3M Medical Department Study: T-6295.22
Analytical Report: FACT TOX-160
LIMS E00-1668

AR226_1103

Study Title

Extended Recovery Study Following a 26-Week Capsule Toxicity Study (FACT-TOX-030) with Perfluorooctane Sulfonate Acid Potassium Salt (PFOS; T-6295.22) in Cynomolgus Monkeys

Analytical Laboratory Report Title

Determination of the Presence and Concentration of PFOS in Serum and Liver Samples of Cynomolgus Monkeys

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

May 3, 2002

Performing Laboratories

Sera and Liver Analyses

3M Environmental Laboratory
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

Sera and Liver Extractions

Pace Analytical Services, Inc.—Tier2 Facility
1700 Elm Street, Suite 100
Minneapolis, MN 55414

Project Identification

3M Medical Department Study: T-6295.22
Covance In-Life Study: 6329-268
Analytical Report: FACT TOX-160
3M LIMS No. E00-1668

Total Number of Pages

113

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This page has been reserved for specific country requirements.

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GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS in Serum and Liver Samples of Cynomolgus Monkeys

Study Identification Numbers: T-6295.22, FACT TOX-160, LIMS-E00-1668

This study was conducted in compliance with United States Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards 40 CFR Part 792, 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 four separate studies. The in-life study phase was considered to end at the generation and shipment of specimens. The analytical study phase 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.
- There were two in-life studies and two analytical studies that utilized the same test system. These studies include in-life studies Covance 6329-223 and Covance 6329-268 and analytical studies FACT-TOX-030 and FACT-TOX-160.
- The purity and stability of the reference standards are not included in this report, they are not known at this time.

Andrew M. Seacat — May 3rd, 2002
Andrew Seacat, Ph.D., *Study Director* — Date

John L. Butenhoff — May 3, 2002
John Butenhoff, Ph.D., *Sponsor Representative* — Date

Lisa A Clemen — 05/01/02
Lisa Clemen, *Principal Analytical Investigator* — Date

[signature] — 05/01/02
William Reagen, Ph.D., *Analytical Laboratory Manager* — Date

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GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS in Serum and Liver Samples of Cynomolgus Monkeys

Study Identification Numbers: T-6295.22, FACT TOX-160, LIMS-E00-1668

This study has been inspected by the 3M Environmental Laboratory 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
06/19/01	Protocol	06/19/01	06/19/01
09/05/01	Extraction	09/05/01	09/05/01
09/11/01	Analysis	09/12/01	09/12/01
02/01/02, 02/06/02-02/08/02, 02/18/02-02/22/02	Data	02/26/02	02/26/02
02/21/02, 02/22/02	Draft report	02/26/02	02/26/02

Tanya K Rude — 5/1/02

QAU Representative — Date

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Study Personnel and Contributors

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Kelly J. Kuehlwein*
Sally A. Linda*
Bob W. Wynne*

*Contract lab professional service employees

Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory and will be retained according to 40 CFR Part 792 requirements. The test substance 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 and will be retained according to 40 CFR Part 792 requirements.

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Introduction and Purpose

The purpose of the study is to determine the presence and concentration of PFOS in cynomolgus monkey sera and liver samples taken from Covance study# 6329-268, "Extended Recovery Study Following a 26-Week Capsule Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295.22) in Cynomolgus Monkeys." The animals from study# 6329-268 were previously assigned to a completed in-life Covance study# 6329-223, "26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295.7) in Cynomolgus Monkeys" and a completed analytical study# FACT-TOX-030, "Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295.7) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples". During study# 6329-223, the animals received 0.15 mg/kg/day of PFOS as a single daily capsule dose for at least 26 weeks followed by a 52-week recovery. At the end of the initial recovery the animals were transferred to a follow-up study (Covance 6329-268, T-6295-22) for evaluation of extended recovery. Animals were not treated in the follow-up study.

The sera and liver samples for this study are the product of the in-life recovery study completed by Covance Analytical Research Laboratories under study# 6329-268 (T-6295.22). Analyses of sera and liver samples were completed by the 3M Environmental Laboratory under study number FACT-TOX-160 (E00-1668), and the results of these analyses are presented in this report. The analytical portion of this study was initiated on 27 August, 2001.

Table 1. Study Timeline

26 Weeks	52 Weeks Recovery	52 Weeks Extended Recovery
In-Life Study: Covance 6329-223		In-Life Study: Covance 6329-268
Analytical Study: FACT-TOX-030		Analytical Study: FACT-TOX-160
3M Medical Study: T-6295.7		3M Medical Study: T-6295.22

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Specimen Receipt and Maintenance

The 3M Environmental Laboratory received cynomolgus monkey specimens collected at the end of the *in-life* study #6329-268 from 09 May, 2000 to 15 March, 2001. All specimens were received frozen in good condition on dry ice and were immediately transferred to storage at -50°C ± 20°C. Specimens that were extracted at Pace Tier2 were shipped frozen on dry ice.

Table 2. Cynomolgus Monkey Specimen Receipt for Study (#6329-223)

Receipt Date	Timepoint	Specimen	Number Received
05/09/00	Week 9	Serum	4
07/06/00	Week 17	Serum/Urine/Feces	4/4/4
08/30/00	Week 25	Serum	4
10/24/00	Week 33	Serum/Urine/Feces	4/4/4
12/21/00	Week 41	Serum	4
03/13/01	Week 53	Serum/Urine/Feces	4/4/4
03/15/01	Week 53	Liver/Lung	4/4
		Kidney/Spleen	4/4
		Thyroid/Brain	4/4
		Abdominal Fat	4
		Heart/Bile	4/4
		Serum	3 additional vials from sample I05552

Control matrices used in sera and liver analyses performed during FACT-TOX-160 were obtained from commercial sources and are presented in Appendix A. Samples analyzed at the 3M Environmental Laboratory will be stored and maintained at the laboratory according to 40 CFR Part 792 requirements.

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Chemical Characterization of the Reference Substance

Potassium perfluorooctanesulfonate (KPFOS)
CAS Number: 2795-39-3

Chemical Formula: $C_8F_{17}SO_3^-K^+$ Molecular Weight: 537.9

Chemical characterization information on the reference substances KPFOS used in this study is presented in tabular form below.

Table 3. Characterization of the Analytical Reference Substances in Study FACT-TOX-160

Location	3M Lab	
Substance	PFOS SD-018	THPFOS (Surrogate Standard) TCR-00017-055
Source	3M	SynQuest
Expiration Date	08/31/06	ND
Storage Conditions	Frozen –20C +/- 10C	Frozen –20C +/- 10C
Chemical Lot Number	217	Q-75-91
Physical Description	White Powder	White Powder
Purity	86.9%*	NA

ND - Not determined
NA - Not available
*See Certificate of Analysis from Centre Analytical Laboratories in Appendix G.

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Sample Preparation and Analysis

Sera Analyses

As per the study protocol, all serum samples were analyzed in this phase study.

Sera samples were extracted beginning on 31 August, 2001 using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). Sample extracts were analyzed using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple reaction mode versus an extracted rabbit sera curve. PFOS levels were quantitated by external calibration.

Liver Analyses

As per the study protocol, all liver samples were analyzed in this phase study.

Liver samples were extracted beginning on 05 September, 2001 using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). Sample extracts were analyzed using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple reaction mode versus an extracted rabbit liver curve. PFOS levels were quantitated by external calibration.

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.

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PREPARATORY METHODS

- **ETS-8-4.2,** "Extraction of Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

  Analytical samples were extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to a laboratory sample and the analyte ion pair was partitioned into methyl-*tert*-butyl-ether (MtBE). The MtBE extract was then removed and put into a nitrogen evaporator until dry. Each extracted laboratory sample was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter using a 3 mL disposable plastic syringe into glass autovials.

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

Liver samples were homogenized in water. An aliquot of each homogenate was spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. 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 passed through a 0.2 µm nylon filter, using a 3 mL disposable plastic syringe into glass autosampler vials.

ANALYTICAL METHODS

- **ETS-8-5.2,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-7.0,** "Analysis of Potassium Perfluorooctane-sulfonate 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 a particular fluorochemical 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 to produce ion 99 (FSO_3-). The characteristic ion 99 was monitored for quantitative analysis.

ANALYTICAL EQUIPMENT

The following is representative of the settings used during the analytical phase of this study.

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50 mm (5 µm)
Column temperature: 30°C
Mobile phase components:
Component A: 2mM ammonium acetate
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 9.0 minutes

Time (minutes)	*%B*
0.0	10%
1.0	10%
5.5	95%
7.5	95%
8.0	10%

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Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.4
Cone Voltage: 30–70 V
Collision Gas Energy: 20–50 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 4. Target Ions Monitored in 3M Laboratory Analyses

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

Deviations/Amendments

There were one amendment and seven deviations from the original protocol. Amendments and deviations from the original protocol and methods are included in the Appendix B.

Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated in the protocol for this study:

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.985 for liver analyses and equal to or greater than 0.990 for sera analyses using 1/x weighting.
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve, defined as the lowest standard that is both 2 times the matrix blank and is calculated within ± 30% of the expected concentration.
- **Acceptable Precision:** Quality control samples are required to meet ± 25% precision for analyses, provided they are quantitated within the selected calibration range.
- **Acceptable Spike Recoveries:** Matrix spikes and matrix spike duplicates required for analysis of liver and sera samples should show spike recoveries within 70–130%.
- **Confirmatory Methods:** If a confirmatory method is used, an amendment to this protocol will be written.
- **Demonstration of Specificity:** PFOS identification will be substantiated by chromatographic retention time (approximately 8.00 minutes), by the characteristic primary ion (499) and characteristic product ion (99).

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Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory protocol for FACT-TOX-160 (see Appendix B) 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 for liver analyses and ≥0.990 for sera analyses.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis, 1/x weighted of one or two extracted matrix curves bracketing each group of samples, except as noted in the deviation summary. High and/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 each analyte was based on the response of one specific product ion(s) 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 that is within ±30% of the theoretical value, and is at least two times the analyte peak area detected in the extraction blanks.

Table 5. Determinations of the LOQ For the Extracted Curve in the Analyses of Serum and Liver Extracts

Analyte	Method LOQ
PFOS—Serum	4.92 ng/mL
PFOS—Liver	12.6 ng/g

- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed monkey sera which were above the lower limit of quantitation, rabbit sera was selected as a suitable surrogate matrix and all rabbit sera blanks were within criteria.
- **Precision:** precision was determined by analysis of CCVs in sera and was reproducible to within 25%, precision was determined by analysis of CCVs in liver and was reproducible to within 30%.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of liver and sera samples and analyzed at the 3M Environmental Laboratory (see tables 5 and 6). Two rabbit liver matrix spikes, extracted 09/05/01, were within criteria and two monkey liver matrix spikes were not within ±30% of the theoretical concentration when analyzed 09/17/01. The monkey liver matrix spikes were not prepared at the appropriate concentration based on endogenous levels present in the samples. Additional monkey liver matrix spikes and one monkey liver sample were re-extracted on 10/02/01 at levels appropriate to the endogenous

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levels. The re-extracted spikes did not meet criteria and the extraction was suspect due to an inconsistent sample concentration, inconsistent matrix spike recoveries, and high surrogate values – refer to deviations attached to this report for more information. Additional monkey liver matrix spikes and one monkey liver sample were re-extracted on 11/07/01. The extracted spikes and sample were in agreement with the initial extraction on 09/05/01. The rabbit liver matrix spike averages extracted with the samples on 09/05/01 and the monkey liver matrix spike averages extracted on 11/07/01 were within ± 35% of the theoretical concentration.

