Study Title

Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

Analytical Laboratory Report Title

Determination of the Concentration of Potassium Perfluorooctanesulfonate (PFOS) Fluorochemical in Rabbit Liver and Serum Specimens

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Analytical Phase Completion Date

at signing

Performing Laboratories

Liver and Serum Extractions

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

Liver and Serum Analyses

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

Project Identification

3M Medical Department Study: T-6295.10
Argus In-Life Study: #418-012
Analytical Report: FACT TOX-099
3M Environmental Laboratory Request No. U2400

Total Number of Pages

127

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Concentration of Potassium Perfluorooctanesulfonate (PFOS) Fluorochemical in Rabbit Liver and Serum Specimens

Study Identification Numbers: T-6295.10, FACT TOX-099, LRN-U2400

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

Exceptions to GLP compliance:

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase was considered to end at the generation and shipment of specimens. The analytical study was considered to start at the receipt of these specimens for analysis. This resulted in having two separate study directors, one for each phase of the same study. However, since the technical performance of each phase was entirely separate, no effect is expected from this exception.
- There is a lack of an electronic data audit trail.
- There is no reference standard stability.
- The surrogate THPFOS was not characterized.
- On at least one occasion, data entries were not signed and dated on the date of entry

Marvin T Case — 9 Feb 2001

Marvin T. Case, D.V.M., Ph.D., *Study Director* — Date

John L. Butenhoff — 02/08/01

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

Kris J. Hansen — 02/08/01

Kris J. Hansen, Ph.D., *Analytical Investigator* — Date

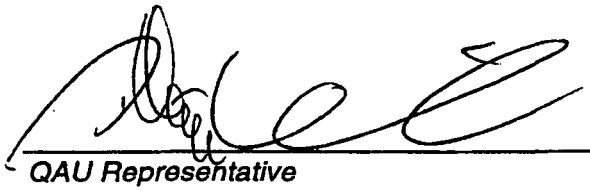
GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Concentration of Potassium Perfluorooctanesulfonate (PFOS) Fluorochemical in Rabbit Serum and Liver Specimens

Study Identification Numbers: T-6295.10, FACT TOX-099, LRN U2400, Argus 418-012

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
10/16/00	Liver Extraction	10/20/00	10/20/00
11/08/00–11/10/00	Data	11/10/00	11/10/00
12/11/00–12/15/00	Draft report	12/15/00	12/15/00
12/20/00–12/21/00 1/11/01–1/12/01, 1/15/01, 1/18/01–1/19/01	Data/Draft Report	1/22/01	1/22/01



QAU Representative

2/08/01

Date

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Study Personnel and Contributors

Study Director
Marvin T. Case, D.V.M. Ph.D.
3M Corporate Toxicology
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000
(651)733-5180

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

John L. Butenhoff, Ph.D., *Sponsor Representative*

Analytical Chemistry Laboratories
Serum and Liver Extractions
Pace Analytical Services, Tier2 Division

Serum and Liver Analyses
3M Environmental Laboratory
Kris J. Hansen, Ph.D., *Principal Analytical Investigator*

3M Environmental Laboratory Contributing Personnel

Lisa A. Clemen
Rhonda Dick*
Kelly J. Dorweiler*
Sarah A. Heimdal*
Marlene M. Heying*
Harold O. Johnson
Kelly J. Kuehlwein*
Sally A. Linda*
Bob W. Wynne*
Richard Youngblom*

*Denotes contract laboratory professional personnel

Location of Archives

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

Introduction and Purpose

The purpose of the study is to determine the concentration of perfluorooctanesulfonate (PFOS) fluorochemical in rabbit liver and serum specimens taken from the Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits. The analytical phase of this study was initiated on 10 June 1999 (date of protocol signing).

Test System

Five groups of female pregnant rabbits (a total of 110 rabbits) were used as the test system for the main part of the study. Additional female pregnant rabbits (a total of 19 rabbits) were assigned to one of the same five dosage groups for the satellite study. It is only the satellite study that provided toxicokinetic samples that were tested and reported here. The test article, PFOS, was administered orally via a stomach tube once daily to female rabbits on days 7 through 20 of presumed gestation (DG7 through DG20). The carrier for the test article was 0.5% Tween® 80 in Reverse Osmosis Membrane Processed Deionized Water. Doses of 0 (carrier only), 0.1, 1.0, 2.5 and 3.75 mg/kg/day were administered at a dosage volume of 5 mL/kg, adjusted daily on the basis of weight.

The test system species and strain selected was the New Zealand White Rabbits [Hra:NZW)SPF)] received from Covance Research Products, Inc., permanently identified using a Monel® self-piercing ear tag. F0 female rabbits were approximately 5 months of age and weighed approximately 2.84–4.21 kg when received. There were 133 female rabbits initially acclimated. The quantity actually used for the study is given in Table 1. The rabbits in the main portion of the study were sacrificed and a gross necropsy of maternal rabbit tissues and fetuses was performed. See the Argus Final Report #418-012 for results. Toxicokinetic samples for laboratory analysis at 3M Environmental Laboratory were only obtained from the satellite study portion.

Table 1. Rabbit Population Demographics for Study (Argus 418-012)

Population	Selected for Main Study	Selected for Satellite Study
Totals	110	19
Group I (Control) 0 mg/kg/day 0 mg/mL	22	3
Group II 0.1 mg/kg/day 0.02 mg/mL	22	5
Group III 1.0 mg/kg/day 0.2 mg/mL	22	3
Group IV 2.5 mg/kg/day 0.5 mg/mL	22	3
Group V 3.75 mg/kg/day 0.75 mg/mL	22	5

Specimen Collection and Analysis

In the analytical study reported here, 19 maternal serum and 19 maternal liver specimens collected from rabbits on DG 21 were sent to the 3M Environmental Laboratory and analyzed for PFOS. In addition, 18 fetuses and 18 placentas were collected on DG21 from sacrificed rabbits. One doe was not pregnant at the time of sacrifice; therefore, no fetuses or placenta were collected from this rabbit. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Satellite Study Groups 1 through 5 (through 9/10/98):
Maternal Serum Specimens—19 specimens
Maternal Liver Specimens—19 specimens
Fetuses—18 specimens
Placentas—18 specimens

Blood specimens were collected and centrifuged. Serum was then harvested and frozen at -60°C to -78°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were frozen and then stored at -60°C to -78°C until shipped to the 3M Environmental Laboratory. Fetuses and placentas were pooled per litter and stored frozen at -60°C to -78°C until shipped to the 3M Environmental Laboratory. Liver, sera, fetuses and placenta specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice. (Note: Although fetal and placenta tissues were collected at the same time as liver and serum, results from these analyses will not be included in this report. A separate report may be issued for fetal tissue data.)

