640	65.4	10	529	0.529			
	C92534M	1.0065	NA	0.9275	0.8640	451	1	359	0.359			
	C92540M	1.0043	NA	0.9275	0.8640	133	1	106	0.106			
	C92562M	1.0017	NA	0.9275	0.8640	440	10	3519	3.52		163	
	C92570M	1.0099	NA	0.9275	0.8640	130	1	103	0.103	0.635	1.04	
	C92862F	1.0085	NA	0.9275	0.8640	712	10	5657	5.66			
	C92876F	0.9937	NA	0.9275	0.8640	163	1	131	0.131			
Females	C92889F	0.9965	NA	0.9275	0.8640	259	10	2081	2.08			
	C92894F	1.0128	NA	0.9275	0.8640	349	1	276	0.276			
	C92899F	0.9979	NA	0.9275	0.8640	271	1	217	0.217			
	C92910F	1.0171	NA	0.9275	0.8640	251	1	198	0.198			
	C92917F	1.0066	NA	0.9275	0.8640	309	1	246	0.246			
	C92922F	1.0083	NA	0.9275	0.8640	73.0	1	58.0	0.0580			
	C92923F	1.0118	NA	0.9275	0.8640	195	1	154	0.154		191	
	C92930F	1.0028	NA	0.9275	0.8640	269	1	215	0.215	0.923	1.77	
Group 5 High Dose 20 ppm in Diet	C92751M	1.0034	NA	0.9275	0.8640	280	2500	558532	559			
	C92754M	0.9907	NA	0.9275	0.8640	240	2500	485592	486			
	C92755M	1.0139	NA	0.9275	0.8640	246	2500	485843	486			
	C92778M	0.9951	NA	0.9275	0.8640	139	2500	279139	279			
	C92790M	1.0111	NA	0.9275	0.8640	204	2500	404861	405			
	C92795M	1.0179	NA	0.9275	0.8640	282	2500	554138	554			
	C92796M	0.9930	NA	0.9275	0.8640	212	2500	426888	427			
	C92817M	0.9950	NA	0.9275	0.8640	200	2500	403197	403		22.3	
	C92820M	1.0019	NA	0.9275	0.8640	158	2500	315897	316	435	96.9	
Group 5 High Dose 20 ppm in Diet	C93112F	1.0040	NA	0.9275	0.8640	148	2500	296060	296			
	C93116F	0.9931	NA	0.9275	0.8640	265	2500	533843	534			
	C93117F	0.9943	NA	0.9275	0.8640	255	2500	513715	514			
	C93120F	1.0004	NA	0.9275	0.8640	215	2500	430218	430			
	C93130F	0.9912	NA	0.9275	0.8640	250	2500	504367	504			
	C93138F	0.9945	NA	0.9275	0.8640	280	2500	564538	565			
	C93147F	1.0040	NA	0.9275	0.8640	310	2500	619098	619			
	C93154F	0.9967	NA	0.9275	0.8640	211	2500	423936	424			
	C93163F	1.0141	NA	0.9275	0.8640	447	2500	883325	883		32.2	
	C93171F	0.9000	NA	0.9275	0.8640	373	2500	830454	830	560	180	

Original PFOS LOQ (26.9 ng/g, 61.3 ng/g, 12.3 ng/g) updated to reflect K+ and purity information on 10/04/00. LAC 10/04/00

Correction Factors not applicable to MS/MSD QC data

PFOS = Perfluorooctanesulfonate

NA = Not Applicable

Date Entered/Analyst: 11/24/99 LAC, 12/2/99 GML, 01/24/00 MMH, 11/20/00 LAC
 Date Verified/Analyst: 10/03/00 KJH, 11/21/00 MMH
 Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
Product Number/Test Substance: T-6295
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 and ETS-8-7.0
Analytical Equipment System Number: Amelia 062498
Instrument Software/Version: Masslynx 3.4
Date of Extraction/Analyst: 05/16/00, 05/18/00 SAL/KJK
Date of Analysis/Analyst: 05/22/00, 05/24/00, 05/25/00, 06/12/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH
Date of Data Reduction/Analyst: 05/30/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH
Sample Data

DAY 719 RAT LIVER

Lot 171

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	05160-H2OBlk-5	NA	1.00	NA	0.9949	0.8640	0.740	1	0.643	D061400077	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
	05160-H2OBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400078	< LOQ (0.0107 ug/g)		
	05180-H2OBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	05180-H2OBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200073	< LOQ (0.0107 ug/g)		
	052200-H2OBlk-3	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400088	< LOQ (0.0107 ug/g)		
	052200-H2OBlk-4	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400089	< LOQ (0.0107 ug/g)		
Matrix Blk	RBL05160-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400079	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
	RBL05160-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400080	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.570	1	0.495	A052200074	< LOQ (0.0107 ug/g)		
	RBL052200-LiverBlk-3	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400090	< LOQ (0.0107 ug/g)		
	RBL052200-LiverBlk-4	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400091	< LOQ (0.0107 ug/g)		
QC	C92503M G1-MSD-250ppb-3	NA	1.0161	NA	NA	NA	357	1	177	A073100018	60%	69%	26%
	C92503M G1-MSD-250ppb-3	NA	1.0161	NA	NA	NA	411	1	230	A073100019	78%		
	C92505M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	496	1	377	A052200020	126%		
	C92505M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	478	1	359	A052200021	120%		
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	125	10	389	A061200060	126%		
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	135	10	488	A061200061	158%		
	C92868F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	379	1	253	D072800055	85%		
	C92868F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	433	1	306	A052200025	102%		
	C92510M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	803	1	217	D072800056	72%		
	C92510M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	966	1	380	A052200076	126%		
	C92991F G3-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	492	100	5430	D061400045	1816%		
	C92991F G3-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	517	100	7923	D061400046	2650%		
Group 3 Mid Dose 2.0 ppm in Diet	C92821M G6-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	319	50	6423	D061400047	2134%	55.1	37%
	C92821M G6-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	309	50	5914	D061400048	1965%		
	C92991F	NA	1.0024	NA	0.9949	0.8640	504	100	43639	A052500015	43.6		
	C93003F	NA	0.9967	NA	0.9949	0.8640	593	100	51692	A052500016	51.7		
	C93007F	NA	1.0010	NA	0.9949	0.8640	1121	100	97210	A052500017	97.2		
	C93011F	NA	1.0018	NA	0.9949	0.8640	153	100	13222	A052500018	13.2		
	C93022F	NA	1.0008	NA	0.9949	0.8640	104	100	9018	A052500019	9.02		
	C93039F	NA	1.0030	NA	0.9949	0.8640	921	100	79737	A052500022	79.7		
	C93040F	NA	0.9967	NA	0.9949	0.8640	1080	100	94108	A052500023	94.1		
	C93047F	NA	1.0016	NA	0.9949	0.8640	615	100	53315	A052500024	53.3	57.2	31.5
	C93048F	NA	1.0020	NA	0.9949	0.8640	620	100	53775	A052500025	53.8		