All sera matrix spike recoveries, evaluated versus extracted and unextracted curves, were within ± 25% of the theoretical concentration. Extraction efficiency and absolute recovery were >100% in the sera matrix, based on average recoveries of 101%, 111%, 111%, and 116% versus an unextracted (solvent) curve.

Table 6. Liver Matrix Spike Recoveries

Matrix	Extraction Date	Analysis Date	Type	% Recovery	Average
Liver	09/05/01	09/10/01	Rabbit	102%	102%
				102%	
		09/17/01	Monkey	298%	294%
				290%	
	10/02/01 Suspect Extraction – inconsistent sample concentration and high surrogate deviation for 10/24/01 analysis	10/04/01 Initial Analysis	Monkey 2 ug/g	55%	35%
				15%	
			Monkey 10 ug/g	60%	NA
				Too Dilute	
		10/16/01 Reanalysis	Monkey 2 ug/g	30%	9%
				-11%	
			Monkey 10 ug/g	65%	54%
				43%	
		10/24/01 Redilution from original sample	Monkey 2 ug/g	178%	119%
				61%	
			Monkey 10 ug/g	268%	242%
				216%	
	11/07/01	11/15/01	Monkey 2 ug/g	100%	95%
				90%	
			Monkey 10 ug/g	73%	67%
				61%	

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Table 7. Sera Matrix Spike Recoveries

Matrix	Extraction Date	Analysis Date	Type	% Recovery	Average
Sera	08/31/01	09/06/01 Evaluated Versus an Unextracted Curve = Absolute Recovery	Monkey 25 ng/mL	118%	101%
				84%	
			Monkey 500 ng/mL	111%	111%
				112%	
	10/02/01	10/04/01 Evaluated Versus an Extracted Curve	Monkey 2.0 ug/mL	104%	102%
				99%	
			Monkey 10 ug/mL	119%	120%
				121%	
		10/04/01 Evaluated Versus an Unextracted Curve = Absolute Recovery	Monkey 2.0 ug/mL	113%	111%
				109%	
			Monkey 10 ug/mL	115%	116%
				117%	

- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. The surrogate response of each analytical run utilizing extracted matrix calibration curves was verified to determine that it did not vary more than ± 50% from the mean within each analytical run. All responses were within ± 50% except for analysis of liver dilutions on 10/24/01. These samples were >50%. These samples were considered suspect and re-extracted. Analysis of these re-extracts met surrogate criteria requirements.

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, indicates that these data are quantitative to 25% or greater in sera and 35% or greater in liver.

Summary of Sample Results

PFOS results (those obtained using lot # 217) have been corrected for purity of the analytical reference material.

- **Samples from Control Animals:** No control animals were included in this study.
- **Samples from Dosed Animals:** In general, PFOS levels found in sera of the test animals decreased over time. PFOS levels in male livers were approximately half that determined in female livers. Detailed sample data tables are presented in Appendices D and E.

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Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix F for example calculations used to generate the liver and serum sample data in FACT-TOX-160.

Statement of Conclusion

Under the conditions of the present studies, the fluorochemical PFOS was observed in the serum and liver of all recovery study cynomolgus monkeys originally dosed with the test substance during the in-life phase of the study #6329-223 (TOX-030).

References

None

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Appendix A: Control Matrices

Table 8. Characterization of the Control Matrices Used for Serum and Liver Analyses in Study FACT-TOX-160

Control Matrix	Monkey Serum TCR-99131-022	Rabbit Serum TN-A-4511	Rabbit Liver TCR-99131-046
Source	Lampire Biological	Sigma-Aldrich	Covance Laboratory
Expiration Date	01/01/2010	09/26/05	NA
Storage Conditions	Frozen -20°C +/- 10°C	Frozen -20°C +/- 10°C	Frozen -20°C +/- 10°C
Chemical Lot #	111022515	99H8400	F04053
Physical Description	Monkey Serum	Rabbit Serum	Rabbit Liver

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Appendix B: Protocol, Amendments, and Deviations

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3M

Study Title

Extended Recovery Study Following a 26-Week Capsule Toxicity Study (FACT-TOX-030) with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

ANALYTICAL PHASE PROTOCOL

Author
Lisa Clemen

Date:
August 27, 2001

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Laboratory Project Identification
FACT TOX-160
Covance In-life Study Number: 6329-268
3M Medical Department Study: T-6295.22
ET&SS LIMS: E00-1668

Study Identification

Extended Recovery Study Following a 26-Week Capsule Toxicity Study (FACT-TOX-030) with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

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Sponsor Representative	John Butenhoff, Ph.D. 3M Corporate Toxicology 3M Medical Department Building 220-2E-02 St. Paul, MN 55144 Telephone: 651-733-1962
Study Director	Andrew Seacat, Ph.D. 3M Corporate Toxicology 3M Medical Department Building 220-2E-02 St. Paul, MN 55144 Telephone: 651-575-3161
Principal Analytical Investigator (PAI)	Lisa Clemen
Phase Locations	
In vivo Testing Facility	Covance Laboratories, Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Analytical Testing Laboratories (sera and liver analyses)	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106
(sera and liver extractions)	Pace Tier2 Facility 1700 Elm Street, Suite 200 Minneapolis, MN 55414
Proposed Study Timetable	
Experimental Start Date	27 August 2001
Experimental Completion Date	29 April 2002

1. STUDY

Extended Recovery Study Following a 26-Week Capsule Toxicity Study (FACT-TOX-030) with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys.

2. PURPOSE

This study is designed to continue the assessment of perfluorooctanesulfonate (PFOS) levels in sera and liver during an extended recovery time of approximately one year following the daily administration of the test material by capsule to cynomolgus monkeys for at least 26 weeks and at least 52 weeks of recovery.

The animals used in this study were previously assigned to a recovery phase of a completed study for at least 52 weeks. At the end of this initial recovery phase study Covance #6329-223, a partial hepatectomy was conducted on the animals, they were allowed to recover from the surgical procedure, then transferred to this follow-up study for evaluation of recovery for an additional 52 weeks.

The serum and liver of cynomolgus monkeys will be analyzed for PFOS. Additional tissues or fluids may be analyzed at the discretion of the PAI or study director. The in-life portion of this extended recovery study was conducted at Covance Laboratories, study #6329-268.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR 792.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and the final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

5. TEST MATERIAL

Refer to Covance Laboratory protocol for study #6329-268. Animals were not treated during this study. The FACT TOX-160 study is an extended recovery study of cynomolgus monkeys from Covance study 6329-223 (analytical work performed under 3M Environmental Study # FACT-TOX-030), where animals received 0.15 mg/kg/day of PFOS lot # 217 in a daily single capsule for at least 26 weeks, followed by a 52-week recovery period.

6. CONTROL MATRICES

Types of control matrices and their source, physical description, storage requirements, and traceability numbers will be recorded in the raw data and included in the final report.

7. REFERENCE MATERIAL FOR FACT TOX-160

Potassium perfluorooctanesulfonate (KPFOS), $C_8F_{17}SO_3^-K^+$, CAS 2795-39-3

The target analyte is perfluorooctanesulfonate (PFOS), $C_8F_{17}SO_3^-$.

8. TEST SYSTEM

Cynomolgus monkeys were used as the test system, and were maintained and dosed as described in the original Covance protocol #6329-223 (TOX-030). Two male and two female monkeys which were dosed during study #6329-223 (TOX-030) were included in this extended recovery study and all were allocated to Group 1. These animals received no further treatment during the recovery study.

9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory received specimens of the following body tissues and fluids from the indicated points in the study from Covance Laboratories at the end of the in-life phase of the study. All specimens were frozen and packed on dry ice for shipping.

Body tissue/fluid	Collected	Expected # of specimens
Serum – all animals	2, 4, 6, 8, 10, and 12 months following initiation of study At scheduled sacrifices	~24–28 serum samples
Liver – all animals	At scheduled sacrifices	4

Total number of test animals: 4

Modifications to the number of samples analyzed may be implemented at the discretion of the PAI and the study director.

Specimens sent to 3M Environmental Laboratories were received and tracked according to the Sample Tracking System Standard Operating Procedure. Details of specimen inspection for damage, receipt, storage, identification, chain of custody, protocols and data will be presented in the phase report.

10. PREPARATORY METHODS

10.1 ETS-8-6, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 ETS-8-4, Extraction of Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

11. ANALYTICAL METHODS

11.1 ETS-8-7, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

11.2 ETS-8-5, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

12. DATA QUALITY OBJECTIVES

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators are included in the analytical methods. In addition, the following criteria will be met:

12.1 Linearity

The coefficient of determination (r^2) of the extracted liver standard curve must be equal to or greater that 0.985 using linear regression or quadratic fit.

The coefficient of determination (r^2) of the extracted serum standard curve must be equal to or greater that 0.990 using linear regression or quadratic fit.

12.2 Limits of Quantitation (LOQ)

The LOQ will be equal to the lowest acceptable standard in the calibration curve, defined as the lowest standard that is both 2 times the matrix blank and is calculated within ± 30% of the expected concentration.

12.3 Acceptable Precision

Quality control samples are required to meet ± 25% precision, provided they are quantitated within the selected calibration range.

12.4 Spike Acceptable Recoveries

Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve.

These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the

sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

For serum matrix spikes, a comparison of extracted (matrix) versus unextracted (solvent) calibration standards will be performed at two concentrations spanning the validated calibration range of the method. This comparison will demonstrate the absolute recovery (recovery + serum matrix affect) of analyte from the matrix for determining the extraction efficiency of serum versus an unextracted calibration curve.

After serum samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as sample into the validated range of the calibration curve.

These serum matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted serum matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

12.5 Use of Confirmatory Methods

If a confirmatory method is used, an amendment to this protocol will be written.

12.6 Demonstration of Specificity

PFOS identification will be substantiated by chromatographic retention time (approximately 8 minutes), by the characteristic primary ion (499) and the characteristic product ion (99).

Minor modifications to the Data Quality Objectives may be implemented at the discretion of the PAI. These will be documented in the raw data and the analytical report.

13. SUB-CONTRACTED ANALYSIS

13.1 All sera and liver extractions as detailed in this protocol will be performed at Pace Tier2, 1700 Elm Street, Suite 200, Minneapolis, MN 55414.

13.2 All sera and liver analyses as detailed in this protocol will be performed at 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

13.3 An amendment to this protocol will be written if extractions and analyses are performed at laboratories other than the 3M Environmental Laboratory or Pace Tier2.

14. STATISTICAL ANALYSIS

Statistical methods will be limited to the calculation of means and standard deviations. Examples of the calculations used in the analyses will be included in the analytical phase report.

15. REPORT

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the phase

15.2 Dates upon which the phase was initiated and completed

15.3 A statement of compliance by the PAI addressing any exceptions to Good Laboratory Practice Standards

15.4 Objectives and procedures as stated in the approved phase protocol, including any amendments to the original phase protocol

15.5 Identity, purity, stability and the solubility of the reference standard under conditions of use

15.6 A description of the methods used to conduct the test(s)

15.7 A description of the specimens

15.8 A description of any circumstances that may have affected the quality or the integrity of the data

15.9 The name of the PAI and the names of other scientists, professionals, and supervisory personnel involved in the phase

15.10 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses

15.11 Statistical methods used to evaluate the data, if applicable

15.12 The signed and dated reports of each of the individual scientists or other professionals involved in the phase, if applicable

15.13 The location where raw data and the final report are to be stored

15.14 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

16. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT

Original data, or copies thereof, will be available at 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the phase report. When the phase report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory. All corresponding

training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained at the 3M Environmental Laboratory.