Sera and liver samples were extracted beginning on 16 October 2000 using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to the extraction procedure. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external calibration. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received serum, liver, fetuses and placenta specimens collected at DG21 of the *in-life* phase of this study FACT TOX-099 on 15 September 1998 from Argus Research Laboratories. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-099 were obtained from commercial sources and are presented in Appendix A (see Table 4). Samples analyzed at the 3M Environmental Laboratory will be maintained for a maximum period of 10 years and will be stored at the laboratory at -20°C ±10°C.

Chemical Characterization of Reference Standard

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 standard, potassium perfluorooctanesulfonate, CAS #2795-39-3, is presented in Table 2 below.

Table 2. Characterization of the Analytical Reference Standard in Study FACT TOX-099

Location	3M Environmental Laboratory
Analytical Reference Standard	Potassium Perfluorooctanesulfonate (KPFOS) SD009
Source	3M Specialty Chemicals
Expiration Date	1/01/2010
Storage Conditions	Ambient temperature*
Chemical Lot Number	171
Physical Description	Light-colored powder
Purity	86.4%

*Kept at ambient until 5/16/00, then moved to cold storage (–10°C)

Method Summaries

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

As the present study progressed, more advanced methods evolved and earlier methods listed in the protocol were not used. Amendments to the protocol were written to cover method changes. These changes only improved the effectiveness of the method. Protocol, protocol amendments and deviations are included in Appendix B.

3M Environmental Laboratory

PREPARATORY METHODS

- **ETS-8-4,** "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry"

Serum samples were 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 MtBE 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, then filtered with a 0.2 µm nylon filter attached to a 3 mL plastic syringe into glass autovials.

- **ETS-8-6,** "Extraction of Potassium Perfluorooctanesulfonate 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,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

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

  The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of 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 analytical equipment settings used in the present analytical phase of this study varied slightly during data collection. 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: Ambient
Mobile phase components:
Component A: 2 mM ammonium acetate
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 10.0 minutes

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

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.4
Cone Voltage: 60 V
Collision Gas Energy: 45–50 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored in 3M Environmental Laboratory Analyses

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

Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Amendments to the protocol were written to address method changes.

Deviations from the original protocol and methods are included in 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 0.98
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve and is at least two times the analyte peak area detected in the extraction blanks.
- **Acceptable Precision:** Precision is better than 30% for the method
- **Acceptable Spike Recoveries:** 70–130%
- **Confirmatory Methods:** None.
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and mass spectral daughter ion characterization.

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-099 (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 ≥0.98.
- **Calibration Standards:** Quantitation of PFOS was based on linear regression analysis (1/x weighted) of a single curve injected before or two extracted matrix curves bracketing, each group of samples. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of PFOS* was based on the response of one specific product ion using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).

  *Isomers of PFOS were not chromatographically resolved. Quantitation was based on the sum of linear and all branched isomers.

- **Limits of Quantitation (LOQ):** The LOQ was equal to 0.00426 µg/mL and 0.0214 µg/mL in sera and 0.00529 µg/g and 0.0529 µg/g in liver. See Table 6 for LOQs on specific analytical dates.

- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest.
- **Precision:** Precision was determined by triplicate analysis of one extracted liver sample injected three times and two sera samples that were reproducible to within 5%.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs at the 3M Environmental Laboratory. Samples were spiked prior to extraction. Rabbit serum and liver samples for MS studies were obtained from commercial sources. After homogenation (liver) and prior to extraction, tissue samples were spiked with PFOS at approximately 250 ng/mL or 250 ng/g. All sera matrix spikes were within ±10% of the theoretical concentration. Matrix spikes prepared in liver were compliant within ±20% with one exception; one liver matrix spike was recovered at 156%. The average matrix spike recovery for the Group 1 samples was 138%.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards prior to extraction. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. The surrogate response of each analytical run was monitored to determine that it did not vary more than ±50% from the mean within each analytical run. No problems were observed with these data.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to approximately ±30%; values for liver analysis may be biased high.

Summary of Sample Results (3M Environmental Laboratory)

- **Samples from Control Animals:** PFOS was detected in the sera and liver of all animals.
- **Samples from Dosed Animals:** In general, PFOS levels found in the sera and liver of the test animals increased with dose group. PFOS levels increased as dosage increased. Detailed sample data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix F for example calculations used to generate the liver and serum sample data in FACT TOX-099.

Statement of Conclusion (3M Environmental Laboratory)

Under the conditions of the present studies, the fluorochemical PFOS was observed in the liver and serum of all rabbits dosed with the test article during the in-life phase of the study.

References

Argus In-Life Final Report #418-012, "Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits"

Appendix A: Chemical Characterization and Control Matrices

Table 4. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT TOX-099

Control Matrix	Rabbit Serum TN-A-4511	Rabbit Liver TCR-99131-45
Source	Sigma Chemicals	Covance Labs
Expiration Date	9/2005	N/R
Storage Conditions	Frozen -20°C (±10°C)	Frozen -20°C (±10°C)
Chemical Lot #	99H8400	F04051
Physical Description	Rabbit Serum	Rabbit Liver

N/R—not recorded

Table 5. Characterization of Test Article

	Test Article[a]
Chemical Name	**KPFOS** (Potassium Perfluorooctanesulfonate)
Source	3M Toxicology Services, Medical Department
Expiration Date	5/2000
Storage Conditions	Room temperature
Chemical Lot #	217
Physical Description	Off-white powder
Purity	86.9%

[a] The test article is defined as the substance gavaged to the test system.