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00

* Reanalyzed with the same results. LAC 08/14/00

a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. KJH 10/03/00

NA = Not applicable

Date Entered/Analyst: 06/01/00, 06/21/00, 06/22/00, 08/14/00 CSH/LAC

Date Verified/Analyst: 10/03/00 KJH

Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295
 Matrix: Rat Liver
 Method/Revision: ETS-8.6.0 and ETS-8.7.0
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: Mawlyn 3.4
 Date of Extraction/Analysis: 05/16/00, 05/18/00, 05/22/00, 05/24/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00, 1AS/MMH
 Date of Analysis/Analyst: 05/19/00, 05/22/00, 05/24/00, 06/12/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00, 11/20/00, 1AS/MMH/KJH
 Date of Data Reduction/Analyst: 05/22/00, 05/23/00, 05/24/00, 05/25/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00, 11/20/00, 1AS/MMH/KJH
 Sample Data

WEEK 105 RAT LIVER														Lot 171	
Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	Original Purity Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ug/g ur % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD		
Method Blk	05160-H2OBlk-5	NA	1.00	NA	0.9949	0.8640	0.740	1	0.643	D061400077	< LOQ (0.0107 ug/g)				
	05160-H2OBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400078	< LOQ (0.0107 ug/g)				
	05180-H2OBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)				
	05180-H2OBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200073	< LOQ (0.0107 ug/g)				
	052200-H2OBlk-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400088	< LOQ (0.0107 ug/g)		NA		
Matrix Blk	052200-H2OBlk-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400089	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA		
	RBL05160-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400079	< LOQ (0.0107 ug/g)				
	RBL05160-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400080	< LOQ (0.0107 ug/g)				
	RBL05180-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	1.14	1	0.990	A052200060	< LOQ (0.0107 ug/g)				
	RBL052200-LiverBlk-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400090	< LOQ (0.0107 ug/g)		NA		
QC	RBL052200-LiverBlk-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400091	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA		
	C92503M GI-MS-250ppb-3	NA	1.0161	NA	NA	NA	357	1	177	A073100018	60%	*			
	C92503M GI-MSD-250ppb-3	NA	1.0161	NA	NA	NA	411	1	230	A073100019	78%		69%		
	C92505M GI-MS-250ppb-5	NA	0.9927	NA	NA	NA	496	1	377	A052200020	126%		26%		
	C92505M GI-MSD-250ppb-5	NA	0.9927	NA	NA	NA	478	1	359	A052200021	120%		5%		
	C92866F GI-MS-250ppb-4	NA	0.9665	NA	NA	NA	125	10	389	A061200060	126%		123%		
	C92866F GI-MSD-250ppb-4	NA	0.9665	NA	NA	NA	135	10	488	A061200061	158%	*	142%		
	C92868F GI-MS-250ppb-6	NA	1.0055	NA	NA	NA	379	1	253	D072800055	85%		22%		
	C92868F GI-MSD-250ppb-6	NA	1.0055	NA	NA	NA	433	1	306	A052200025	102%		20%		
	C92510M GI-MS-250ppb-6	NA	0.9973	NA	NA	NA	803	1	217	D072800056	72%		94%		
	C92510M GI-MSD-250ppb-6	NA	0.9973	NA	NA	NA	965	1	380	A052200076	126%	*	55%		
	C92991F GI-MS-250 ppb-5	NA	1.0024	NA	NA	NA	492	100	5430	D061400045	1816%	* a			
	C92991F GI-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	517	100	7923	D061400046	2650%	* a	2233%		
	C92821M GI-MS-250 ppb-5	NA	0.9900	NA	NA	NA	319	50	6423	D061400047	2134%	* a			
	C92821M GI-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	309	50	5914	D061400048	1965%	* a	2049%		
	C92503M	NA	1.0161	NA	0.9949	0.8640	177	1	152	A052400025	0.152				
	C92505M	NA	0.9927	NA	0.9949	0.8640	122	1	106	A052200026	0.106				
	C92510M	NA	0.9973	NA	0.9949	0.8640	587	1	511	A052200077	0.511				
	C92513M	NA	0.9947	NA	0.9949	0.8640	75.9	1	66.3	A052200080	0.0663				
	Males	C92514M	NA	0.9873	NA	0.9949	0.8640	265	1	233	A052400026	0.233			
C92518M		NA	0.9939	NA	0.9949	0.8640	8.00	1	6.99	D061400081	< LOQ (0.0107 ug/g)				