16.1 The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

16.1.1 Approved protocol and amendments

16.1.2 Study correspondence

16.1.3 Shipping records

16.1.4 Raw data

16.1.5 Approved final report (original signed copy)

16.1.6 Electronic copies of data

16.2 The following supporting records will be retained separately from the study folder according to 3M Environmental Laboratory Standard Operating Procedures:

16.2.1 Training records

16.2.2 Calibration records

16.2.3 Instrument maintenance logs

16.2.4 Standard Operating Procedures, Equipment Procedures, and Methods

17. Specimen Retention

Specimens remaining after the analytical phase is completed will be sent to and maintained by:

Lisa Clemen
3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106
Telephone: (651) 778-6176

18. Protocol Amendments and Deviations

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and Sponsor Representative and filed with the raw data.

19. ATTACHMENTS

19.1 Attachment A:

Material Safety Data Sheets (MSDS) for Reference Standard

20. SIGNATURES

Andrew Seacat

Andrew Seacat, Ph.D., *Study Director* — 8/27/01 Date

John Z. Butenhoff

John Butenhoff, Ph.D., *Sponsor Representative* — 27 August 2001 Date

Lisa A. Clemen

Lisa Clemen, *Principal Analytical Investigator* — 08/23/01 Date

Study Title

Extended Recovery Study Following a 26-Week Capsule Toxicity Study (FACT-TOX-030) with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 1

Amendment Date:

April 4, 2002

Performing Laboratory

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

Laboratory Project Identification

FACT TOX-160
Covance In-life Study Number: 6329-268
3M Medical Department Study: T-6295.22
ET&SS LIMS: E00-1668

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

1. ***PROTOCOL READS:***
Section 10 states that method ETS-8-6.0 will be used for extracting liver samples and section 12 of method ETS-8-6.0 references the use of equipment procedure AMDT-EP-22 for cleaning the tissue grinder.

AMEND TO READ:
Section 12 of method ETS-8-6.0 changed to reference equipment procedure ETS-9-52.0 as of 11/07/01.

REASON:
Equipment procedure AMDT-EP-22 was replaced by equipment procedure ETS-9-52.0 on 11/07/01. Both cover the tissue grinder's cleaning procedure.

2. ***PROTOCOL READS:***
Section 11 states that method ETS-8-7.0 will be used for analyzing liver extracts using HPLC-Electrospray/Mass Spectrometry
AMEND TO READ:
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: Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read: 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: Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

Modify method to read: One continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

Section 14.3.2 Method reads: NA

Modify method to read: 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.

Section 14.3.3 Method reads: NA

Modify method to read: 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: 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: 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: A valid calibration curve must contain at least 5 active points.

REASON:
Method modifications are improvements/clarifications to the current method.

Amendment Approval

Andrew Seacat 4/12/02

Andrew Seacat, Ph.D., *Sponsor Representative/Study Director* Date

Lisa A. Clemen 04/09/02

Lisa A. Clemen., *Principal Analytical Investigator* Date

04/09/02

William K. Reagen, Ph.D., *Performing Laboratory Management* Date

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-160, E00-1668

Deviation type (Check one): . SOP X Protocol Method Other: Equipment Procedure

Document number
FACT-TOX-160, E00-1668

Date(s) of occurrence
09/05/01

II. Description

Required procedure/process:

Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:

The MKL090401 – 250 ppb – I05505M matrix spike/matrix spike duplicate (MS/MSD) average recovery was 294%. These samples were not spiked at the appropriate level in relation to endogenous levels present in the sample.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

New matrix spikes were prepared at 2 ug/g & 10 ug/g then extracted on 10/02/01.

Recorded by Lisa A. Clemen Date 12/10/01

Authorized by John Z. Butenhoff Date 12/13/01

Andrew Seacat 12/13/01

Sponsor Representative: John Butenhoff
Study Director: Andrew Seacat

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.
FACT-TOX-160, E00-1668

Deviation type (Check one): SOP / X Protocol / Method / Other: / Equipment Procedure

Document number: FACT-TOX-160, E00-1668

Date(s) of occurrence: 10/04/01

II. Description

Required procedure/process:

Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:

The 2 ug/g matrix spike/matrix spike duplicate (MS/MSD) average recovery was 35% and the 10 ug/g MS/MSD was too dilute using a 1:500 dilution factor.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

The 10 ug/g MS/MSD was re-diluted using a 1:50 dilution and analyzed on 10/16/01. The 1:50 dilution of the 2 ug/g MS/MSDs was also reanalyzed on 10/16/01 to confirm the original low recovery.

Recorded by: Lisa A. Clemen — Date: 12/10/01

Authorized by: John Butenhoff — Date: 12/13/01

Andrew M. Seacat — 12/13/01

Sponsor Representative: John Butenhoff
Study Director: Andrew Seacat

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.
FACT-TOX-160, E00-1668

Deviation type (Check one)	SOP	Method	Equipment Procedure
	X Protocol	Other:	

Document number	Date(s) of occurrence
FACT-TOX-160, E00-1668	10/16/01

II. Description

Required procedure/process:

Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:

The 2 ug/g and 10 ug/g matrix spike/matrix spike duplicate (MS/MSD) average recoveries were 9% and 52%.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

The MS/MSD and I05505M sample were all re-diluted from the original extracts at 1:50 then analyzed on 10/24/01.

Recorded by Lisa A. Clemen — Date 12/10/01

Authorized by John T. Butterhoff — Date 12/13/01

Andrew M. Seacat — 12/13/01

Sponsor Representative: John Butenhoff
Study Director: Andrew Seacat

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.
FACT-TOX-160, E00-1668

Deviation type (Check one)	SOP	Method	Equipment Procedure
	X Protocol	Other:	

Document number	Date(s) of occurrence
FACT-TOX-160, E00-1668	10/24/01

II. Description

Required procedure/process:

Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:

The 2 ug/g and 10 ug/g matrix spike/matrix spike duplicate (MS/MSD) average recoveries were 119% and 242%. The sample recovery was not consistent with the previous analyses.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

The sample set was re-extracted on 11/07/01, then diluted 1:50 and analyzed.

Recorded by Lisa A. Clemen — Date 12/10/01

Authorized by John R. Butenhoff — Date 12/13/01

Andrew Seacat — 12/13/01

Sponsor Representative: John Butenhoff
Study Director: Andrew Seacat

Deviation No. 4
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-160, E00-1668

Deviation type (Check one)	SOP	Method	Equipment Procedure
	X Protocol	Other:	

Document number
FACT-TOX-160, E00-1668

Date(s) of occurrence
11/16/01

II. Description

Required procedure/process:

Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.

After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:

The 10 ug/g matrix spike/matrix spike duplicate (MS/MSD) average recovery was 67%.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

The 10 ug/g MS/MSD was not reanalyzed to confirm recovery since the 2 ug/g MS/MSD average recovery, analyzed during the same run, was 95% and within the stated criteria. Also, all CCVs and other data quality objectives were within criteria for this analytical run. The accuracy for the liver data in this study will be changed from +/- 30% to +/- 35% in the final report.

Recorded by Lisa A. Clemen — Date 12/10/01

Authorized by [signature] — Date 12/13/01

Andrew M. Seacat — 12/13/01

Sponsor Representative: John Butenhoff
Study Director: Andrew Seacat

Deviation No. 5
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.
Tox 160

Deviation type (Check one): SOP / Protocol / X Method / Other: / Equipment Procedure

Document number: Ets 8-7.0

Date(s) of occurrence: 11-16-01

II. Description

Required procedure/process:
According to section 11.1 The average of two standard curves will be plotted by linear regression.

Actual procedure/process:
For the data set m011116a, the unextracted curve was plotted using a quadratic fit. A linear curve that met the r^2 criteria and extended to 250 ppb (the level of the CCVs) could not be generated.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded by: [signature] Date: 11-28-01

IV. Impact on Study / Project

(completed by Study Director or Project Lead)

No Adverse impact on the outcome of this study. Study samples were evaluated versus Extracted Matrix Curves. LAC 11/28/01

Authorized by: [signature] Date: 12-03-01

Study Director: Andrew Seacat [signature] 12/3/01 **Deviation No.** 6
(assigned by Study Director or Project Lead at the end of study or project)
Sponsor Representative: John Butenhoff

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-160, E00-1668

Deviation type (Check one)	SOP	Method	Equipment Procedure
	X Protocol	Other:	

Document number	Date(s) of occurrence
FACT-TOX-160, E00-1668	11/16/01

II. Description

Required procedure/process:
Protocol FACT-TOX-160, E00-1668 states: Matrix spikes and matrix spike duplicates required for analysis of liver samples should show spike recoveries within 70%–130%.
After liver samples have been analyzed, sample concentrations will be evaluated. If a measured sample (s) concentration exceeded the calibration range of the method and was diluted with methanol into the validated calibration range, then additional matrix spikes will be prepared at the approximate measured concentrations of the samples then subsequently diluted with the same volume of methanol as the sample into the validated range of the calibration curve. These liver matrix spikes will be evaluated versus an extracted matrix calibration curve and an unextracted calibration curve. If the diluted liver matrix spikes do not show recoveries within 70-130% versus the extracted matrix calibration curve, then the sample concentrations, at the same dilution factor as the matrix spikes, will be corrected for the spike recovery.

Actual procedure/process:
1) The 10 ug/g matrix spike/matrix spike duplicate (MS/MSD) average recovery was 67%.

2) No 1/500 liver dilution was reported during the course of this study. The 1/500 liver dilution was too dilute because the wrong liver sample was used for preparing that MS/MSD level.

III. Actions Taken

(such as amendment issued, SOP revision, etc.)

1) No liver values were corrected for this low recovery since one set of MS/MSDs were within criteria and the average recovery for this set of MS/MSDs were within the extended 65-135% criteria as stated in an earlier deviation.

2) Dilution was not reextracted/rediluted using the correct sample since the 1/50 dilution was within criteria and this 1/50 dilution was used to show that no effect was observed when liver dilutions were needed.

Recorded by
Lisa A. Clemen Lisa A. Clemen

Date
04/09/02 04/09/02

Authorized by
Andrew Seacat

Date
4/12/02

Sponsor Representative/ Study Director : Andrew Seacat

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

John 2. Butterhoff May 3, 2002

3M Medical Department Study: T-6295.22
Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

ETS-8-4.2, "Extraction of Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry," (16 pages)

ETS-8-6.0, "Extraction of Potassium Perfluorooctane-sulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry," (14 pages)

ETS-8-5.2, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry," (11 pages)

ETS-8-7.0, "Analysis of Potassium Perfluorooctane-sulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry," (10 pages)

3M Environmental Laboratory

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF FLUOROCHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-4.2 **Adoption Date:** 03/01/99

Effective Date: 2/2/01

Approved By:

02/02/01
Laboratory Manager Date

02/02/01
Group Leader Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of 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 performance-based method describes the procedure for extracting perfluorooctane-sulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). 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 0.2 µm nylon filter using a 3-mL plastic

syringe into glass autovials. (Application of this method to seven fluorochemicals was demonstrated: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and a surrogate standard {see *3.0 **Definitions***}).