Appendix B: Protocol, Protocol Amendments, Deviations

3M Environmental Technology and Services
PO Box 33331
St. Paul, MN 55133-3331
612 778 6442

Protocol #FACT-TOX-099

3M

Study Title

Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

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

Laboratory Project Identification
FACT-TOX-099
U2400

Study Identification

Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Telephone: 651-733-5180 Facsimile: 651-733-1773
Study Director	Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

Sub-Contract Laboratory	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850
	Battelle Memorial Institute 505 King Avenue Columbus, Ohio 43201-2693

Proposed Study Timetable

Analytical Start Date	June 08, 1999
Analytical Completion Date	August 08, 2000

1. STUDY

Oral (stomach tube) developmental toxicity study of PFOS in rabbits

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rabbits. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-012. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. . If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Food and Drug Administration, Good Laboratory Practices Standards, Final Rule 21 CFR 58, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

4. QUALITY ASSURANCE

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

The QA Unit at the sub-contract laboratory will audit their study conduct, data, and results report prior to submitting to the 3M Environmental Laboratory.

5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-012.

6. CONTROL MATRICES

6.1 *Identification* Rabbit liver and serum, traceability numbers will be recorded in the raw data and included in the final report

6.2 *Source* Argus Reseacrh Laboratories and/or Sigma Chemical

6.3 *Physical Description* Rabbit liver and serum

6.4 *Purity and Stability* Not applicable

6.5 *Storage Conditions* Frozen at -20 °C ± 10 °C or –50 °C ± 10 °C

6.6 *Reserve Matrix* A portion of the control matrix will be retained in the 3M archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 *Disposition* Matrices will be retained at the 3M Environmental Laboratory per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

6.8 *Safety Precautions* Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

7. REFERENCE MATERIAL

7.1 *Identification* Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

7.2 *Source* 3M Specialty Chemicals

7.3 *Physical Description* White powder

7.4 *Purity and Stability* Purity = 99%, Stability to be determined by the Sponsor

7.5 *Storage Conditions* Room temperature

7.6 *Reserve Material* A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

7.7 *Disposition* Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

7.8 *Safety Precautions* Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

8. TEST SYSTEM

Rabbits were used as the test system and were maintained and dosed as described in ArgusLaboratories protocol #418-012. The rabbits will be given the test material or control once daily on days 7 through 20 of presumed gestation. See table 1 for dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of rabbits	Dosage mg/kg/day	Concentration mg/mL	Dosage Volume mL/kg
1	22+3[a]	0.0	0.0	5
2	22+5[a]	0.1	0.02	5
3	22+3[a]	1.0	0.2	5
4	22+3[a]	2.5	0.5	5
5	22+5[a]	3.75	0.75	5

[a] Rabbits assigned to toxicokinetic evaluation.

9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory will receive specimens of the following body tissues and fluids from the indicated points in the study. All specimens will be packed on dry ice for shipping. See table 2 for specimen information.

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam animals	Toxicokinetic Collection – Day 21	19
Liver – Dam animals	Toxicokinetic Collection – Day 21	19
Fetus – Pooled litter animals	Toxicokinetic Collection – Day 21	19
Placenta – Pooled litter animals	Toxicokinetic Collection – Day 21	19

Total number of expected specimens: 76
Total number of test animals: 104
Total number of control animals: 25

Specimens sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

10. PREPARATORY METHODS

10.1 FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

10.2 ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid 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.

10.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

11. ANALYTICAL METHODS

11.1 FACT-M-2.1, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

11.2 ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid 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.

11.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

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 $r^2 \geq 0.98$

12.2 Limits of detection / quantitation

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

12.2.2 Limit of Quantitation (LOQ) – Equal to the lowest acceptable standard in the calibration curve

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

12.5 Use of confirmatory methods Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

12.6 Demonstration of specificity Chromatographic retention time, mass spectral daughter ion characterization.

13. Sub-Contracted Analysis

13.1 All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106, at Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, or at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than 3M Environmental Laboratories, Advanced Bioanalytical Services, Inc., or Battelle Memorial Institute.

14. Statistical Analysis

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

14.1 Data transformations and analysis Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

14.2 Statistical analysis Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

15. Report

A report containing all the results of the study will be prepared by the 3M Environmental Laboratory. If analyses are sub-contracted to other laboratories, each laboratory will prepare a report and submit it to the 3M Environmental Laboratory for inclusion in the 3M Environmental Laboratory report. Each report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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

15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol

15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor

15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor

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

15.8 A description of the test system

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

15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study

15.11 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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

15.15 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 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 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 the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

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 in the archives 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 will be maintained in the 3M Environmental Laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

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 filed with the raw data.

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T. Case 9 June 1999

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

Kristen H 6/10/99

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director Date

Study Title
Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 04, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX099
LIRN U2400

3M Environmental Laboratory

Protocol FACT-TOX099
Amendment No. 1

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

1. ***PROTOCOL READS:*** The sub-contract laboratory assigned to extract and analyze the rabbit serum specimens is listed as:

   Advanced Bioanalytical Services, Inc. (ABS)
   15 Catherwood Road
   Ithaca, NY 14850

AMEND TO READ: The extraction and analysis of the rabbit serum specimens will be sub-contracted to:

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414

Note: The serum specimens were sent to ABS on 6/15/99 and will be shipped directly from ABS to Pace Analytical Services in c/o Jon Berdahl. The specimens will be shipped on dry ice, overnight and will include the original chain of custody along with all paperwork listing storage conditions while at ABS. Also, Jon Berdahl at (612) 607-6451 will be notified prior to shipping these specimens.

REASON: Since ABS hasn't performed a rabbit serum validation, the contract lab extracting and analyzing the serum specimens was changed to Pace Analytical Services who has completed a rabbit serum validation.