C92541M		NA	1.0035	NA	0.9949	0.8640	32.7	1	28.3	A052200081	0.0283				
C92542M		NA	0.9861	NA	0.9949	0.8640	38.9	1	34.2	A052400027	0.0342				
C92544M		NA	0.9913	NA	0.9949	0.8640	33.2	1	29.1	A052200082	0.0291				
C92551M		NA	1.0077	NA	0.9949	0.8640	0.00	1	0.00	D061400084	< LOQ (0.0107 ug/g)		130		
C92569M		NA	1.0067	NA	0.9949	0.8640	80.0	1	69.0	A052200029	0.0690	0.114	0.148		
C92866F		NA	0.9665	NA	0.9949	0.8640	101	10	907	A061200062	0.907				
C92867F		NA	0.9915	NA	0.9949	0.8640	419	1	367	A052200083	0.367				
C92868F		NA	1.0055	NA	0.9949	0.8640	125	1	108	A052200032	0.108				
Females	C92869F	NA	1.0065	NA	0.9949	0.8640	290	1	250	A052200084	0.250				
	C92871F	NA	1.0005	NA	0.9949	0.8640	25.0	1	21.7	D061400085	< LOQ (0.0107 ug/g)				
	C92873F	NA	1.0150	NA	0.9949	0.8640	297	1	254	A052400031	0.254				
	C92874F	NA	0.9957	NA	0.9949	0.8640	173	1	151	A052200034	0.151				
	C92877F	NA	0.9906	NA	0.9949	0.8640	309	1	271	A052200087	0.271				
	C92881F	NA	0.9994	NA	0.9949	0.8640	104	1	90.4	A052400032	0.0904				
	C92882F	NA	1.0042	NA	0.9949	0.8640	264	1	228	A052400033	0.228				
	C92885F	NA	1.0344	NA	0.9949	0.8640	0.00	1	0.00	D061400092	< LOQ (0.0107 ug/g)				
	C92890F	NA	0.9635	NA	0.9949	0.8640	275	1	248	A052400035	0.248				
	C92892F	NA	0.9984	NA	0.9949	0.8640	56.3	1	49.0	A052200088	0.0490				
Group 2 Low Dose 0.5 ppm in Diet	C92895F	NA	1.0074	NA	0.9949	0.8640	131	1	113	A052200089	0.113				
	C92896F	NA	1.0100	NA	0.9949	0.8640	135	1	116	A052200035	0.116				
	C92897F	NA	0.9983	NA	0.9949	0.8640	182	1	158	A052200036	0.158				
	C92900F	NA	1.0148	NA	0.9949	0.8640	384	1	328	A052200039	0.328				
	C92907F	NA	0.9758	NA	0.9949	0.8640	38.9	1	34.6	A052400038	0.0346				
	C92909F	NA	1.0083	NA	0.9949	0.8640	131	1	113	A052200090	0.113				
	C92911F	NA	1.0094	NA	0.9949	0.8640	239	1	206	A052200091	0.206				
	C92912F	Confirmed Low	0.9960	NA	0.9949	0.8640	76.5	1	66.7	A052200092	0.0667				
	C92913F	NA	1.0035	NA	0.9949	0.8640	211	1	183	A052200040	0.183				
	C92925F	NA	1.0722	NA	0.9949	0.8640	6.16	1	4.99	D061400095	< LOQ (0.0107 ug/g)		99.4		
	C92927F	NA	0.9973	NA	0.9949	0.8640	185	1	161	A052200041	0.161	0.185	0.184		
	Males	C92577M	NA	1.0121	NA	0.9949	0.8640	186	10	1596	A061200098	1.60			
		C92583M	NA	0.9943	NA	0.9949	0.8640	188	10	1641	A051900015	1.64			
		C92590M	NA	1.0076	NA	0.9949	0.8640	90.2	50	3888	D061400032	3.89			
		C92597M	NA	1.0092	NA	0.9949	0.8640	200	100	17190	A061300015	17.2			
C92598M		NA	1.0050	NA	0.9949	0.8640	233	10	2016	A051900017	2.02				
C92601M		NA	0.9814	NA	0.9949	0.8640	244	100	21574	A061200089	21.6				
C92602M		NA	1.0729	NA	0.9949	0.8640	124	100	10022	A061200090	10.0				
C92619M		NA	1.0018	NA	0.9949	0.8640	32.1	50	1390	D061400033	1.39				
C92623M		NA	1.0866	NA	0.9949	0.8640	167	100	13353	A061200091	13.4				
C92626M		NA	1.0069	NA	0.9949	0.8640	136	50	5606	D061400034	5.61	7.83	93.8		
Females	C92936F	NA	0.9944	NA	0.9949	0.8640	120	100	10455	D061400037	10.5				
	C92938F	NA	1.0011	NA	0.9949	0.8640	20	100	1719	D061400038	1.72				
	C92946F	NA	1.0030	NA	0.9949	0.8640	176	100	15281	D061400039	15.3				
	C92953F	NA	1.0082	NA	0.9949	0.8640	234	100	20115	A061200092	20.1				
	C92957F	NA	1.0272	NA	0.9949	0.8640	172	100	14523	A061200093	14.5				
	C92960F	NA	1.0800	NA	0.9949	0.8640	614	10	4938	A061200099	4.94				
	C92964F	NA	0.9955	NA	0.9949	0.8640	230	100	20038	A051900011	20.1				
	C92970F	NA	1.0078	NA	0.9949	0.8640	77.7	100	6691	A051900019	6.69				
	C92973F	NA	1.0016	NA	0.9949	0.8640	58.6	100	5084	A051900023	5.08				
	C92974F	NA	0.9968	NA	0.9949	0.8640	181	100	15787	D061400040	15.8				
	C92975F	NA	0.9967	NA	0.9949	0.8640	155	100	13476	A061200096	13.5				
	C92983F	NA	0.9980	NA	0.9949	0.8640	310	100	26999	A051900024	27.0				
	C92984F	NA	1.0062	NA	0.9949	0.8640	99	100	8576	D061400041	8.58				
	C92986F	NA	0.9951	NA	0.9949	0.8640	151	100	13153	A061200097	13.2		52.7		
	C92988F	NA	1.0099	NA	0.9949	0.8640	198	100	17189	A051900025	17.2	12.9	6.81		