2.2 These sample extracts are analyzed following method ETS-8-5.2 or other appropriate method.

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 THPFOS: 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 Su and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes, plastic or glass (or equivalent)

7.3 Polypropylene bottles, capable of holding 250 mL and 1 L (Nalgene or equivalent)

7.4 Volumetric flasks, glass, type A

7.5 40 mL glass vials (I-CHEM or equivalent)

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Bottle-top Dispenser – 3.0 to 10.0 mL or a 5–10 mL graduated pipette, glass

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 mL (B&D or equivalent)

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap glass autovials and caps

7.15 Crimpers

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

8.0 Reagents and Standards

8.1 Reagent grade water, Milli-Q™, Nanopure II or equivalent

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

8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.9.3 PFOSAA+H (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 $C_8F_{13}SO_3H$, [THPFOS]), molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing different 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 water, mix until all solids are dissolved. Store in a 1 L polypropylene (or equivalent) 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 bring to volume using water. Store in a 125 mL polypropylene (or equivalent) bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH. (Add the last 1.0 mL of NaOH slowly, as the pH changes abruptly.) Dilute to volume with water. Store in a 1 L polypropylene (or equivalent) 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 water. Store in a 1 L polypropylene (or equivalent) 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 approximately 1.00 µg/mL of PFOS, PFOSA, PFOSAA, PFOSEA, M556 and EtFOSE-OH).

8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight. For standards with K^+ or other salts, multiply by a correction factor. For example, the molecular weight of KPFOS = 538, and the molecular weight of PFOS = 499. Calculate the correction factor by using the following equation:

$$\frac{\textit{molecular wt. PFOS (499)}}{\textit{molecular wt. KPFOS (538)}} = 0.9275 \quad \textit{(Correction factor)}$$

8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 µg/mL.

8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 µg/mL.

$$\left(\frac{1000\ \mu g/mL \times 5\ mL}{100\ mL}\right) = 50\ \mu g/mL$$

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 µg/mL.

$$\left(\frac{50\ \mu g/mL \times 10\ mL}{100\ mL}\right) = 5.0\ \mu g/mL$$

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 µg/mL.

$$\left(\frac{50\ \mu g/mL \times 1.0\ mL}{100\ mL}\right) = 0.500\ \mu g/mL$$

8.12 Surrogate THPFOS stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ (THPFOS) 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 µg/mL.

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 µg/mL. 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 at room temperature or in lukewarm water prior to extraction.

10.0 Quality Control

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

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

10.1.2 Method Blanks: Following this procedure, extract two 1.0 mL aliquots of water and use as method blanks.

10.1.3 Matrix Blanks: Matrix blanks are prepared from one of three sources: 1) a study control matrix from a study control animal received with each sample set; 2) a commercially obtained sample of the same species as the study animals; or 3) a surrogate matrix, also obtained commercially, but of a different species than the study animal (e.g. if rabbit is used to generate standard curves and CCVs for a monkey or rat sera study). The matrix to use depends on what matrix is used for the curve.

10.1.3.1 Study control matrix curve—If the study control matrix is used for the curve, prepare two (2) matrix blanks using the study control matrix.

10.1.3.2 Commercially obtained (same species) matrix curve—If the curve is prepared using commercially obtained matrix in the same species as the study animal, prepare two matrix blanks using this same commercially available matrix.

10.1.3.3 Surrogate matrix curve—If a surrogate matrix is used for the curve and continuing calibration verification samples:

a) prepare two (2) matrix blanks using the surrogate matrix.

b) Also, prepare a matrix blank with each set of matrix spikes (a set is a matrix spike/matrix spike duplicate at one level) using a commercially available matrix of the same species as the study animals.

10.2 Matrix spikes

10.2.1 Study Control Matrix Curve

No matrix spikes are prepared.

10.2.2 Commercially obtained matrix curve (same species)

No matrix spikes are prepared.

10.2.3 Surrogate matrix curve

Matrix spikes are necessary if matrix is not available in the same species as the study animal and a surrogate matrix is used for the curve and CCV samples (e.g., rabbit sera may be used to generate standard curves for a monkey sera study, due to measurable levels of endogenous analyte in the monkey sera). Prepare and analyze matrix spike and matrix spike duplicate samples to verify the accuracy of the extraction for target analytes.

10.2.4 Prepare each MS and MSD at two (2) levels (usually a low and mid-range concentration) using a sample chosen by the analyst, typically sera from a control group animal received with each sample set.

10.2.4.1 If there is not sufficient sera available from the control group, prepare matrix spikes using commercially available matrix from the same species as the study animal.

10.2.5 Prepare one matrix spike and matrix spike duplicate at each level listed in **10.2.4** per 20 samples, with a minimum of 2 matrix spikes per level per batch. If a batch includes more than 20 samples, additional spikes may be prepared at the same, low, or high range levels.

10.2.6 If more than 25% of the samples are ≤LOQ, two matrix spikes should be prepared at approximately 2–5 times the expected LOQ.

10.2.7 If the majority of the samples are at or above the high range of the curve, additional matrix spikes should be prepared to approximate sample concentrations.

10.3 Continuing calibration verifications (CCVs)

10.3.1 Prepare continuing calibration verification samples for instrument stability verification during analysis. Prepare and analyze CCVs for every assay run, regardless of the matrix type used to prepare the standard curve.

10.3.2 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve (i.e., either the study control matrix, commercial matrix of same species, or surrogate matrix).

10.3.3 The expected concentrations of the CCVs will fall within the range of the initial calibration curve; typically, the low to mid-range of the curve.

10.3.4 Prepare, at a minimum, two continuing calibration verifications, each at a different concentration, per group of 10 samples. For example, if a sample set = 34, eight (8) checks are prepared; four (4) at a low range and four (4) at a mid-range concentration.

10.3.5 Additional continuing calibration verifications may be included at the same, low, mid or high-range concentration.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Transfer 1 mL of appropriate serum to a 15 mL centrifuge tube. If commercially available sera is used, prepare two matrix blanks, using the volume of sera available for most study animals.

11.1.1.1 Depending upon study goals or if analyte-free commercial sera is not available, a surrogate matrix may be used for generation of the calibration standard. For example, rabbit sera may be used as a surrogate matrix for rat studies if quantitation of extremely low levels of the analyte is required.

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 thirteen aliquots in 15-mL centrifuge tubes, mix or shake sera between aliquots.

11.1.4 Two 1-mL aliquots, or other appropriate volume, serve as curve matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1 at the end of this section to spike one standard curve, for a total of nine 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 See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, continuing check, and sample add an appropriate amount of surrogate working standard to achieve a constant concentration that falls within the calibration curve range of 2.5 ng/mL–1000 ng/mL. Usually, samples are spiked for a constant concentration in all samples at approximately 500 ng/mL or other concentration as determined by the analyst.

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

*ppm=µg/mL

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw at room temperature or in lukewarm water.

12.2 Label a 15 mL polypropylene centrifuge tube with the study number, sample ID, date and analyst initials. See attached worksheet (Attachment A or similar worksheet) for documenting the remaining steps.

12.3 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to the 15 mL polypropylene centrifuge tube.

12.4 Return unused samples to freezer after extraction amounts have been removed.

12.5 Record the initial volume on the extraction worksheet (Attachment A or similar worksheet).

12.6 Spike all samples, blanks and standards, that are ready for extraction with surrogate standard as described in **11.2.**

12.7 Spike each calibration standard 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 if necessary 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 bottle top dispenser or 5–10 mL graduated glass pipette, 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 (top) 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, or longer if needed.

12.19 Label a 1.5 mL glass autovial (or low-volume autovial when necessary) with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.20 Attach a 25 mm, 0.2 µm nylon mesh filter to a 3 mL syringe and transfer the sample from step 12.18 to this syringe. Filter into the labeled autovial.

12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.22 Complete the extraction worksheet (Attachment A or similar worksheet) and include in the study binder.

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:

$$\frac{mL\ of\ std \times concentration\ of\ std\ (\mu g / mL)}{mL\ of\ std + mL\ of\ surrogate\ std + initial\ matrix\ volume\ (mL)} = Final\ concentration\ (\mu g / mL)\ of\ PFOS\ in\ matrix$$

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**). At the discretion of the PAI, MDL may not be defined for some studies.

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 If surrogate matrix is used for curve and QC, matrix spike and matrix spike duplicate samples to verify extraction efficiency.

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-8-5.2 for method performance criteria.

15.0 Pollution Prevention and Waste Management

15.1 Dispose of sample waste, flammable solvent waste and used glass pipette waste using proper methods and by placing each in their designated waste container.

16.0 Records

16.1 Complete the extraction worksheet attached to this method (or a similar worksheet), and include in the study binder.

17.0 Attachments

17.1 Attachment A, Extraction worksheet (a similar worksheet may be substituted for Attachment A)

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Ion Pair Standard Curves worksheet (a similar worksheet may be substituted for Attachment C)

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"

18.3 ETS-8-5.2, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

19.0 Affected Documents

19.1 ETS-8-5.2, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

20.0 Revisions

Revision Number	Reason For Revision	Revision Date
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
2	Section 1.1, 2.1 Eliminate 'potassium' from perfluorooctanesulfonate 8.9.1 Add K to PFOS 8.9.3 Add H to PFOSAA 10.2-10.2.4, 14.2.2 Add information about using surrogate matrix, clarify control matrix and spiking the curve, change spikes to every 20 samples 10.3 Clarify purpose of continuing calibration checks 11.1 Added wording about control animal sera, using and not using commercially available tissue In general, make less specific for equipment/supplies. Substitute water for Milli-Q, Nalgene or equivalent bottle may be used.	

Extraction Worksheet ETS-8-4.2

Study #_____ Matrix_____ Box #_____ Wk/Day_____ Date Spiked/ Analyst:	Surrogate Std actual µg/mL #_____	FC-Mix approx. 0.5 µg/mL #_____	FC-Mix approx. 5 µg/mL #_____	FC-Mix approx. 50 µg/mL #_____	Comments	Verified by:
MS						
MSD						
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
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		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		
		-	-	-		

Blank ID # amount = mL	
Serum Extraction Method : **Date & Initials**	
Vortex 15 sec.	
Pipette Matrix, spike with appropriate surrogate or FCMix Volume mL	
Pipette 1 mL of 0.5 M TBA, pH 10. pH = _____ Std. #_____	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer Std. #_____	
Dispense/pipette 5 mL of methyl-t-butyl ether TN-A-_____	
Dispenser ID #:	
Shake 20 min. Shaker ID #: Shaker speed:_____	
Centrifuge 20-25 min. Centrifuge ID#: Centrifuge speed:	
Remove a 4 mL aliquot of organic layer	
Put on Nitrogen Evaporator to dryness	
Add methanol Volume_____ mL TN-A-_____	
Vortex 30 sec.	
Filter using a 3mL B-D syringe with a 0.2um filter into a 1.5 mL autosample vial	

Cont. Cal. Verifications used same matrix as for std curve.

MDL/LOQ values for rabbit serum

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

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 (ng/mL)	LCR from curve (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 (ng/mL)	LCR from curve (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 (ng/mL)	LCR from curve (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 (ng/mL)	LCR from curve (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 (ng/mL)	LCR from curve (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 (ng/mL)	LCR from curve (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

EXAMPLE

Ion Pair Standard Curves – Fluids

Analyst(s) **Study number:**

Prep date(s): **Final solvent and TN:**

Analyte(s): **Blank fluid/identifier:** {Record matrix used to prepare curves, (e.g., 'Rabbit,' ID #XXX)}

Study Animal Matrix :

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: {Record the standards used to prepare the extracted curves.}

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix in methanol

PFOS Std conc µg/mL	PFOSA Std conc µg/mL	PFOSAA Std conc µg/mL	EtFOSE Std conc µg/mL	PFOSEA Std conc µg/mL	M556 Std conc µg/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 sera matrix: Rabbit

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		
24.8	25.1	26.3	24.8	25.2	25.8	Surrogate	
49.3	50.0	52.4	49.4	50.1	51.3	Final conc ng/mL	
97.6	98.9	104	97.8	99.3	102	500	
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 ng/mL	5.00-1000 ng/mL	5.00-1000 ng/mL	5.00-1000 ng/mL	5.00-1000 ng/mL	5.00-1000 ng/mL
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.					