2. ***PROTOCOL READS:*** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"
FACT-M-2.1 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ: The extraction and analytical methods are:

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

3M Environmental Laboratory

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

REASON: The FACT-M-1.1 and FACT-M-2.1, respectively were updated on 7/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

3. ***PROTOCOL READS:*** Sections 10.4 and 11.4 state that if analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

AMEND TO READ: The extraction and analytical methods for rabbit serum at Pace Analytical Services, Inc. are:

CAG-SP-01.0 "Extraction of Potassium Perfluorooctanesulfonate and Other Fluorochemical compounds from Serum for Analysis by LC/MS"

CAG-CHR-01.0 "Analysis of Potassium Perfluorooctanesulfonate and Other Fluorochemicals in Serum Extracts Using LC/MS"

The extraction and analytical methods for rabbit liver at Battelle Memorial Institute are:

"Method for Analysis of Prefluorooctane Sulfonate (PFOS) in Rat Liver by LC/MS/MS, Version 1.0"

REASON: The methods listed previously were for extraction and analysis performed at the 3M Environmental Laboratory. This amendment was written to include the methods to be followed at the Pace Analytical Services, Inc laboratory and Battelle Memorial Institute.

4. ***PROTOCOL READS:*** Section 12.2.1 b) lists liver method detection limit as 15 ppb.

AMEND TO READ: The liver method detection limit is 8.50 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 included a lower method detection limit for PFOS.

Protocol FACT-TOX099
Amendment No. 1

Amendment Approval

Marvin Case 10 August 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative Date

Kris Hansen 8/11/99
Kris J. Hansen, Ph.D., Study Director Date

3M Environmental Laboratory

Study Title

Oral (Stomach Tube) Developmental Toxicity Study of PFOS in Rabbits

PROTOCOL AMENDMENT NO. 2

Amendment Date:

20 January 2000

Performing Laboratories

Liver Analyses	Serum Analyses
Battelle Memorial Institute	Pace Analytical Services, Inc.
505 King Avenue	1700 Elm Street, Suite 200
Columbus, OH 43201-2693	Minneapolis, MN 55414

Laboratory Project Identification

ET&SS LRN-U2400
FACT TOX-099
Argus Study: 418-012
3M Medical Department Study: T-6295.10

3M Environmental Laboratory

Protocol LRN-U2400
Amendment Number 2

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

1. ***PROTOCOL READS:***
The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D..
AMEND TO READ:
The role of study director for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of 20 January 2000. The previous study director, Kristen Hansen, has been reassigned to the role of Principle Analytical Investigator.
REASON:
The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58.

2. ***PROTOCOL READS:***
The sponsor for the present study was identified as Marvin T. Case, D.V.M., Ph.D.
AMEND TO READ:
The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.
REASON:
To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

3. ***PROTOCOL READS:***
17. Specimen Retention:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.
AMEND TO READ:
17. Specimen Retention:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Any specimens sent to sub-contract laboratories will be returned to the 3M Environ- mental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. Specimens analyzed at sub-contract laboratories will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.
REASON:
To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

3M Environmental Laboratory

4. ***PROTOCOL READS:***
Section 16 states that the following raw data and records will be retained in the study folder in the archives according to AMDT-S-8: Approved protocol and amendments; study correspondence; shipping records; raw data; approved final report (original signed copy); and electronic copies of data. Additionally, Section 16 states that supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following: Training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.
AMEND TO READ:
Section 16 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.
REASON:
To direct subcontract laboratories in the disposition of the items listed above.

5. ***PROTOCOL READS:***
2. Purpose
This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rabbits.
AMEND TO READ:
2. Purpose
This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver, serum, and fetal tissues of rabbits.
REASON:
The study sponsor requested the addition of fetal tissue analyses.

Note: At the time that this amendment was produced, a method for the determination of PFOS in fetal tissue is under development. When the method is approved and validated, another amendment will be written to address the use of the additional method.

Protocol LRN-U2400
Amendment Number 2

Amendment Approval

John L. Butenhoff February 10, 2000
John L. Butenhoff, Ph.D., Sponsor Representative Date

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

Marvin T Case 10 February 2000
Marvin T. Case, D.V.M., Ph.D., Incoming Study Director Date

3M Environmental Laboratory

Study Title

Oral (Stomach Tube) Development Toxicity Study of PFOS in Rabbits

PROTOCOL AMENDMENT NO. 3

Amendment Date:

August 16, 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX-099
U2400

3M Environmental Laboratory

Protocol FACT-TOX-099
Amendment #3

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

1. ***PROTOCOL READS:***
3M FACT TOX-099 PROTOCOL: Sub-Contract Laboratory heading under Study Location lists Battelle Memorial Institute.
2. PURPOSE—All liver samples will be extracted and analyzed at Battelle Memorial Institute.
13. SUB-CONTRACTED ANALYSIS—All analyses as detailed in this protocol will be performed at 3M Environmental Laboratory, Advanced Bioanalytical Services, or Battelle Memorial Institute.

AMEND TO READ:
Remove Battelle Memorial Institute as a subcontract laboratory under the Study Location heading.
2. Change to state that all liver samples will be extracted and analyzed at 3M Environmental Laboratory.
13. Change to state that all analyses as detailed in this protocol will be performed at 3M Environmental Laboratory or Advanced Bioanalytical Services.

REASON:
3M Environmental Laboratory is able to meet the analytical needs of the study.

2. ***PROTOCOL READS:***
3M FACT TOX-099 PROTOCOL:
10. PREPARATORY METHODS & 11. ANALYTICAL METHODS—The amended protocol states the liver methods are ETS-8-6.0 and ETS-8-7.0.

AMEND TO READ:
Change to specify: Use the most current version of the methods listed in both sections.

REASON:
To specify that the most current version of the preparatory and analytical methods should be used during the course of the study.