AMDT# 112296.1
Covance# 6329-183

3M Study Title: 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Covance Study Title: 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats
 Product Number/Test Substance: T-6295
 Matrix: Rat Liver
 Method/Revision: ETS-8-6.0 and ETS-8-7.0
 Analytical Equipment System Number: Amelia 062498, Davey 070799
 Instrument Software/Version: Masslynx 3.4
 Date of Extraction/Analysis: 5/19/00, 5/22/00, 5/24/00, 5/26/00, 06/12/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH
 Date of Analysis/Analyst: 5/19/00, 5/22/00, 5/24/00, 5/26/00, 06/12/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH
 Date of Data Reduction/Analyst: 5/22/00, 5/23/00, 5/24/00, 5/25/00, 5/30/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH
 Sample Data

WEEK 105 RAT LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	Original Purity Factor	PFOS Purity Factor	PFOS Conc. ug/g	PFOS Dilution Factor	PFOS Calc. Conc. ug/g	Filename (optional)	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	05160-H20Blk-5	NA	1.00	NA	0.9949	0.8640	0.740	1	0.643	D061400077	< LOQ (0.0107 ug/g)		
	05160-H20Blk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400078	< LOQ (0.0107 ug/g)		
	05180-H20Blk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	05180-H20Blk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200073	< LOQ (0.0107 ug/g)		
	05200-H20Blk-3	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400088	< LOQ (0.0107 ug/g)		NA
	05200-H20Blk-4	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400089	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
Matrix Blk	RBL05160-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400079	< LOQ (0.0107 ug/g)		
	RBL05160-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400080	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.570	1	0.495	A052200074	< LOQ (0.0107 ug/g)		
	RBL05200-LiverBlk-3	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400090	< LOQ (0.0107 ug/g)		NA
	RBL05200-LiverBlk-4	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400091	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
QC	C92503M G1-MSD-250ppb-3	NA	1.0161	NA	NA	NA	357	1	177	A073100019	69%	69%	26%
	C92503M G1-MSD-250ppb-3	NA	1.0161	NA	NA	NA	411	1	230	A073100019	78%		
	C92505M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	496	1	377	A052200020	126%		
	C92505M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	478	1	359	A052200021	120%	123%	5%
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	125	10	389	A061200060	126%		
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	135	10	488	A061200061	158%	142%	22%
	C92868F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	379	1	253	D072800055	85%		
	C92868F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	433	1	306	A052200045	102%	94%	20%
	C92510M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	803	1	217	D072800056	72%		
	C92510M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	966	1	380	A052200076	126%	99%	55%
	C92991F G3-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	492	100	5430	D061400045	1816%	* a	
	C92991F G3-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	517	100	7923	D061400046	2650%	* a	2233%
	C92821M G6-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	319	50	6423	D061400047	2134%	* a	
	C92821M G6-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	309	50	5914	D061400048	1965%	* a	2049%
Group 3 Mid Dose 2.0 ppm in Diet Males	C92631M	NA	1.0036	NA	0.9949	0.8640	480	100	41522	A051900026	41.5		
	C92633M	NA	0.9973	NA	0.9949	0.8640	588	100	51167	A052500026	51.2		
	C92637M	NA	0.9931	NA	0.9949	0.8640	43.7	100	3823	A051900027	3.82		
	C92641M	NA	1.0076	NA	0.9949	0.8640	38.9	100	3352	A052500029	3.35		
	C92642M	NA	0.9928	NA	0.9949	0.8640	273	100	23890	A051900031	23.9		
	C92643M	NA	0.9943	NA	0.9949	0.8640	212	100	18505	A052600015	18.5		
	C92644M	NA	1.0045	NA	0.9949	0.8640	50.0	100	4318	A051900032	4.32		
	C92649M	NA	1.0021	NA	0.9949	0.8640	623	100	54004	A052500030	54.0		
	C92653M	NA	0.9991	NA	0.9949	0.8640	131	25	2855	A061200100	2.85		
	C92657M	NA	1.0731	NA	0.9949	0.8640	245	100	19795	D052600017	19.8		
	C92659M	NA	1.0111	NA	0.9949	0.8640	715	100	61389	A052500031	61.4		
	C92660M	NA	0.9929	NA	0.9949	0.8640	121	100	10581	A052500032	10.6		
	C92669M	NA	1.0099	NA	0.9949	0.8640	202	100	17354	A052500033	17.4		
	C92674M	NA	1.004	NA	0.9949	0.8640	682	100	59020	A051900033	59.0		
Group 4 Mid-High Dose 5.0 ppm in Diet Males	C92682M	NA	0.9935	NA	0.9949	0.8640	437	100	38218	A051900034	38.2		
	C92683M	NA	1.0362	NA	0.9949	0.8640	202	100	16893	D052600018	16.9		77.5
	C92690M	NA	0.9111	NA	0.9949	0.8640	226	100	21542	D052600019	21.5	26.4	20.4
	C92691M	NA	0.9909	NA	0.9949	0.8640	317	100	27748	A051900035	27.7		
	C92693M	NA	0.9722	NA	0.9949	0.8640	136	250	30480	D052600022	30.5		
	C92694M	NA	1.0031	NA	0.9949	0.8640	240	1000	207597	A061300017	208		
	C92695M	NA	0.9918	NA	0.9949	0.8640	223	1000	194911	A061300018	195		
	C92696M	NA	1.0070	NA	0.9949	0.8640	139	1000	120002	A061300019	120		
	C92698M	NA	1.0065	NA	0.9949	0.8640	87.6	250	18896	A052500036	18.9		
	C92699M	NA	1.0000	NA	0.9949	0.8640	189	1000	163768	A061300022	164		
	C92700M	NA	1.0539	NA	0.9949	0.8640	335	250	69067	D052600023	69.1		
	C92702M	NA	0.9997	NA	0.9949	0.8640	60.1	250	13052	A052500037	13.1		
	C92704M	NA	1.0042	NA	0.9949	0.8640	756	100	65347	A051900043	65.3		
	C92705M	NA	0.9979	NA	0.9949	0.8640	311	250	67702	D072800048	67.7		
Group 4 Mid-High Dose 5.0 ppm in Diet Females	C92708M	NA	1.0022	NA	0.9949	0.8640	595	100	51567	A051900047	51.6		
	C92716M	NA	0.9955	NA	0.9949	0.8640	393	250	85794	A052500039	85.8		
	C92717M	NA	1.0129	NA	0.9949	0.8640	638	25	13674	A061200103	13.7		
	C92724M	NA	1.0017	NA	0.9949	0.8640	531	100	46056	A051900048	46.1		
	C92726M	NA	1.0698	NA	0.9949	0.8640	468	250	95019	D052600025	95.0		
	C92730M	NA	1.0073	NA	0.9949	0.8640	83.7	250	18049	A052500040	18.0		
	C92735M	NA	1.0086	NA	0.9949	0.8640	856	100	73730	A051900049	73.7		
	C92736M	NA	0.9886	NA	0.9949	0.8640	68.8	250	15111	D052600026	15.1		
	C92739M	NA	1.0888	NA	0.9949	0.8640	179	250	35603	D052600029	35.6		
	C92740M	NA	0.9962	NA	0.9949	0.8640	711	250	154965	D052600030	155		
	C92742M	NA	1.0035	NA	0.9949	0.8640	33.1	250	7159	A052500043	7.16		
	C92747M	NA	0.9921	NA	0.9949	0.8640	776	250	169815	A052500044	170		
	C92748M	NA	1.0012	NA	0.9949	0.8640	52.9	250	11473	A052500045	11.5		89.4
	C92750M	NA	1.0072	NA	0.9949	0.8640	31.5	250	6796	A052500046	6.80	70.5	63.1
Group 4 Mid-High Dose 5.0 ppm in Diet Females	C93060F	NA	0.9952	NA	0.9949	0.8640	941	250	183396	A052500047	183		
	C93062F	NA	1.0789	NA	0.9949	0.8640	660	250	132866	D052600031	133		
	C93063F	NA	1.0068	NA	0.9949	0.8640	112	2500	241755	A061300016	242		
	C93065F	NA	0.9976	NA	0.9949	0.8640	611	250	133009	A051900051	133		
	C93072F	NA	0.9991	NA	0.9949	0.8640	771	250	167486	A052500050	167		
	C93077F	NA	1.0544	NA	0.9949	0.8640	740	250	152272	D052600032	152		
	C93078F	NA	1.0089	NA	0.9949	0.8640	287	250	61653	A051900055	61.7		
	C93079F	NA	0.9979	NA	0.9949	0.8640	519	250	112894	A052500051	113		
	C93080F	NA	1.0316	NA	0.9949	0.8640	709	250	149290	D052600033	149		
	C93081F	NA	0.9939	NA	0.9949	0.8640	539	250	117702	A052500052	118		
	C93090F	NA	1.0008	NA	0.9949	0.8640	858	250	186045	A051900056	186		
	C93098F	NA	0.9906	NA	0.9949	0.8640	87.6	250	19199	A052500053	19.2		
	C93101F	NA	0.9962	NA	0.9949	0.8640	888	250	194266	A052500054	193		
	C93102F	NA	1.0888	NA	0.9949	0.8640	201	250	40012	D052600036	40.0		46.7
	C93107F	NA	1.0096	NA	0.9949	0.8640	371	250	79802	A051900057	79.8	131	61.4