Exact Copy of Original
Initial LWW Date 10/19/01

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: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

JA Bruno — 7/22/99
Laboratory Manager — Date

Kristen Hansen — 7/14/99
Group Leader — Date

Lisa A Clemen — 07/14/99
Technical Reviewer — 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 µm 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 sampule 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 (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 (µg/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.)	µl	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 sampule 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 sampule 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 sampule 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 sampule 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 matirx 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.

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

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:

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

* 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:

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

*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

Study #_____ Matrix_____ Box #_____ Wk/Day_____ Date Spiked/Analyst	Surrogate Std approx. ppm actual ppm #_____	FC Mix Std approx. 0.5 ppm actual ppm #_____	FC Mix Std approx. 5 ppm actual ppm #_____	FC Mix Std approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank	Liver Homogenate: Std #	Liver amount = g
Liver Extraction Method	:	**Date & Initials**
Spike surrogate and Standard mix, Vortex 15 sec.		
Pipette-1 mL of Liver Solution		
Pipette 1 mL of †0.5 M TBA, pH 10. pH =	Std. #	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. #	
Dispense 5ml of Methyl-t-Butyl Ether	TN-A-	
Shake 20 min.	Shaker Speed	
Centrifuge 20-25 min.	Centrifuge Speed	
Remove a 4 mL aliquot of organic layer		
Put on Nitrogen Evaporator to dryness	Evaporator Temperature	
Add 1.0 mL of Methanol	TN-A-	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial		

Cont. Cal. Verifications used the same matrix as for the standard curve.

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			
29.9	29.9	29.9	29.9	29.9	29.9		Surrogate	
59.9	59.9	59.9	59.9	59.9	59.9		Final conc	
120	120	120	120	120	120		ng/mL	
299	299	299	299	299	299		0.500	
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 POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-5.2

Adoption Date: 03/01/99

Revision Date: 2/1/01

Author: Lisa Clemen, Kris Hansen

Approved By:

Laboratory Manager	Date 02/01/01
Group Leader	Date 02/01/01
Technical Reviewer	Date 02-01-01

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 Although supported by a validation for most commonly used matrices, this is a performance-based method. Careful attention should be paid to method QC as there is great variability in sera. 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 and Ultima 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 and Ultima 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 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 or Ultima 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 HPLC 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 or Ultima triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 or Agilent 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 or nitrogen 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 and 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-8-4.2.**

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 at least once during the course of the study to determine if contamination occurred during sample prep.

10.1.2 Analyze at least one solvent blank prior to each calibration curve.

10.1.3 Matrix blanks should be analyzed with each sample list that includes undiluted extracts.

10.2 Matrix Spikes

10.2.1 If curves and method QC are prepared in a surrogate matrix (e.g. curves in rabbit sera, samples are monkey sera), matrix spikes and matrix spike duplicates are prepared in blank sample matrix (e.g., monkey sera) and analyzed to verify extraction efficiency.

10.2.2 If curves and method QC are prepared in the same matrix as samples, no additional matrix spikes are required.

10.3 Continuing Calibration Verifications (CCVs)

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

10.3.2 Analyze two calibration standards (one at each of 2 levels) after every one to ten samples, with a minimum of two per batch and always finishing an injection sequence with at least two calibration standards.

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix calibration standards prior to each set of extracts. The curve will be plotted by linear regression, 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, reextract samples, or reanalyze the standard curve.

11.3 For purposes of accuracy when quantitating levels of analyte at the limits of the curve range, it may be necessary to use either the low end or the high end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, it may be beneficial to 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. It is also acceptable to break the linear range into a low curve and a high curve. If this is done, no more than one point should be used in common between the curves. For example, the low curve may include the following points: 1, 5, 25, 100, 250 ppb and the high curve may include the points: 250, 500, 750, 1000, 1250 ppb.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 On the MassLynx main page, set up a sample list name. Save the list as instrument designator letter, last 2 digits of test year-mo-day, and a letter that will increase through the alphabet with each additional list for that day.

Example Sample List: IYYMMDDa or D010712a

I=instrument name (D for "Davey")
YY=year of test (01)
MM=month of test (07)
DD=day of test (12)
a=first sample list (run) of the day (the next sample list will end with 'b,' the next 'c' and so on.)

12.1.2 Assign a filename using the instrument designator letter, the last 2 digits of year-mo-day, and a 3-digit sequential file number that starts with 1 and increases by one for each filename.

Example File Name: IYYMMDD### or D010712001

I=instrument name (D for "Davey")
YY=year of test (01)
MM=month of test (07)
DD=day of test (12)
###=3-digit sequential file number starting with 1 through 999 (001)

Also, as part of the sample list, assign a method (MS) for acquiring, an inlet file, a bottle number, an injection volume and sample descriptions.

12.1.3 To create a method, click on Method Editor button in the MS Status Pane and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. Also set the acquisition start and stop times. 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.

12.1.4 Typically the analytical batch run sequence begins with solvent blanks and a set of extracted matrix standards.

12.1.5 Sample extracts are analyzed with two CCVs injected every one to ten samples. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered sample extracts but may be included as such.

12.2 Using the HPLC

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

12.2.2 Set-up the HPLC to 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 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 10.0 minutes

12.2.2.4 Flow rate = 300 µL/min

12.2.2.5 Mobile Phase (program)

Time	MeOH	2.0 mM Ammonium acetate (in H_2O)
0.00 min.	10%	90%
1.00 min.	10%	90%
5.50 min.	95%	5%
7.50 min.	95%	5%
8.00 min.	10%	90%

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24** 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. The probe should be checked weekly.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 µL/min or as appropriate. Observe droplets coming out of the tip of the probe.

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, MS file, HPLC parameters, sample list and the Microsoft Word summary page and store in the study binder with a copy taped into the instrument log.

12.3.9 Click on start button in the MassLynx main page (this may vary among MassLynx versions, see 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:

13.1.1 Calculate matrix spike percent recoveries using the following equation:

$$\%\ Recovery = \frac{Observed\ Result - Matrix\ Blank\ Result}{Spiking\ Level} \times 100$$

13.1.2 Calculate percent difference using the following equation:

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

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

$$\frac{(Conc.\ of\ PFOS\ Calc.\ from\ Std.\ Curve\ (ng/mL) \times Dilution\ Factor)}{\left\{\frac{(Initial\ Volume\ of\ Matrix\ (mL) + mL\ of\ Surrogate\ Standard)}{Final\ Volume\ (mL)}\right\}} \times \frac{1\ \mu g}{1000\ ng}$$

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-4.2, 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 must be below the lowest active standard in the calibration curve.

14.3 Calibration Curves

14.3.1 The coefficient of determination value for the calibration curve must be 0.990 or better.

14.3.2 All active calibration curve points must be within 25% of the theoretical value with the exception of the LOQ point, which may deviate up to 30%.

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

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

14.3.5 Not including low or high points dropped to optimize the linear range, curve points may be deactivated if they deviate more than 25% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

14.3.6 One point below the LOQ may remain active even if it deviates more than ±30% or has a peak area less than two times the matrix blank; however, the LOQ will be defined at the lowest point with acceptable deviation.

14.3.7 A valid calibration curve must contain at least 5 active points above and including the LOQ.

14.4 Matrix Spikes

14.4.1 The average matrix spike percent recoveries should be within ± 30% of the spiked concentration. Recoveries outside of this range should be discussed in the report.

14.5 Continuing Calibration Verifications (CCVs)

14.5.1 Continuing calibration samples within the linear range of the run must show a percent recovery within ±25% of the spiked concentration. If a CCV is outside of this recovery, subsequent data should not be accepted. Acceptable data must be bracketed by the curve and passing CCVs.

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 The first page of each data packet generated for a study must have the following information included either in the header, in the footer or hand written on the page: study or project number, instrument, sample matrix and time point, date, and analyst.

16.2 A data packet includes the following: data review summary form, MassLynx quantify compound summary report for each target analyte, quantify calibration report for each target analyte (curve), method report, Word document listing set-up parameters, tune

method report, MassLynx scanning method report, HPLC method report, sample list, and quantity sample report (chromatogram).

16.3 For each analysis, after printing the tune method report, Word document listing, set-up parameters, MassLynx scanning method report, and sample list, copy and tape into the instrument runlog. The original is maintained in the data packet.

16.4 On each page of the quantify compound report, quantify calibration report (curve), and quantify sample report (chromatogram), the following information must be included either in the header, the footer, or hand written on the page: study or project number, instrument, method, calibration (the method and calibration are usually assigned the same name), analyst, and date.

16.5 The analyst must date and initial the first page in a packet as long as their initials and date are electronically included on each page. If initials and date are not electronically included, they must date and initial each page.

16.6 Summarize data using suitable software (e.g., Excel) for inclusion in the final report. See **Attachment A** for an example of a summary spreadsheet.

16.7 Back up electronic data to appropriate medium. Record the file name and location of backup electronic data in instrument log book.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: 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-8-4.2, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
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
2	10.1 Clarified when blanks are run. 10.2 Added instructions for when surrogate matrix is used. 10.3 Specify requirements for CCVs. 11.1 Requires only a calibration curve before the samples. 11.2 Clarify what to do if curve does not meet requirements. 11.3 Allow to truncate the curve. 12.1.1 Clarify sample list ID. 12.1.3 Clarify typical run. 12.2 Changes to mobile phase gradient and specify flow rate. 14.3 Change acceptable limits. Add specifics for acceptance of calibration curve. 14.3.3 When to deactivate calibration standards based on blank response. 14.3.5 When to deactivate calibration standards within the linear range. 14.4 Modifies evaluation and use of matrix spikes. 14.5 Describes evaluation and use of CCVs. 14.5.1 Evaluation of CCVs. 16.0 Add requirements for records and documentation.	

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 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:

DJ Bruner — 7/22/99
Laboratory Manager — Date

Kristen Hansen — 7/14/99
Group Leader — Date

Lisa A Clemen — 07/14/99
Technical Reviewer — 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 µL 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:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. 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 ± 30% of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within ± 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

Revision Number	Reason For Revision	Revision 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 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

3M Medical Department Study: T-6295.22

Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix D: Data Summary Tables

Table 9. Data Summary for PFOS in Serum FACT-TOX-160—µg/mL

Group	Timepoint	Sex	PFOS µg/mL Average ± SD
Group 1	Week 9	Male	17.2 ± .824 n=2
		Female	26.6 ± 5.88 n=2
	Week 17	Male	13.7 ± 1.13 n=2
		Female	20.3± 2.37 n=2
	Week 25	Male	8.7 ± 1.93 n=2
		Female	15.8 ± 5.27 n=2
	Week 33	Male	8.10 ± 0.768 n=2
		Female	12.4 ± 4.2 n=2
	Week 41	Male	5.65 ± 0.303 n=2
		Female	10.4 ± 4.37 n=2
	Week 53	Male	4.26 ± 0.052 n=2
		Female	7.95 ± 1.76 n=2

NOTE: 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, indicates that the data are quantitative to 30% or greater.