3M Environmental Laboratory

Protocol FACT-TOX-099
Amendment #3

3. PROTOCOL READS:
The amended ***3M ANALYTICAL PROTOCOL FACT-TOX-099*** (Amendment No. 2) states that Marvin T. Case replaces Kristen J. Hansen as the study director. Kristen J. Hansen was reassigned the role of *Principle* Analytical Investigator. John L. Butenhoff was reassigned the role of Sponsor Representative.

AMEND TO READ:
Kristen J. Hansen was reassigned to the role of *Principal* (corrected spelling) Analytical Investigator.

REASON:
Corrected spelling of *Principal.*

Amendment Approval

John L. Butenhoff 15/Sept/2000
John L. Butenhoff, Ph.D., Sponsor Representative Date

Marvin T Case 7 Sept 2000
Martin T. Case, D.V.M., Ph.D., Study Director Date

Study Title

Oral (Stomach Tube) Development Toxicity Study of PFOS in Rabbits

PROTOCOL AMENDMENT NO. 4

Amendment Date:

September 25, 2000

Performing Laboratory

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

Laboratory Project Identification

Analytical Report: FACT TOX-099
3M Laboratory Request No. U2400

3M Environmental Laboratory

Protocol #FACT TOX-099
Amendment 4

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

1. ***PROTOCOL READS:***
SUB-CONTRACT LABORATORIES: The amended protocol (Amendment No. 1) lists Pace Analytical Services, Inc. as the sub-contract laboratory for the extraction and analysis of rabbit sera specimens.
The amended protocol (Amendment No. 3) states that all liver samples will be extracted and analyzed at 3M Environmental Laboratory.
Section 2. Purpose—The amended protocol states that all serum samples will be extracted and analyzed at Pace Analytical Services, Inc. and all liver samples will be extracted and analyzed at 3M Environmental Laboratory.
SECTION 13. SUB-CONTRACTED ANALYSIS: The amended protocol states that all analyses as detailed in this protocol will be performed at 3M Environmental Laboratories or at Pace Analytical, Inc.

AMEND TO READ:
Page 3, Study Locations, Sub-Contract Laboratories: Add Pace Tier II as a sub-contract laboratory.
2. Purpose: Change to state that all liver and sera samples will be *extracted* at the Pace Tier II facility. All liver and sera samples will be *analyzed* at 3M Environmental Laboratory.
13.1 Sub-Contracted Analysis: Change to say that liver and sera extractions for this protocol will be performed at the Pace Tier II facility. Change to say that all liver and sera analyses as detailed in this protocol will be performed at 3M Environmental Laboratory.

REASON:
Pace Tier II and 3M Environmental Laboratory are able to meet the needs of the study.

2. ***PROTOCOL READS:***
SECTION 13. SUB-CONTRACTED ANALYSIS: No SOPs are listed for the sub-contract laboratory.

AMEND TO READ:
Add to Section 13: Pace Analytical Services, Inc. (Pace—Tier II Facility, Minneapolis, MN) will be performing the extraction of samples for analyses of liver and sera specimens. All extracted samples will be shipped to the 3M Environmental Laboratory for analysis. Pace will follow all applicable 3M Environmental Laboratory Standard Operating Procedures and the following Pace Standard Operating Procedures:

PSS-Admin-01	Quality System
PSS-DC-05	Lab Notebooks, Logbooks, and Phone Logs
PSS-OPS-01	Use and Maintenance of Ultra-Turrax T25 Homogenizer
PSS-OPS-02	Raw Data
PSS-OPS-03	Hazardous Waste Disposal

3M Environmental Laboratory

PSS-OPS-04	Use and Maintenance of N-Evap Analytical Evaporator
PSS-OPS-05	Use of Compressed Gases in the Laboratory
PSS-OPS-06	Use and Maintenance of NANOpure II Water Purification System
PSS-OPS-07	Cleaning Non-disposable Volumetric Pipettes
PSS-OPS-08	Laboratory Calculations
PSS-OPS-13	Use and Maintenance of Lab Refrigerators, Freezers, and Ovens
PSS-OPS-15	Glassware Cleaning
PSS-OPS-16	Use and Maintenance of the Fisher Scientific Isotemp Freezer
PSS-OPS-31	Use and Maintenance of Shakers (Use for Shaker Only)
PSS-OPS-32	Operation, Maintenance and Calibration of Laboratory Centrifuges
ETS-9-40 (3M Lab)	Operation and Maintenance of Shakers and Vortex Mixers (Use for Vortex Mixer Only)
PSS-OPS-37	Operation, Maintenance, and Calibration of pH Meters and pH Electrodes
PSS-MTR-01	Calibration of Certified Weight Set
PSS-MTR-02	Calibration of Certified Thermometers/Thermocouples
PSS-MTR-06	Verification of Calibration of the Eppendorf Pipettor
PSS-MTR-08	Verification of Calibration and Use of Analytical Balances
PSS-MC-01	Purchasing of Laboratory Supplies
PSS-ARC-02	Disposition of Archive Materials
PSS-DM-03	Statistical Evaluation of Data

REASON: All extractions for liver and sera samples will be performed at Pace Tier II, using the above SOPs.

3. ***3. PROTOCOL READS:***
SECTION 13. SUB-CONTRACTED ANALYSIS: No methods are listed for the sub-contract laboratory. Under Preparatory Methods, ETS-8-4.1 is listed as the method for serum extraction. Under Analytical Methods, ETS-8-5.1 is listed as the method for serum analysis. The amended protocol (Amendment No. 1) lists ETS-8-6.0 and ETS-8-7.0 as the methods to use for extraction and analysis of liver samples.

AMEND TO READ:
The following 3M Environmental Laboratory methods will be used for liver and sera extractions and analyses:

ETS-8-4	Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical

Protocol #FACT TOX-099
Amendment 4

	Electrospray/Mass Spectrometry
ETS-8-5	Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry
ETS-8-6	Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
ETS-8-7	Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

REASON:
To clarify the methods to use for extraction and analyses of TOX-099 liver and sera samples.