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00

* Reanalyzed with the same results. LAC 08/14/00

* Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. KJH 10/03/00

NA = Not Applicable

Date Entered/Analyst: 5/24/00, 5/30/00, 5/31/00, 6/2/00, 06/20/00, 06/21/00, 06/22/00, 08/14/00 MMH/CSH/LAC

Date Verified/Analyst: 10/03/00 KJH

Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

AMDT# 112296.1
Covance# 6329-183

3M Study Title:

Covance Study Title:

Procter Number/Test Substance:

Matrix:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Date of Extraction/Analysis:

Date of Analysis/Analysis:

Date of Data Reduction/Analysis:

Sample Data

104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

T-6295

Rat Liver

ETS-8-6.0 and ETS-8-7.0

Amelia 062498, Davey 070799

Mastlynx 3.4

5/1600-5/1800, 5/2200 SAL/CK

5/1900, 5/2200, 5/2400, 5/2600, 06/1200, 06/1300, 06/1400, 07/2800, 07/3100 IAS/MMH

5/2200, 5/2300, 5/2400, 5/2500, 5/3000, 06/1300, 06/1400, 06/1500, 06/1600, 07/3100, 08/0100 IAS/MMH

Fluorname:

R-Squared Value:

Slope:

Y-Intercept:

See Below

See Attachments

See Attachments

See Attachments

WEEK 105 RAT LIVER

Lot 171													
Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	Original Purity Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	05160-H2OBLK-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400077	< LOQ (0.0107 ug/g)		
	05160-H2OBLK-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400078	< LOQ (0.0107 ug/g)		
	05180-H2OBLK-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	05180-H2OBLK-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200073	< LOQ (0.0107 ug/g)		
	052200-H2OBLK-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400088	< LOQ (0.0107 ug/g)		
Matrix Blk	052200-H2OBLK-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400089	< LOQ (0.0107 ug/g)		
	RBL05160-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400079	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
	RBL05160-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	D061400080	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-5	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200060	< LOQ (0.0107 ug/g)		
	RBL05180-LiverBlk-6	NA	1.00	NA	0.9949	0.8640	0.00	1	0.00	A052200074	< LOQ (0.0107 ug/g)		
QC	RBL052200-LiverBlk-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400090	< LOQ (0.0107 ug/g)		
	RBL052200-LiverBlk-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400091	< LOQ (0.0107 ug/g)	< LOQ (0.0107 ug/g)	NA
	C92505M G1-MS-250ppb-3	NA	1.0161	NA	NA	NA	357	1	177	A073100018	60%		
	C92505M G1-MS-250ppb-3	NA	1.0161	NA	NA	NA	411	1	230	A073100019	78%	69%	26%
	C92505M G1-MS-250ppb-5	NA	0.9927	NA	NA	NA	496	1	377	A052200020	120%		
	C92505M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	478	1	359	A052200021	120%	123%	5%
	C92866F G1-MS-250ppb-4	NA	0.9665	NA	NA	NA	125	10	389	A061200060	126%		
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	135	10	488	A061200061	158%	142%	22%
	C92866F G1-MS-250ppb-6	NA	1.0055	NA	NA	NA	159	1	253	D072800055	85%		
	C92866F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	433	1	306	A052200025	102%	94%	20%
	C92510M G1-MS-250ppb-6	NA	0.9973	NA	NA	NA	803	1	217	D072800056	72%		
	C92510M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	966	1	380	A052200076	126%	99%	55%
	C92991F G3-MS-250 ppb-3	NA	1.0024	NA	NA	NA	492	100	5430	D061400045	1816%	* a	
	C92991F G3-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	512	100	7938	D061400046	2650%	* a	37%
Group 5 High Dose 20 ppm in Diet Males	C92821M G6-MS-250 ppb-5	NA	0.9900	NA	NA	NA	319	50	6423	D061400047	2134%	* a	
	C92821M G6-MSD-250 ppb-5	NA	0.9900	NA	NA	NA	309	50	5914	D061400048	1965%	* a	2049%
	C92753M	NA	1.0013	NA	0.9949	0.8640	261	500	131104	D052600037	113		
	C92753M	NA	1.0100	NA	0.9949	0.8640	765	500	329066	A051900058	329		
	C92761M	NA	1.0065	NA	0.9949	0.8640	186	500	80173	A052500057	80.2		
	C92762M	NA	0.9941	NA	0.9949	0.8640	400	500	174603	A051900059	175		
	C92774M	NA	0.9768	NA	0.9949	0.8640	88.6	500	39390	D052600038	39.4		
	C92775M	NA	1.0108	NA	0.9949	0.8640	81.4	500	34980	A052500058	35.0		
	C92776M	NA	0.9953	NA	0.9949	0.8640	734	500	320170	A052500059	320		
	C92780M	NA	0.9985	NA	0.9949	0.8640	375	500	163214	D052600039	163		
	C92782M	NA	0.9923	NA	0.9949	0.8640	121	500	53101	A052500060	53.1		
	C92786M	NA	1.0004	NA	0.9949	0.8640	227	500	98636	A051900063	98.6		
	C92788M	NA	1.0336	NA	0.9949	0.8640	915	500	384592	D052600040	385		
	C92791M	NA	1.0100	NA	0.9949	0.8640	160	500	68954	A051900064	69.0		
Group 5 High Dose 20 ppm in Diet Females	C92792M	NA	1.0046	NA	0.9949	0.8640	127	500	54772	A052500061	54.8		
	C92800M	NA	1.0003	NA	0.9949	0.8640	366	500	158784	A051900065	159		
	C92802M	NA	0.9946	NA	0.9949	0.8640	93.6	5000	408544	A061300023	409		
	C92803M	NA	1.0428	NA	0.9949	0.8640	987	500	411159	D052600043	411		
	C92804M	NA	1.0029	NA	0.9949	0.8640	762	500	330049	A051900067	330		
	C92808M	NA	0.9908	NA	0.9949	0.8640	271	500	118664	A051900071	119		
	C92809M	NA	1.0161	NA	0.9949	0.8640	1091	25	23316	A061200106	23.3		
	C92814M	NA	1.0275	NA	0.9949	0.8640	847	500	357873	D052600045	358		
	C92815M	NA	0.9995	NA	0.9949	0.8640	166	500	71998	A052500064	72.0		
	C92818M	NA	1.0013	NA	0.9949	0.8640	812	500	351038	A052500065	352	189	74.9
	C93113F	NA	0.9987	NA	0.9949	0.8640	85.8	5000	373041	A061300024	373		141
	C93115F	NA	1.0021	NA	0.9949	0.8640	872	500	377647	A052500066	378		
	C93124F	NA	1.0015	NA	0.9949	0.8640	401	500	173786	A052500067	174		
	C93125F	NA	1.0051	NA	0.9949	0.8640	103	5000	444108	A061300025	444		
	C93133F	NA	0.9705	NA	0.9949	0.8640	900	500	402766	D052600046	403		
Group 5 High Dose 20 ppm in Diet Females	C93135F	NA	1.0082	NA	0.9949	0.8640	135	5000	581680	A061300026	582		
	C93137F	NA	0.9941	NA	0.9949	0.8640	643	500	280805	A052500068	281		
	C93139F	NA	0.9735	NA	0.9949	0.8640	999	500	445629	D052600047	446		
	C93140F	NA	0.9965	NA	0.9949	0.8640	21.1	500	9198	A052500071	9.20		
	C93146F	NA	1.0991	NA	0.9949	0.8640	304	500	138983	A052500072	131		
	C93149F	NA	0.9962	NA	0.9949	0.8640	132	5000	579132	A061300029	575		
	C93151F	NA	1.0219	NA	0.9949	0.8640	687	500	291713	D052600050	292		
	C93153F	NA	1.0057	NA	0.9949	0.8640	541	500	233544	A052500073	234		
	C93157F	NA	0.9904	NA	0.9949	0.8640	143	5000	76847	D052600051	76.8		
	C93158F	NA	1.0034	NA	0.9949	0.8640	143	5000	617048	A061300030	617		
	C93161F	NA	0.9938	NA	0.9949	0.8640	486	500	212218	A051900080	212		
	C93162F	NA	1.0044	NA	0.9949	0.8640	161	5000	694812	A061300031	695		
	C93164F	NA	1.0078	NA	0.9949	0.8640	118	5000	509959	A061300032	510		
	C93165F	NA	1.0317	NA	0.9949	0.8640	976	500	410860	D052600052	411		
	C93166F	NA	0.9959	NA	0.9949	0.8640	903	500	393880	A052500074	394		
	C93168F	NA	0.9930	NA	0.9949	0.8640	1056	500	461592	A052500075	462		
	C93174F	NA	0.9921	NA	0.9949	0.8640	113	5000	493038	A061300033	493		
	C93177F	NA	1.0740	NA	0.9949	0.8640	154	5000	622698	A061200105	623		
	C93178F	NA	0.9730	NA	0.9949	0.8640	886	500	395515	D052600054	396		
	C93179F	NA	1.0472	NA	0.9949	0.8640	792	500	328294	D052600057	328	381	46.1