Table 10. Data Summary for PFOS in Liver FACT-TOX-160—µg/g

Group	Sex	PFOS µg/g Average ± SD
Group 1	Male	3.56 ± 0.436 n=2
	Female	9.23 ± 2.04 n=2

NOTE: 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, indicates that the data are quantitative to 35% or greater.

3M Medical Department Study: T-6295.22
Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix E: Data Spreadsheets

FACT-TOX-160
Covance# 6329-268

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an extracted rabbit sera curve
Analytical Equipment System Number:	Amelia062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	09/07/01 RWW
Dates of Analysis/Analyst:	09/13/01 MMH
Date of Data Reduction/Analyst:	09/14/01 MMH
Box:	01-042, 01-043

Sample Data

MONKEY SERUM

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	WB090601-H2O Blk-1	0.00	<LOQ (0.00492 ug/mL)		
	WB090601-H2O Blk-2	0.00	<LOQ (0.00492 ug/mL)	<LOQ	NA
Matrix Blk	RBS090601-Sera Blk-1	0.00	<LOQ (0.00492 ug/mL)		
	RBS090601-Sera Blk-2	0.00	<LOQ (0.00492 ug/mL)	<LOQ	NA
	MKS090601-Sera Blk-1	10.2	0.0102		
	MKS090601-Sera Blk-2	7.71	0.00771	0.00897	0.00177
QC - 50 ppb	MKS090601-50 ppb-MS	72.6	129%		
	MKS090601-50 ppb-MSD	64.8	113%	121%	13%
250 ppb	MKS090601-250 ppb-MS	279	110%		
	MKS090601-250 ppb-MSD	258	101%	105%	8%
Group 1 Week 9	I05505M	44.6	17.8		
	I05523M	45.8	16.7	17.2	0.824
	I05539F	76.8	30.7		
	I05552F	44.8	22.4	26.6	5.88
Group 1 Week 17	I05505M	45.8	14.52		
	I05523M	37.5	12.93	13.7	1.13
	I05539F	48.4	22.0		
	I05552F	77.3	18.6	20.3	2.37
Group 1 Week 25	I05505M	36.7	10.1		
	I05523M	20.9	7.33	8.70	1.93
	I05539F	50.8	19.5		
	I05552F	24.8	12.1	15.8	5.27
Group 1 Week 33	I05505M	33.3	8.64		
	I05523M	29.1	7.56	8.10	0.768
	I05539F	48.5	15.4		
	I05552F	47.3	9.46	12.4	4.20
Group 1 Week 41	I05505M	17.6	5.86		
	I05523M	12.5	5.43	5.65	0.303
	I05539F	31.0	13.5		
	I05552F	20.0	7.28	10.4	4.37
Group 1 Week 53	I05505M	14.0	4.30		
	I05523M	11.4	4.22	4.26	0.052
	I05539F	14.7	9.19		
	I05552F	19.1	6.70	7.95	1.76

PFOS = Perfluorooctanesulfonate

Date Entered/By: 09/26/01 LAC
Date Verified/ By: 12/6/01 mmh LAC 04/25/02

FACT-TOX-160
Covance# 6329-268

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an extracted rabbit sera curve
Analytical Equipment System Number:	Amelia062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	09/07/01 RWW
Dates of Analysis/Analyst:	09/13/01 MMH
Date of Data Reduction/Analyst:	09/14/01 MMH
Box:	01-042, 01-043

Sample Data

MONKEY SERUM

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	WB090601-H2O Blk-1	1	NA	1	0.00	A010913049	<LOQ (0.00492 ug/mL)		
	WB090601-H2O Blk-2	1	NA	1	0.00	A010913050	<LOQ (0.00492 ug/mL)	<LOQ	NA
Matrix Blk	RBS090601-Sera Blk-1	1	NA	1	0.00	A010913051	<LOQ (0.00492 ug/mL)		
	RBS090601-Sera Blk-2	1	NA	1	0.00	A010913052	<LOQ (0.00492 ug/mL)	<LOQ	NA
	MKS090601-Sera Blk-1	1	NA	1	10.22	A010913053	0.0102		
	MKS090601-Sera Blk-2	1	NA	1	7.71	A010913054	0.00771	0.00897	0.00177
QC - 50 ppb	MKS090601-50 ppb-MS	1	NA	1	72.55	A010913055	129%		
	MKS090601-50 ppb-MSD	1	NA	1	64.80	A010913056	113%	121%	13.0%
250 ppb	MKS090601-250 ppb-MS	1	NA	1	279.46	A010913057	110%		
	MKS090601-250 ppb-MSD	1	NA	1	258.06	A010913058	101%	105%	8.24%
Group 1 Week 9	I05505M	0.50	NA	200	44.56	A010913088	17.8		
	I05523M	0.55	NA	200	45.81	A010913089	16.7	17.2	0.824
	I05539F	0.50	NA	200	76.79	A010913090	30.7		
	I05552F	0.40	NA	200	44.81	A010913091	22.4	26.6	5.88
Group 1 Week 17	I05505M	0.63	NA	200	45.75	A010913081	14.5		
	I05523M	0.58	NA	200	37.49	A010913082	12.9	13.7	1.13
	I05539F	0.44	NA	200	48.36	A010913083	22.0		
	I05552F	0.83	NA	200	77.32	A010913084	18.6	20.3	2.37
Group 1 Week 25	I05505M	0.73	NA	200	36.72	A010913077	10.1		
	I05523M	0.57	NA	200	20.90	A010913078	7.33	8.70	1.93
	I05539F	0.52	NA	200	50.76	A010913079	19.5		
	I05552F	0.41	NA	200	24.75	A010913080	12.1	15.8	5.27
Group 1 Week 33	I05505M	0.77	NA	200	33.28	A010913070	8.64		
	I05523M	0.77	NA	200	29.10	A010913071	7.56	8.10	0.768
	I05539F	0.63	NA	200	48.51	A010913075	15.4		
	I05552F	1.00	NA	200	47.32	A010913076	9.46	12.4	4.20
Group 1 Week 41	I05505M	0.60	NA	200	17.59	A010913066	5.86		
	I05523M	0.46	NA	200	12.50	A010913067	5.43	5.65	0.303
	I05539F	0.46	NA	200	30.97	A010913068	13.5		
	I05552F	0.55	NA	200	20.02	A010913069	7.28	10.4	4.37
Group 1 Week 53	I05505M	0.65	NA	200	13.96	A010913062	4.30		
	I05523M	0.54	NA	200	11.40	A010913063	4.22	4.26	0.052
	I05539F	0.32	NA	200	14.71	A010913064	9.19		
	I05552F	0.57	NA	200	19.09	A010913065	6.70	7.95	1.76

PFOS = Perfluorooctanesulfonate

Date Entered/By: 09/26/01 LAC
Date Verified/ By: 12/6/01 mmh LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an unextracted curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	08/31/01 RWW
Dates of Analysis/Analyst:	09/05/01, 09/06/01 MMH
Date of Data Reduction/Analyst:	09/06/01, 09/07/01 MMH
Box:	01-042

Sample Data

MONKEY SERUM

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	MKS083101-H2O Blk-1	0.18	<LOQ (0.0012 ug/mL)		
	MKS083101-H2O Blk-2	0.23	<LOQ (0.0012 ng/mL)	<LOQ	NA
Matrix Blank	MKS083101-Sera Blk-1	9.69	0.00775		
	MKS083101-Sera Blk-2	9.05	0.00724	0.00750	0.000362
QC	MKS083101-MS-25 ppb	32.6	118%		
	MKS083101-MSD-25 ppb	25.9	84%	101%	34%
	MKS083101-MS-500 ppb	448	111%		
	MKS083101-MSD-500 ppb	449	112%	111%	0%

PFOS = Perfluorooctanesulfonate
LOQ = 1.0 ng/mL in standard calculates to 1.2 ng/mL in serum.
LOQ = (1.0 ng/mL unext standard * 1.25 ext dil factor * 1 dilution factor)/1000 = 0.0012 ug/mL in serum

Date Entered/By: 09/12/01 LAC
Date Verified/ By: 16/6/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an unextracted curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	08/31/01 RWW
Dates of Analysis/Analyst:	09/05/01, 09/06/01 MMH
Date of Data Reduction/Analyst:	09/06/01, 09/07/01 MMH
Box:	01-042

Sample Data

MONKEY SERUM

Group Dose	Sample #	Initial Vol. mL	Extraction Dilution Factor	Surrogate Verified	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	MKS083101-H2O Blk-1	1	1.25	Confirmed High	1	0.18	A010906019	<LOQ (0.0012 ug/mL)		
	MKS083101-H2O Blk-2	1	1.25	Confirmed High	1	0.23	A010906020	<LOQ (0.0012 ng/mL)	<LOQ	NA
Matrix Blank	MKS083101-Sera Blk-1	1	1.25	Confirmed High	1	9.69	A010906021	0.00775		
	MKS083101-Sera Blk-2	1	1.25	Confirmed High	1	9.05	A010906022	0.00724	0.00750	0.000362
QC	MKS083101-MS-25 ppb	1	1.25	Confirmed High	1	32.62	A010906023	118%		
	MKS083101-MSD-25 ppb	1	1.25	Confirmed High	1	25.87	A010906024	84%	101%	34%
	MKS083101-MS-500 ppb	1	1.25	2nd analysis OK	1	447.77	A010906025	111%		
	MKS083101-MSD-500 ppb	1	1.25	Confirmed High	1	448.57	A010906026	112%	111%	0%

PFOS = Perfluorooctanesulfonate
LOQ = 1.0 ng/mL in standard calculates to 1.2 ng/mL in serum.
LOQ = (1.0 ng/mL unext standard * 1.25 ext dil factor * 1 dilution factor)/1000 = 0.0012 ug/mL in serum

Date Entered/By: 09/12/01 LAC
Date Verified/ By: 16/6/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an unextracted curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/02/01 RWW
Dates of Analysis/Analyst:	10/04/01 MMH
Date of Data Reduction/Analyst:	10/05/01 MMH
Box:	01-046

Sample Data

MONKEY SERUM

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	WB100201-H2O Blk-1	0.00	<LOQ (0.00313 ug/mL)		
	WB100201-H2O Blk-2	0.00	<LOQ (0.00313 ug/mL)	<LOQ	NA
Matrix Blank	RBS100201-Sera Blk-1	0.09	<LOQ (0.00313 ug/mL)		
	RBS100201-Sera Blk-2	0.13	<LOQ (0.00313 ug/mL)	<LOQ	NA
	MKS100201-Sera Blk-3	7.23	0.00904		
	MKS100201-Sera Blk-4	7.14	0.00893	0.00898	0.0000795
QC	MKS100201-MS-2 ug/mL	8.69	113%		
	MKS100201-MSD-2 ug/gmL	8.34	109%	111%	4%
	MKS100201-MS-10 ug/mL	45.6	115%		
	MKS100201-MSD-10 ug/mL	46.5	117%	116%	2%

PFOS = Perfluorooctanesulfonate
LOQ = 2.50 ng/mL in standard calculates to 3.13 ng/mL in serum.
LOQ = (2.50 ng/mL unext standard * 1.25 ext dil factor * 1 dilution factor)/1000 = 0.00313 ug/mL in serum