3M Environmental Laboratory

Protocol #FACT TOX-099
Amendment 4

Amendment Approval

John L Butenhoff October 9, 2000

John L. Butenhoff, Ph.D., Sponsor Representative Date

Marvin T Case 9 October 2000

Marvin T. Case, D.V.M., Ph.D., Study Director Date

3M Environmental Laboratory

Record of Deviation

I. Identification

Study / Project No.
TOX-099 Argus 418-012

Deviation Type (Check one) ☐ SOP ☒ Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number: ETS-8-4.1, ETS-8-6.0

Date(s) of occurrence: 10/16/00

II. Description:

Required Procedure/process:
Type I water is required for GLP Studies.

Actual Procedure/process:
The type of nanopure water used for this study at Tier2 is unknown.

III. Actions Taken:
(such as amendment issued, SOP revision, etc.)

The Tier 2 nanopure water was sent for testing. The results will be added to this study when they are received.

Recorded By: [signature] Date: 10/31/00

IV. Impact on Study / Project

Type 2 water will not adversely affect the study.
kh 11/17/00

Authorized By *(Study Director / Project Lead)* Date

Marvin T Case 21 Nov 2000 / John Z. Butenhoff 4 DEC 00

Sponsor: John Butenhoff Study Director: Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

October 30, 2000

Mr. Wade Scheil
3M Environmental Laboratory
935 Bush Ave.
Bldg. 2-3E-09
St. Paul, MN 55144

RE: Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

Dear Mr. Scheil:

Enclosed are the analytical results for sample(s) received by the laboratory on October 18, 2000. If you have any questions concerning this report, please feel free to contact me.

Sincerely,

Andrea Nemitz
Project Manager

Enclosures

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

DATE: 10/30/00
PAGE: 1

.M Environmental Laboratory
'35 Bush Ave.
.ldg. 2-3E-09
.t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

.ttn: Mr. Wade Scheil
'hone: 612-778-5233

Solid results are reported on a wet weight basis

'ace Sample No: 102339926 Date Collected: 10/18/00 Matrix: Water
:lient Sample ID: TIER II WATER SAMPLE Date Received: 10/18/00

'arameters	Results	Units	PRL	Analyzed	Analyst	CAS#	Footnotes
letals							
Metals, Trace ICP		Method: EPA 6010			Prep Method: EPA 3010		
Sodium	ND	ug/l	1000	10/28/00	BDA	7440-23-5	
Date Digested				10/19/00			
let Chemistry							
Specific Conductance		Method: EPA 120.1			Prep Method: EPA 120.1		
Specific Conductance	2	umhos/cm	1	10/18/00	JM1		
pH, Water		Method: EPA 150.1			Prep Method: EPA 150.1		
pH	5.5		0.1	10/18/00	JM1		
Anions, Ion Chromatography		Method: EPA 300.0			Prep Method: EPA 300.0		
Chloride	ND	mg/l	1	10/24/00	BKR		
Total Organic Carbon		Method: SM 5310			Prep Method: SM 5310		
Total Organic Carbon	ND	mg/l	1	10/20/00	BKR	7440-44-0	

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

DATE: 10/30/00
PAGE: 2

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

ARAMETER FOOTNOTES

D	Not Detected
C	Not Calculable
RL	Pace Reporting Limit

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

QUALITY CONTROL DATA

DATE: 10/30/00
PAGE: 3

M Environmental Laboratory
35 Bush Ave.
dg. 2-3E-09
t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

ttn: Mr. Wade Schell
hone: 612-778-5233

C Batch ID: 51031 QC Batch Method: EPA 120.1
nalysis Method: EPA 120.1 Analysis Description: Specific Conductance
ssociated Pace Samples: 102339926

ETHOD BLANK: 102340106
ssociated Pace Samples:
102339926

Parameter	Units	Method Blank Result	PRL	Footnotes
Specific Conductance	umhos/cm	1	1	

AMPLE DUPLICATE: 102340114

Parameter	Units	102339926	Dup. Result	RPD	Footnotes
Specific Conductance	umhos/cm	2.000	2.000	0	

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

QUALITY CONTROL DATA

DATE: 10/30/00
PAGE: 4

:M Environmental Laboratory
'35 Bush Ave.
.ldg. 2-3E-09
:t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

\ttn: Mr. Wade Scheil
'hone: 612-778-5233

)C Batch ID: 51032
'nalysis Method: EPA 150.1
\ssociated Pace Samples: 102339926

QC Batch Method: EPA 150.1
Analysis Description: pH, Water

LABORATORY CONTROL SAMPLE: 102340130

'arameter	Units	Spike Conc.	LCS Result	Spike % Rec	Footnotes
)H		5.000	5.000	101	

SAMPLE DUPLICATE: 102340122

'arameter	Units	102338563	Dup. Result	RPD	Footnotes
)H		8.200	8.200	0	

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

QUALITY CONTROL DATA

DATE: 10/30/00
PAGE: 5

4 Environmental Laboratory
35 Bush Ave.
Bldg. 2-3E-09
t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

ttn: Mr. Wade Scheil
hone: 612-778-5233

C Batch ID: 51059
nalysis Method: EPA 6010
ssociated Pace Samples: 102339926

QC Batch Method: EPA 3010
Analysis Description: Metals, Trace ICP

ETHOD BLANK: 102340494
ssociated Pace Samples:
102339926

arameter	Units	Method Blank Result	PRL	Footnotes
odium	ug/l	ND	1000	

ATRIX SPIKE & MATRIX SPIKE DUPLICATE: 102340510 102340528

arameter	Units	102339124	Spike Conc.	Matrix Spike Result	Spike % Rec	Matrix Sp. Dup. Result	Spike Dup % Rec	RPD	Footnotes
odium	ug/l	0	50000	61210	122	58410	117	5	1

ABORATORY CONTROL SAMPLE: 102340502

arameter	Units	Spike Conc.	LCS Result	Spike % Rec	Footnotes
odium	ug/l	50000	59230	118	