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 1004400

* Reanalyzed with the same results. LAC 08/01/00

* Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. Kjh 10/03/00

NA = Not Applicable

Date Entered/Analysis:

Date Verified/Analysis:

Purity Entered/Verified:

05/24/00, 05/30/00, 05/31/00, 06/02/00, 06/20/00, 06/21/00, 06/22/00, 08/14/00 MMH/CSH/LAC

10/03/00 KJH

10/04/00 LAC, 10/04/00 KJH

AMDT# 12296.1
Covance# 6329-183

3M Study Title:

Covance Study Title:

Product Number(Test Substance):

Matrix:

Method/Revision

Analytical Equipment System Number:

Instrument Software/Version

Date of Extraction/Analyst:

Date of Analysis/Analyst:

Date of Data Rec

Sample Note

104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Bis(2-Ethylhexyl) Sebacate (DEHS) in F344/N Rats and B6C3F1 Mice

104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS) in B6C3F₁ Mice

T-6295

Rat Liver

ETS-8-6.0 and ETS-8-7.0

Amelia 062498. Davey 07

Masslynx 3.4

5/16/00 5/18/00 SAI /KTB

\$/2500, 06/1300, 06/1400, 07/1800, 07/2100, 1A S/B MUI

5/25/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00 LAS/MMH
5/30/00, 06/14/00, 06/15/00, 07/31/00, 08/01/00, 11/01/00