Date Entered/By: 10/11/01 LAC
Date Verified/ By: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an unextracted curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/02/01 RWW
Dates of Analysis/Analyst:	10/04/01 MMH
Date of Data Reduction/Analyst:	10/05/01 MMH
Box:	01-046

Sample Data

MONKEY SERUM

Group Dose	Sample #	Initial Vol. mL	Extraction Dilution Factor	Surrogate Verified	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	WB100201-H2O Blk-1	1	1.25	NA	1	0.00	A011004082	<LOQ (0.00313 ug/mL)		
	WB100201-H2O Blk-2	1	1.25	NA	1	0.00	A011004083	<LOQ (0.00313 ug/mL)	<LOQ	NA
Matrix Blank	RBS100201-Sera Blk-1	1	1.25	NA	1	0.09	A011004084	<LOQ (0.00313 ug/mL)		
	RBS100201-Sera Blk-2	1	1.25	NA	1	0.13	A011004085	<LOQ (0.00313 ug/mL)	<LOQ	NA
	MKS100201-Sera Blk-3	1	1.25	NA	1	7.23	A011004086	0.00904		
	MKS100201-Sera Blk-4	1	1.25	NA	1	7.14	A011004087	0.00893	0.00898	0.0000795
QC	MKS100201-MS-2 ug/mL	1	1.25	Out High	200	8.69	A011004088	113%		
	MKS100201-MSD-2 ug/gmL	1	1.25	NA	200	8.34	A011004089	109%	111%	4%
	MKS100201-MS-10 ug/mL	1	1.25	NA	200	45.57	A011004090	115%		
	MKS100201-MSD-10 ug/mL	1	1.25	NA	200	46.54	A011004091	117%	116%	2%

PFOS = Perfluorooctanesulfonate
LOQ = 2.50 ng/mL in standard calculates to 3.13 ng/mL in serum.
LOQ = (2.50 ng/mL unext standard * 1.25 ext dil factor * 1 dilution factor)/1000 = 0.00313 ug/mL in serum

Date Entered/By: 10/11/01 LAC
Date Verified/ By: 12/10/01 mmh LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an extracted rabbit sera curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/02/01 RWW
Dates of Analysis/Analyst:	10/04/01 MMH
Date of Data Reduction/Analyst:	10/05/01 MMH
Box:	01-046

Sample Data

MONKEY SERUM

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	WB100201-H2O Blk-1	0.00	<LOQ (0.00492 ug/mL)		
	WB100201-H2O Blk-2	0.00	<LOQ (0.00492 ug/mL)	<LOQ	NA
Matrix Blank	RBS100201-Sera Blk-1	0.00	<LOQ (0.00492 ug/mL)		
	RBS100201-Sera Blk-2	0.00	<LOQ (0.00492 ug/mL)	<LOQ	NA
	MKS100201-Sera Blk-3	8.02	0.00802		
	MKS100201-Sera Blk-4	7.90	0.00790	0.00796	0.0000849
QC	MKS100201-MS-2 ug/mL	9.96	104%		
	MKS100201-MSD-2 ug/mL	9.49	99%	102%	5%
	MKS100201-MS-10 ug/mL	59.0	119%		
	MKS100201-MSD-10 ug/mL	60.3	121%	120%	2%

PFOS = Perfluorooctanesulfonate

Date Entered/By: 10/11/01 LAC
Date Verified/ By: 12/10/01 mmh LAC 04/25/02

FACT-TOX-160
Covance# 6329-268

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295.22
Matrix:	Monkey Serum
Method/Revision:	ETS-8-4.2 & ETS-8-5.2 versus an extracted rabbit sera curve
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	Masslynx 3.4
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/02/01 RWW
Dates of Analysis/Analyst:	10/04/01 MMH
Date of Data Reduction/Analyst:	10/05/01 MMH
Box:	01-046

Sample Data

MONKEY SERUM

Group Dose	Sample #	Initial Vol. mL	Extraction Dilution Factor	Surrogate Verified	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blank	WB100201-H2O Blk-1	1	1.00	NA	1	0.00	A011004082	<LOQ (0.00492 ug/mL)		
	WB100201-H2O Blk-2	1	1.00	NA	1	0.00	A011004083	<LOQ (0.00492 ug/mL)	<LOQ	NA
Matrix Blank	RBS100201-Sera Blk-1	1	1.00	NA	1	0.00	A011004084	<LOQ (0.00492 ug/mL)		
	RBS100201-Sera Blk-2	1	1.00	NA	1	0.00	A011004085	<LOQ (0.00492 ug/mL)	<LOQ	NA
	MKS100201-Sera Blk-3	1	1.00	NA	1	8.02	A011004086	0.00802		
	MKS100201-Sera Blk-4	1	1.00	NA	1	7.90	A011004087	0.00790	0.00796	0.0000849
QC	MKS100201-MS-2 ug/mL	1	1.00	NA	200	9.96	A011004088	104%		
	MKS100201-MSD-2 ug/gmL	1	1.00	NA	200	9.49	A011004089	99%	102%	5%
	MKS100201-MS-10 ug/mL	1	1.00	NA	200	58.97	A011004090	119%		
	MKS100201-MSD-10 ug/mL	1	1.00	NA	200	60.27	A011004091	121%	120%	2%

PFOS = Perfluorooctanesulfonate

Date Entered/By: 10/11/01 LAC
Date Verified/ By: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Attachments
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	09/05/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	09/10/01, 09/13/01, 09/17/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	09/11/01, 09/14/01, 09/18/01 MMH	Box #:	01-042

Sample Data

MONKEY LIVER

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL090501-H2O Blk-1	0.00	<LOQ (0.00629 ug/g)		
	RBL090501-H2O Blk-2	0.00	<LOQ (0.00629 ug/g)	<LOQ	NA
Matrix Blk	RBL090501-Liver Blk-1	0.00	<LOQ (0.00629 ug/g)		
	RBL090501-Liver Blk-2	0.00	<LOQ (0.00629 ug/g)	<LOQ	NA
QC	RBL090501-300 ppb-MS	322	102%		
	RBL090501-300 ppb-MSD	323	102%	102%	0%
	MKL090501-300 ppb-I05505M-MS	879	298%		
	MKL090501-300 ppb-I05505M-MSD	854	290%	294%	3%
Group 1	I05505M	3251	3.25		
	I05523M	3868	3.87	3.56	0.436
	I05539F	10675	10.7		
	I05552F	7788	7.79	9.23	2.04

PFOS = Perfluorooctanesulfonate
* Matrix spikes weren't spiked at the appropriate level. Sample will be re-spiked, at the appropriate levels, at a later date.

Date Entered/Analyst: 09/12/01, 09/26/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668

Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys

Product Number(Test Substance): T-6295.22
Matrix: Monkey Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve
Analytical Equipment System Number: Amelia 062498
Instrument Software/Version: Masslynx 3.4
Date of Extraction/Analyst: 09/05/01 RWW
Date of Analysis/Analyst: 09/10/01, 09/13/01, 09/17/01 MMH
Date of Data Reduction/Analyst: 09/11/01, 09/14/01, 09/18/01 MMH

Filename: See Below
R-Squared V See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Box #: 01-042

Sample Data

MONKEY LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL090501-H2O Blk-1	NA	1.000	NA	0.00	1	0.00	A010910016	<LOQ (0.00629 ug/g)		
	RBL090501-H2O Blk-2	NA	1.000	NA	0.00	1	0.00	A010910017	<LOQ (0.00629 ug/g)	<LOQ	NA
Matrix Blk	RBL090501-Liver Blk-1	NA	1.000	NA	0.00	1	0.00	A010910018	<LOQ (0.00629 ug/g)		
	RBL090501-Liver Blk-2	NA	1.000	NA	0.00	1	0.00	A010910019	<LOQ (0.00629 ug/g)	<LOQ	NA
QC	RBL090501-300 ppb-MS	NA	1.000	NA	321.51	1	322	A010910022	102%		
	RBL090501-300 ppb-MSD	NA	1.000	NA	322.78	1	323	A010910023	102%	102%	0%
	MKL090501-300 ppb-I05505M-MS	NA	1.0160	NA	83.93	50	879	A010917016	298% *		
	MKL090501-300 ppb-I05505M-MSD	NA	1.0160	NA	83.43	50	854	A010917017	290% *	294%	3%
Group 1	I05505M	NA	1.0160	NA	66.07	50	3251	A010917018	3.25		
	I05523M	NA	1.0650	NA	82.38	50	3868	A010917019	3.87	3.56	0.436
	I05539F	NA	1.0155	NA	21.68	500	10675	A010913020	10.7		
	I05552F	NA	1.0664	NA	16.61	500	7788	A010913021	7.79	9.23	2.04

PFOS = Perfluorooctanesulfonate
* Matrix spikes weren't spiked at the appropriate level. Sample will be re-spiked, at the appropriate levels, at a later date.

Date Entered/Analyst: 09/12/01, 09/26/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Attachments
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	10/02/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	10/04/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	10/05/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	PFOS Calc. Conc. ug/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	0.00	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	0.00	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	0.00	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	0.00	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	4364	55%		
	MKL100201-2 ug/g-I05505M-MSD	3554	15%	35%	112%
	MKL100201-10 ug/g-I05505M-MS	9441	60%		
	MKL100201-10 ug/g-I05505M-MSD	5761	<LOQ (0.0126 ug/g)	60%	NA
Group 1	I05505M	3237	3.24		

PFOS = Perfluorooctanesulfonate
@ 1:500 dilution was too dilute, these extracts were re-diluted at 1:50 and analyzed 10/16/01. LAC 11/28/01

Date Entered/Analyst: 10/11/01 LAC
Date Verified/Analyst: 12/10/01 mmh

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Attachments
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	10/02/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	10/16/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	10/16/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	PFOS Calc. Conc. ug/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	0.00	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	0.00	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	1.33	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	0.25	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	3804	29%		
	MKL100201-2 ug/g-I05505M-MSD	2995	-11%	9%	438%
	MKL100201-10 ug/g-I05505M-MS	9719	63%		
	MKL100201-10 ug/g-I05505M-MSD	7466	41%	52%	42%
Group 1	I05505M	3215	3.21		

PFOS = Perfluorooctanesulfonate
NOTE: Data were not within criteria, all diluted samples were rediluted 1:50 from original extract and analyzed on 10/24/01. LAC 11/28/01

Date Entered/Analyst: 10/29/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Attachments
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	10/02/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	10/24/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	10/26/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	0.00	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	0.00	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	0.00	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	0.00	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	14945	178%		
	MKL100201-2 ug/g-I05505M-MSD	12530	61%	119%	98%
	MKL100201-10 ug/g-I05505M-MS	38873	268%		
	MKL100201-10 ug/g-I05505M-MSD	33522	216%	242%	21%
Group 1	I05505M	11282	11.28		

PFOS = Perfluorooctanesulfonate

Date Entered/Analyst: 11/06/01 LAC
Date Verified/Analyst: 12/10/01 mmh

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Madeline041098	Filename:	See Attachments
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	11/07/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	11/15/01, 11/16/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	11/16/01, 11/19/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	PFOS Calc. Conc. ug/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL110701-H2O Blk-1	0.00	<LOQ (0.00629 ug/g)		
	RBL110701-H2O Blk-2	0.00	<LOQ (0.00629 ug/g)		
	RBL110701-H2O Blk-3	0.00	<LOQ (0.00629 ug/g)		0
	RBL110701-H2O Blk-4	0.00	<LOQ (0.00629 ug/g)	<LOQ	NA
Matrix Blk	RBL110701-Liver Blk-1	0.00	<LOQ (0.00629 ug/g)		
	RBL110701-Liver Blk-2	0.00	<LOQ (0.00629 ug/g)		
	RBL110701-Liver Blk-3	0.00	<LOQ (0.00629 ug/g)		0
	RBL110701-Liver Blk-4	0.00	<LOQ (0.00629 ug/g)	<LOQ	NA
QC	MKL110701-2 ug/g-I05505M-MS	6328.44	100%		
	MKL110701-2 ug/g-I05505M-MSD	6134.39	90%	95%	10%
	MKL110701-10 ug/g-I05505M-MS	11836.78	73%		
	MKL110701-10 ug/g-I05505M-MSD	10657.49	61%	67%	17%
Group 1	I05505M	4241.25	4.24		