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

QUALITY CONTROL DATA

DATE: 10/30/00
PAGE: 6

M Environmental Laboratory
35 Bush Ave.
ldg. 2-3E-09
t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

ttn: Mr. Wade Schell
hone: 612-778-5233

QC Batch ID: 51065
nalysis Method: EPA 300.0
ssociated Pace Samples: 102339926

QC Batch Method: EPA 300.0
Analysis Description: Anions, Ion Chromatography

ETHOD BLANK: 102340734
ssociated Pace Samples:
102339926

arameter	Units	Method Blank Result	PRL	Footnotes
hloride	mg/l	1.1	1	

ATRIX SPIKE & MATRIX SPIKE DUPLICATE: 102340742 102340759

arameter	Units	102339710	Spike Conc.	Matrix Spike Result	Spike % Rec	Matrix Sp. Dup. Result	Spike Dup % Rec	RPD	Footnotes
hloride	mg/l	48.68	50	95.38	93.4	93.66	90.0	4	2,2

ABORATORY CONTROL SAMPLE & LCSD: 102340767 102340775

arameter	Units	Spike Conc.	LCS Result	Spike % Rec	LCSD Result	Spike Dup % Rec	RPD	Footnotes
hloride	mg/l	5.000	5.320	106	5.200	104	2	2,2

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
1700 Elm Street, Suite 200
Minneapolis, MN 55414
Phone: 612.607.1700
Fax: 612.607.6444

QUALITY CONTROL DATA

DATE: 10/30/00
PAGE: 7

H Environmental Laboratory
35 Bush Ave.
Bldg. 2-3E-09
t. Paul, MN 55144

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

Attn: Mr. Wade Scheil
Phone: 612-778-5233

QC Batch ID: 51099
Analysis Method: SM 5310
Associated Pace Samples: 102339926

QC Batch Method: SM 5310
Analysis Description: Total Organic Carbon

METHOD BLANK: 102343464
Associated Pace Samples:
102339926

Parameter	Units	Method Blank Result	PRL	Footnotes
Total Organic Carbon	mg/l	ND	1	

MATRIX SPIKE & MATRIX SPIKE DUPLICATE: 102343472 102343480

Parameter	Units	603882358	Spike Conc.	Matrix Spike Result	Spike % Rec	Matrix Sp. Dup. Result	Spike Dup % Rec	RPD	Footnotes
Total Organic Carbon	mg/l	6.320	2.000	8.850	126	8.980	133	5	3,3

LABORATORY CONTROL SAMPLE & LCSD: 102343506 102343514

Parameter	Units	Spike Conc.	LCS Result	Spike % Rec	LCSD Result	Spike Dup % Rec	RPD	Footnotes
Total Organic Carbon	mg/l	5.000	5.280	106	5.290	106	0	

SAMPLE DUPLICATE: 102343498

Parameter	Units	102339926	Dup. Result	RPD	Footnotes
Total Organic Carbon	mg/l	ND	ND	NC	

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PAGE: 8

Pace Project Number: 1037874
Client Project ID: Water Quality Checks-T2

UALITY CONTROL DATA PARAMETER FOOTNOTES

onsistent with EPA guidelines unrounded concentrations are displayed and have been used to calculate % Rec and RPD values.

D	Not Detected
C	Not Calculable
RL	Pace Reporting Limit
PD	Relative Percent Difference
1]	The MS was out of our QC limits but the MSD was acceptable.
2]	Analyte is found in the associated blank as well as in the sample (CLP B-Flag).
3]	The spike recovery was outside acceptance limits for the MS and /or MSD due to matrix interference. The LCS and/or LCSD were within acceptance limits showing that the laboratory is in control and the data is acceptable.

REPORT OF LABORATORY ANALYSIS

Pace Analytical Services, Inc.
9608 Loiret Blvd.
Lenexa, KS 66219
Phone: 913.599.5665
Fax: 913.599.1759

DATE: 10/24/00
PAGE: 1

INNESOTA LABORATORY
ACE ANALYTICAL SERVICES
700 ELM STREET SUITE 200
INNEAPOLIS, MN 55414

Pace Project Number: 6045897
Client Project ID: SCIENCE SOLUTIONS/1037874

ttn: PROJECT MANAGER
hone: (612)544-3435

olid results are reported on a wet weight basis

ace Sample No: 603917352 Date Collected: 10/18/00 Matrix: Water
lient Sample ID: TIER II WATER SAMPLE Date Received: 10/21/00

arameters	Results	Units	PRL	Analyzed	Analyst	CAS#	Footnotes
et Chemistry							
Silica, Total	Method: EPA 375.3				Prep Method: EPA 375.3		
Silica	ND	mg/l	1	10/23/00	HMG	7631-86-9	
Resistivity	Method:				Prep Method:		
Resisitivity	150000	ohms/cm	100	10/22/00	JLC		

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Lenexa, KS 66219
Phone: 913.599.5665
Fax: 913.599.1759

DATE: 10/24/00
PAGE: 2

Pace Project Number: 6045897
Client Project ID: SCIENCE SOLUTIONS/1037874

ARAMETER FOOTNOTES

ID	Not Detected
IC	Not Calculable
RL	Pace Reporting Limit

REPORT OF LABORATORY ANALYSIS

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Phone: 913.599.5665
Fax: 913.599.1759

QUALITY CONTROL DATA

DATE: 10/24/00
PAGE: 3

MINNESOTA LABORATORY
PACE ANALYTICAL SERVICES
1700 ELM STREET SUITE 200
MINNEAPOLIS, MN 55414

Pace Project Number: 6045897
Client Project ID: SCIENCE SOLUTIONS/1037874

Attn: PROJECT MANAGER
Phone: (612)544-3435

QC Batch ID: 93019
Analysis Method: EPA 375.3
Associated Pace Samples: 603917352

QC Batch Method: EPA 375.3
Analysis Description: Silica, Total

METHOD BLANK: 603919127
Associated Pace Samples: 603917352

Parameter	Units	Method Blank Result	PRL	Footnotes
Silica	mg/l	ND	1	

MATRIX SPIKE: 603919135

Parameter	Units	603886300	Spike Conc.	Matrix Spike Result	Spike % Rec	Footnotes
Silica	mg/l	20.91	10	29.12	82.1	