5/30/00, 06/14/00, 06/15/00, 07/31/00, 08/01/00 LAS/MMH

Filename: See list to right

R-Squared Value: See Attachments

R-Squared Value: See Attachments
Slope: See Attachments

Slope: See Attachments
Y-Intercept: See Attachments

Y-Intercept: See Attachments

/21/DOJAS/MU

/31/00 LAS/MMH
01/00 LAS/MMH

00100 LAS/MMH

WEEK 106 RAT LIVER

WEEK 106 RAT LIVER			Lot 171													RSD	
Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	Original Purity Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Fluorene (optional)	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	Std. Dev. MS/MSD RPD				
Method Blk	05160-H2OBtl-5	NA	1.0000	NA	0.9949	0.8640	0.740	1	0.643	D061400077	<LOQ (0.0107 ug/g)						
	05160-H2OBtl-6	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400078	<LOQ (0.0107 ug/g)						
	05180-H2OBtl-5	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A052200060	<LOQ (0.0107 ug/g)						
	05180-H2OBtl-6	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A052200073	<LOQ (0.0107 ug/g)						
	052200-H2OBtl-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400088	<LOQ (0.0107 ug/g)		NA				
Matrix Blk	052200-H2OBtl-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400089	<LOQ (0.0107 ug/g)	<LOQ (0.0107 ug/g)	NA				
	RBL05160-LiverBlk-5	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400079	<LOQ (0.0107 ug/g)						
	RBL05180-LiverBlk-5	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400080	<LOQ (0.0107 ug/g)						
	RBL05180-LiverBlk-6	NA	1.0000	NA	0.9949	0.8640	0.570	1	0.495	A052200074	<LOQ (0.0107 ug/g)						
	RBL052200-LiverBlk-3	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400090	<LOQ (0.0107 ug/g)		NA				
QC	RBL052200-LiverBlk-4	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D061400091	<LOQ (0.0107 ug/g)	<LOQ (0.0107 ug/g)	NA				
	C92903M G1-MS-250ppb-3	NA	1.0161	NA	NA	NA	357	1	177	A073100018	60%						
	C92903M G1-MSD-250ppb-3	NA	1.0161	NA	NA	NA	411	1	177	A073100019	78%						
	C92905M G1-MS-250ppb-5	NA	0.9927	NA	NA	NA	496	1	177	A052200020	126%	69%	26%				
	C92905M G1-MSD-250ppb-5	NA	0.9927	NA	NA	NA	478	1	359	A052200021	120%	123%	5%				
	C92866F G1-MS-250ppb-4	NA	0.9665	NA	NA	NA	125	10	389	A061200060	126%						
	C92866F G1-MSD-250ppb-4	NA	0.9665	NA	NA	NA	135	10	488	A061200061	158%						
	C92866F G1-MS-250ppb-6	NA	1.0055	NA	NA	NA	379	1	253	D072800055	85%	142%	22%				
	C92866F G1-MS-250ppb-6	NA	1.0055	NA	NA	NA	433	1	306	A052200025	102%						
	C92866F G1-MSD-250ppb-6	NA	1.0055	NA	NA	NA	217	1	724	D072800056	72%						
	C92910M G1-MS-250ppb-6	NA	0.9973	NA	NA	NA	803	1	380	A052200076	126%	94%	25%				
	C92910M G1-MSD-250ppb-6	NA	0.9973	NA	NA	NA	966	1	380	A052200076	126%						
	C92991F G3-MS-250 ppb-5	NA	1.0024	NA	NA	NA	492	100	5430	D061400045	1816%						
	C92991F G1-MSD-250 ppb-5	NA	1.0024	NA	NA	NA	517	100	7923	D061400046	2650%						
	Group 6	C92821M G6-MS-250 ppb-5	NA	0.9900	NA	NA	NA	319	50	6423	D061400047	2134%					
C92821M G6-MSD-250 ppb-5		NA	0.9900	NA	NA	NA	309	50	5914	D061400048	1958%						
C92821M		NA	0.9900	NA	0.9949	0.8640	110	100	9680	D061400020	9.68%						
C92823M		NA	1.0378	NA	0.9949	0.8640	22.9	100	1915	D061400023	1.91						
C92827M		NA	0.9974	NA	0.9949	0.8640	165	1	144	D061300032	0.144						
C92829M		NA	1.0069	NA	0.9949	0.8640	136	1	117	D061300033	0.117						
C92833M		NA	1.0462	NA	0.9949	0.8640	460	1	382	D061300034	0.382						
C92834M		NA	1.0129	NA	0.9949	0.8640	236	1	203	D061300037	0.203						
C92848M		NA	0.9561	NA	0.9949	0.8640	197	100	17890	D061300016	1739						
C92851M		NA	1.0102	NA	0.9949	0.8640	423	1	363	D061300038	0.363						
C92853M		NA	1.0350	NA	0.9949	0.8640	514	1	431	D061300039	0.431						
C92856M		NA	1.0274	NA	0.9949	0.8640	39.3	1	33.2	D061400016	0.0332						
C93181F		NA	1.0108	NA	0.9949	0.8640	50.2	100	4316	D061400017	4.32						
C93182F		NA	1.0429	NA	0.9949	0.8640	25.3	100	2103	D061400018	2.103						
Group 6		C93183F	NA	0.9968	NA	0.9949	0.8640	104	100	9079	D061300019	9.08					
	C93184F	NA	0.9740	NA	0.9949	0.8640	18.9	100	1688	D061400019	1.69						
	C93185F	NA	1.0186	NA	0.9949	0.8640	186	100	15896	D061300023	15.9						
	C93190F	NA	1.0540	NA	0.9949	0.8640	27.7	1000	22831	A052000085	22.8						
	C93192F	NA	1.0134	NA	0.9949	0.8640	175	100	15018	D061300024	15.0						
	C93197F	NA	1.0425	NA	0.9949	0.8640	104	100	8650	D061300025	8.65						
	C93198F	NA	1.0660	NA	0.9949	0.8640	75.9	100	6184	D061300026	6.18						
	C93201F	NA	1.0054	NA	0.9949	0.8640	222	100	19198	D061300027	19.2						
	C93202F	NA	1.0631	NA	0.9949	0.8640	209	100	17050	D061400024	17.0						
	C93205F	NA	1.0013	NA	0.9949	0.8640	152	1	132	D061300044	0.132						
	C93209F	NA	0.9948	NA	0.9949	0.8640	170	100	14854	D061400025	14.9						
	C93211F	NA	1.0991	NA	0.9949	0.8640	49.5	1000	42582	A052001009	42.6						
	C93216F	NA	1.0096	NA	0.9949	0.8640	171	100	14687	D061400027	14.7						
	C93219F	NA	1.0009	NA	0.9949	0.8640	57.2	100	4962	D061400030	4.96						
	C93220F	NA	0.9938	NA	0.9949	0.8640	22.3	1000	19469	A052001114	19.5						
											12.9	10.4					

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00

* Reanalyzed with the same results. LAC 08/14/00

■ Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range): do not include results in the body of the final report. Kib 10/03/00

NA = Not Applicable

Date Entered/Analyst: 06/01/00, 06/21/00, 06/22/00, 08/14/00 CSH/AC

Date Verified/Analyst: 10/03/00, KTH

Purity Entered/Verified: 10/04/00 LAC 10/04/00 KJM

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-002

$$\text{AR (ng/mL)} \times \text{DF} \times \text{SC} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} \times \frac{\text{PC}}{\text{OPC}} = \text{Reported Concentration (}\mu\text{g/mL)}$$

Calculation Used for Group 3, Day 719, Animal ID C92991F

$$348 \text{ ng/mL} \times 50 \times 0.9275 \times \frac{1 \text{ mL}}{1 \text{ mL}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} \times \frac{0.864}{0.9949} = 14.0 \mu\text{g/mL}$$

AR— Analytical result from MassLynx summary

DF— Dilution factor

SC—PFOS salt correction constant (0.9275)

FV—Final extract volume (1.0 mL unless otherwise noted)

EV—Volume of sera extracted

PC—PFOS purity correction factor (86.4% for lot 171)

OPC—Original PFOS purity correction factor (99.49%)

Formula Used for Liver Analyses in Study FACT TOX-002

$$\text{AR (ng/g)} \times \frac{\partial \text{ curve}^{(1)}}{\partial \text{ specimen}} \times \text{SC} \times \text{DF} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} \times \text{PC} = \text{Reported Concentration (}\mu\text{g/g)}$$