PFOS = Perfluorooctanesulfonate

Date Entered/Analyst: 11/26/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295 22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-5.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Below
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	10/02/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	10/04/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	10/05/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	NA	1.0000	NA	0.00	1	0.00	A011004030	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	NA	1.0000	NA	0.00	1	0.00	A011004031	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	NA	1.0000	NA	0.00	1	0.00	A011004032	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	NA	1.0000	NA	0.00	1	0.00	A011004033	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	NA	1.0232	NA	89.30	50	4364	A011004038	55%		
	MKL100201-2 ug/g-I05505M-MSD	NA	1.0232	NA	72.72	50	3554	A011004039	15%	35%	112%
	MKL100201-10 ug/g-I05505M-MS	NA	1.0232	NA	19.32	500	9441	A011004040	60%		
	MKL100201-10 ug/g-I05505M-MSD	NA	1.0232	NA	11.79	500	5761	A011004041	<LOQ (0.0126 ug/g)	60%	NA
Group 1	I05505M	NA	1.0232	NA	66.24	50	3237	A011004037	3.24		

PFOS = Perfluorooctanesulfonate
* 1:500 dilution was too dilute, these extracts were re-diluted at 1:50 and analyzed 10/16/01. LAC 11/28/01

Date Entered/Analyst: 10/11/01 LAC
Date Verified/Analyst: 12/10/01 mmh

Study: FACT-TOX-160, E00-1668	Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys		
Product Number(Test Substance):	T-6295.22		
Matrix:	Monkey Liver		
Method/Revision:	ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve		
Analytical Equipment System Number:	Amelia 062498	Filename:	See Below
Instrument Software/Version:	Masslynx 3.4	R-Squared Value:	See Attachments
Date of Extraction/Analyst:	10/02/01 RWW	Slope:	See Attachments
Date of Analysis/Analyst:	10/16/01 MMH	Y-Intercept:	See Attachments
Date of Data Reduction/Analyst:	10/16/01 MMH	Box #:	01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	NA	1.0000	NA	0.00	1	0.00	A011016030	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	NA	1.0000	NA	0.00	1	0.00	A011016031	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	NA	1.0000	NA	1.33	1	1.33	A011016032	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	NA	1.0000	NA	0.25	1	0.25	A011016033	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	NA	1.0232	NA	77.84	50	3804	A011016038	29%		
	MKL100201-2 ug/g-I05505M-MSD	NA	1.0232	NA	61.29	50	2995	A011016039	-11%	9%	438%
	MKL100201-10 ug/g-I05505M-MS	NA	1.0232	NA	198.88	50	9719	A011016040	63%		
	MKL100201-10 ug/g-I05505M-MSD	NA	1.0232	NA	152.79	50	7466	A011016041	41%	52%	42%
Group 1	I05505M	NA	1.0232	NA	65.79	50	3215	A011016037	3.21		

PFOS = Perfluorooctanesulfonate
NOTE: Data were not within criteria, all diluted samples were redilluted 1:5) from original extract and analyzed on 10/24/01. LAC 11/28/01

Date Entered/Analyst: 10/29/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

Study: FACT-TOX-160, E00-1668 — Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance): T-6295.22
Matrix: Monkey Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve
Analytical Equipment System Number: Amelia 062498 — Filename: See Below
Instrument Software/Version: Masslynx 3.4 — R-Squared Value: See Attachments
Date of Extraction/Analyst: 10/02/01 RWW — Slope: See Attachments
Date of Analysis/Analyst: 10/24/01 MMH — Y-Intercept: See Attachments
Date of Data Reduction/Analyst: 10/26/01 MMH — Box #: 01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL100201-H2O Blk-3	Out High	1.0000	NA	0.00	1	0.00	A011024030	<LOQ (0.0126 ug/g)		
	RBL100201-H2O Blk-4	Out High	1.0000	NA	0.00	1	0.00	A011024031	<LOQ (0.0126 ug/g)	<LOQ	NA
Matrix Blk	RBL100201-Liver Blk-1	NA	1.0000	NA	0.00	1	0.00	A011024032	<LOQ (0.0126 ug/g)		
	RBL100201-Liver Blk-2	NA	1.0000	NA	0.00	1	0.00	A011024033	<LOQ (0.0126 ug/g)	<LOQ	NA
QC	MKL100201-2 ug/g-I05505M-MS	Out High	1.0232	NA	305.83	50	14945	A011024038	178%		
	MKL100201-2 ug/g-I05505M-MSD	Out High	1.0232	NA	256.41	50	12530	A011024039	61%	119%	98%
	MKL100201-10 ug/g-I05505M-MS	Out High	1.0232	NA	795.50	50	38873	A011024040	268%		
	MKL100201-10 ug/g-I05505M-MSD	Out High	1.0232	NA	686.00	50	33522	A011024041	216%	242%	21%
Group 1	I05505M	Out High	1.0232	NA	230.87	50	11282	A011024037	11.3		

PFOS = Perfluorooctanesulfonate
NOTE: Data were not consistent with previous analyses. Samples were re-extracted, diluted 1:50, and analyzed on 11/16/01. LAC 11/28/01

Date Entered/Analyst: 11/06/01 LAC
Date Verified/Analyst: 12/10/01 mmh

Study: FACT-TOX-160, E00-1668 — Extended Recovery Study Following a 26-Week Capsule Toxicity Stdy with Perfluorooctane Sulfonic Acid Potassium Salt(PFOS; T-6295) in Cynomolgus Monkeys
Product Number(Test Substance): T-6295.22
Matrix: Monkey Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0 versus an extracted rabbit liver curve
Analytical Equipment System Number: Madeline041098 — Filename: See Below
Instrument Software/Version: Masslynx 3.4 — R-Squared Value: See Attachments
Date of Extraction/Analyst: 11/07/01 RWW — Slope: See Attachments
Date of Analysis/Analyst: 11/15/01, 11/16/01 MMH — Y-Intercept: See Attachments
Date of Data Reduction/Analyst: 11/16/01, 11/19/01 MMH — Box #: 01-046

Sample Data

MONKEY LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL110701-H2O Blk-1	NS	1.0000	NA	0.00	1	0.00	M011116031	<LOQ (0.00629 ug/g)		
	RBL110701-H2O Blk-2	NS	1.0000	NA	0.00	1	0.00	M011116032	<LOQ (0.00629 ug/g)		
	RBL110701-H2O Blk-3	NA	1.0000	NA	0.00	1	0.00	M011116033	<LOQ (0.00629 ug/g)		
	RBL110701-H2O Blk-4	NA	1.0000	NA	0.00	1	0.00	M011116034	<LOQ (0.00629 ug/g)	<LOQ	NA
Matrix Blk	RBL110701-Liver Blk-1	NS	0.9425	NA	0.00	1	0.00	M011116038	<LOQ (0.00629 ug/g)		
	RBL110701-Liver Blk-2	NS	0.9425	NA	0.00	1	0.00	M011116039	<LOQ (0.00629 ug/g)		
	RBL110701-Liver Blk-3	NA	0.9425	NA	0.00	1	0.00	M011116040	<LOQ (0.00629 ug/g)		
	RBL110701-Liver Blk-4	NA	0.9425	NA	0.00	1	0.00	M011116041	<LOQ (0.00629 ug/g)	<LOQ	NA
QC	MKL110701-2 ug/g-I05505M-MS	NA	1.0023	NA	126.86	50	6328	M011116046	100%		
	MKL110701-2 ug/g-I05505M-MSD	NA	1.0023	NA	122.97	50	6134	M011116047	90%	95%	10%
	MKL110701-10 ug/g-I05505M-MS	NA	1.0023	NA	237.28	50	11837	M011116048	73%		
	MKL110701-10 ug/g-I05505M-MSD	NA	1.0023	NA	213.64	50	10657	M011116049	61%	67%	17%
Group 1	I05505M	NA	1.0023	NA	85.02	50	4241	M011116045	4.24		

PFOS = Perfluorooctanesulfonate
NS = Not Spiked with Surrogate
NOTE: Average recovery of the 10 ug/g matrix spikes was not within the +/- 30% recovery as listed in the protocol. A deviation will be written.

Date Entered/Analyst: 11/26/01 LAC
Date Verified/Analyst: 12/10/01 mmh

LAC 04/25/02

3M Medical Department Study: T-6295.22
Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-160

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

Calculation Used for Group 1, Week 17, Animal ID 105505M

$$45.75\ \text{ng/mL} \times 200 \times \frac{1.0\ \text{mL}}{0.63\ \text{mL}} \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{14.5 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted

Formula Used for Liver Analyses in Study FACT TOX-160

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

(1) ∂ curve is assumed to be: $\frac{1\ \text{g liver}}{5\ \text{mL}\ H_2O}$

Calculation Used for Group1, Week 53, Animal ID 105505M

$$66.07\ \text{ng/g} \times \frac{1\ \text{g/}\ 5\ \text{mL}}{1.0160\ \text{g/}\ 5\text{mL}} \times 50 \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{3.25 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (g sample/ 5 mL H_2O)
DF— Dilution factor

3M Medical Department Study: T-6295.22
Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix G: Interim Certificate(s) of Analysis

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801 www.centrelab.com
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Exact Copy of Original
LAC 04/24/02
Initial Date

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 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.91 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 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Revision 3

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/06

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/06

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.905%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.07%
Purity = 100% - 13.07% = 86.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur 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	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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INTERIM CERTIFICATE OF ANALYSIS

Revision 3

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 result from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average result from the C6 and C8 standard curves.

Prepared By: Chas Simons 10/11/01
Charles Simons Date
Scientist, Centre Analytical Laboratories

Reviewed By: John M Flaherty 10/11/01
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

3M ENVIRONMENTAL LABORATORY

Note to File

Project or Study Number: FACT-TCR-001

Associated Study Number: LIMS # E00-1682

The expiration dates for PFOS lots 171 and 217 (SD009 and SD018) may be extended 5 years (08/31/06) as stability was demonstrated by studies W1878 (hydrolysis), W2775 (photolysis), and E01-0444 (biodegradation).

LAC 08/03/01

Recorded By: Lisa Clemen Lisa A Clemen

Date 08/03/01 08/03/01

Form ETS-4-15.0

Exact Copy of Original
LAC 10/19/01
Initial Date

3M Medical Department Study: T-6295.22

Analytical Report: FACT TOX-160
LIMS E00-1668

Appendix H: Report Signature Page

Andrew Seacat — May 3, 2002

Andrew Seacat, Ph.D., *Study Director* — Date

John T. Butenhoff — May 3, 2002

John Butenhoff, Ph.D., *Sponsor Representative* — Date

Lisa A. Clemen — 05/01/02

Lisa Clemen, *Principal Analytical Investigator* — Date

05/01/02

William Reagen, Ph.D., *Analytical Laboratory Manager* — Date