⁽¹⁾ $\partial \text{ curve}$ is assumed to be: $\frac{1 \text{ g liver}}{5 \text{ mL H}_2\text{O}}$

Calculation Used for Group 3, Week 105, Animal ID C92631M

$$480 \text{ ng/g} \times \frac{1 \text{ g/ 5 mL}}{1.0036 \text{ g/ 5mL}} \times 100 \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} \times \frac{0.864}{0.9949} = 41.5 \mu\text{g/g}$$

AR— Analytical result from MassLynx summary

$\partial \text{ curve}$ —Density of the liver standard curve, assumed to be 1g liver/ 5 ml water

$\partial \text{ specimen}$ —Density of the liver specimen (g specimen/ 5 mL H₂O)

DF— Dilution factor

PC—PFOS purity correction factor (86.4% for lot 171)

OPC—Original PFOS purity correction factor (99.49%)

Standard deviations were calculated using the equation:

$$SD = \sqrt{\frac{n\sum x^2 - (\sum x)^2}{n(n-1)}}$$

Appendix G: Interim Certificates of Analysis



Centre Analytical Laboratories, Inc.

3048 Research Drive
Phone: (814) 231-8032

State College, PA 16801
Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018B

3M Product: PFOS, Lot 171

Reference #: SD-009

Purity: 86.4%

Test Name	Specifications	Result
Purity ¹		86.4%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.017 wt./wt.%
2. Magnesium		2. 0.007 wt./wt.%
3. Sodium		3. 1.355 wt./wt.%
4. Potassium ²		4. 6.552 wt./wt.%
5. Nickel		5. 0.003 wt./wt.%
6. Iron		6. 0.004 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.00 wt./wt.%
Total % Impurity (LC/MS)		10.60 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.30 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable ³
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. 0.27 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.007 wt./wt.%
7. Sulfate ⁴		7. 8.82 wt./wt.%
Organic Acids ⁵ (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. <0.1 wt./wt.%
4. NFPA		4. <0.25 wt./wt.%
Elemental Analysis ⁶ :		
1. Carbon	1. Theoretical Value = 17.8%	1. 12.08 wt./wt.%
2. Hydrogen	2. Theoretical Value = 0%	2. 0.794 wt./wt.%
3. Nitrogen	3. Theoretical Value = 0%	3. 1.61 wt./wt.%
4. Sulfur	4. Theoretical Value = 5.95%	4. 10.1 wt./wt.%
5. Fluorine	5. Theoretical Value = 60%	5. 50.4 wt./wt.%

COA023-018B

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**Centre Analytical Laboratories, Inc.**3048 Research Drive
Phone: (814) 231-8032State College, PA 16801
Fax: (814) 231-1253 or (814) 231-1580***INTERIM CERTIFICATE OF ANALYSIS*****Centre Analytical Laboratories COA Reference #: 023-018B**

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen $\leq -10^{\circ}\text{C}$

Re-assessment Date: 08/31/01

¹Purity = 100% - (sum of metal impurities, 1.39% + LC/MS impurities, 10.60% + Inorganic Fluoride, 0.27% + NMR impurities, 1.00% + POAA, 0.30%)

Total impurity from all tests = 13.56%

Purity = 100% - 13.56% = 86.4%

²Potassium is expected in this salt form and is therefore not considered an impurity.

³Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

⁴Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

⁵ TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, $\text{C}_8\text{F}_{17}\text{SO}_3\text{K}^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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INTERIM CERTIFICATE OF ANALYSIS**Centre Analytical Laboratories COA Reference #: 023-018B**

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.03
C5	1.56
C6	6.38
C7	1.63
Total	10.60

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By:


David S. Bell

Scientist, Centre Analytical Laboratories

Date

9/6/00

Reviewed By:


John Flaherty

Laboratory Manager, Centre Analytical Laboratories

Date

9/11/00

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INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A

3M Product: PFOS, Lot 217

Reference #: SD-018

Purity: 86.9%

Test Name	Specifications	Result
Purity ¹		86.9%
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. 0.005 wt./wt.%
2. Magnesium		2. 0.001 wt./wt.%
3. Sodium		3. 1.439 wt./wt.%
4. Potassium ²		4. 6.849 wt./wt.%
5. Nickel		5. <0.001 wt./wt.%
6. Iron		6. 0.005 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable ³
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. 0.59 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.007 wt./wt.%
7. Sulfate ⁴		7. 8.76 wt./wt.%
Organic Acids ⁵ (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. 0.10 wt./wt.%
4. NFPA		4. 0.28 wt./wt.%
Elemental Analysis ⁶ :		
1. Carbon	1. Theoretical Value = 17.8%	1. 12.48 wt./wt.%
2. Hydrogen	2. Theoretical Value = 0%	2. 0.244 wt./wt.%
3. Nitrogen	3. Theoretical Value = 0%	3. 1.74 wt./wt.%
4. Sulfur	4. Theoretical Value = 5.95%	4. 8.84 wt./wt.%
5. Fluorine	5. Theoretical Value = 60%	5. 54.1 wt./wt.%

COA023-018A

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INTERIM CERTIFICATE OF ANALYSIS**Centre Analytical Laboratories COA Reference #: 023-018A**

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen $\leq -10^{\circ}\text{C}$

Re-assessment Date: 08/31/01

¹Purity = 100% - (sum of metal impurities, 1.45% + LC/MS impurities, 8.41% + Inorganic Fluoride, 0.59% + NMR impurities, 1.93% + organic acid impurities, 0.38% + POAA, 0.33%)

Total impurity from all tests = 13.09%

Purity = 100% - 13.09% = 86.9%

²Potassium is expected in this salt form and is therefore not considered an impurity.

³Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

⁴Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

⁵ TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

⁶Theoretical value calculations based on the empirical formula, $\text{C}_8\text{F}_{17}\text{SO}_3\text{K}^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	8.41

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: David S. Bell
David S. Bell
Scientist, Centre Analytical Laboratories

9/10/00
Date

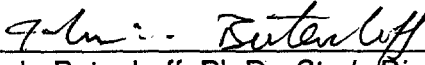
Reviewed By: John M. Flaherty
John Flaherty
Laboratory Manager, Centre Analytical Laboratories

9/11/00
Date

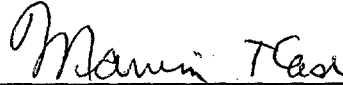
COA023-018A

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Appendix H: Report Signature Page

 21 FEB 01

John L. Butenhoff, Ph.D., *Study Director* Date

 21 Feb 2001

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative* Date

 02/14/01

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* Date

 02/15/01

William K. Reagen, Ph.D., *Laboratory Manager* Date