LABORATORY CONTROL SAMPLE: 603919150

Parameter	Units	Spike Conc.	LCS Result	Spike % Rec	Footnotes
Silica	mg/l	10	5.830	58.3	

SAMPLE DUPLICATE: 603919143

Parameter	Units	603886300	Dup. Result	RPD	Footnotes
Silica	mg/l	20.90	19.30	8	

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DATE: 10/24/00
PAGE: 4

Pace Project Number: 6045897
Client Project ID: SCIENCE SOLUTIONS/1037874

ALITY CONTROL DATA PARAMETER FOOTNOTES

nsistent with EPA guidelines unrounded concentrations are displayed and have been used to calculate % Rec and RPD values.

	Not Detected
	Not Calculable
L	Pace Reporting Limit
D	Relative Percent Difference

REPORT OF LABORATORY ANALYSIS

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-099 Argus 418-012

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

Document Number(s): ETS-8-7.0

Date(s) of occurrence: 12/21/00

II. Description:

Required Procedure/process:

Section 14.4.1 states the matrix spike percent recoveries must be within +/- 30% of the spiked concentration.

Actual Procedure/process:

The liver matrix spike (8553F-MS-250 ppb-3) percent recovery was 156% and the mean matrix spike/matrix spike duplicate percent recovery was 138%.

III. Actions Taken:

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

This deviation was written.

Recorded By: Tina A Clemer Date: 01/02/01

IV. Impact on Study / Project

GI extracts may be biased high. A statement will be added to the final report. tjh 01/02/01

Authorized By *(Study Director / Project Lead)*: John T. Butenhoff JTB 10 JAN 2001 Marvin Case 10 Jan 2001 Date

Sponsor Representative: John Butenhoff Study Director: Marvin Case

4a

3M Environmental Laboratory
Form ETS-4-8.0

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-099 Argus 418-012

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

Document Number(s): ETS-8-7.0

Date(s) of occurrence: Entire study

II. Description:

Required Procedure/process:

Section 13.1.6 describes the calculations that should be used to convert extract concentration to matrix concentration.

Actual Procedure/process:

In order to accommodate purity information, the actual calculation used varied somewhat from that written in section 13.1.6. Two additional factors, salt correction and standard purity, were added. The first accommodates the mass difference between the analytical standard (C8F17SO3K) and the target analyte (C8F17SO3-), while the second addresses the purity of the analytical reference material, determined after the study was completed.

III. Actions Taken:

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

This deviation was written.

Recorded By: kjh Date: 12/20/00

IV. Impact on Study / Project

The updated calculations accommodate new information and are an improvement. No adverse affect on the study. kjh 12/20/00

Authorized By *(Study Director / Project Lead)*: John R. Butenhoff 12-22-00 Marvin T Case 27Dec00 Date

Sponsor Representative: John Butenhoff Study Director: Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 3
(assigned by Study Director or Project Lead at the end of study or project)

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

- **ETS-8-4,** "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry," (14 pages)
- **ETS-8-6,** "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry", (14 pages)
- **ETS-8-5,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry" (9 pages)
- **ETS-8-7**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry" (10 pages)

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-4.1 **Adoption Date:** 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

[signature]	4/27/99
Laboratory Manager	Date
[signature]	4/26/99
Group Leader	Date
[signature]	04/26/99
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 serum.

1.2 Applicable compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 SUMMARY OF METHOD

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

2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 DEFINITIONS

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and safety warnings

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

5.0 INTERFERENCES

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Nalgene bottles, capable of holding 250 mL and 1 L

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 Reagents and Standards

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

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

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

8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

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

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric 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.

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

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

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

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

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

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

8.12 Surrogate stock standard preparation

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

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

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Solvent Blanks, Method blanks and matrix blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.

11.1.5 Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1**, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.

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

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

12.0 PROCEDURE

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

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

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

12.4 Record the initial volume on the extraction worksheet.

12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

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

12.7 Spike each matrix with the appropriate amount of standard as described in **11.1,** or **Table** 1 in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.14 Label a fresh 15 mL centrifuge tube with the same information as in 12.5.

12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.

12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

12.18 Vortex mix for 30 seconds.

12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

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

12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

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

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

14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

Blank	Std #	amount = mL
Serum Extraction Method	:	**Date & Initials**
Vortex 15 sec.		
Pipette Matrix	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 5 mL 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	Temperature:	
Add methanol Volume mL	TN-A-	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2µm 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 (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Fluids

Prep date(s):
Analyte(s):
Sample matrix:

Standard number:
Equipment number:
Final solvent and TN:
Blank fluid/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-6.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

[signature]	7/22/99
Laboratory Manager	Date
[signature]	7/14/99
Group Leader	Date
[signature]	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:

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

* refer to **13.1.1** for details.

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

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

*refer to **13.1.2** for details.

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1.**

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

18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

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-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

[signature]	4/26/99
Laboratory Manager	Date
[signature]	4/26/99
Group Leader	Date
Lisa A Clemen	04/26/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

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

3.0 Definitions

3.1 Atmospheric Pressure Ionization (API): The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).

3.2 Electrospray Ionization (ES, ESI): a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS): The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 Conventional vs. Z-spray probe interface: The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)

3.5 Mass Lynx Software: System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

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

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 Interferences

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

6.0 Equipment

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 Supplies and Materials

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 Reagents and Standards

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

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

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

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

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

10.2 Matrix Spikes

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

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

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

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

10.3 Continuing Calibration Verifications

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

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

11.0 Calibration and Standardization

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

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0** for more details.

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

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

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

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

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

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

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

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

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

14.0 METHOD PERFORMANCE

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

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

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

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be ± 30% of the spiked concentration.

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

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

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

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

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

16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Laboratory Study

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

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

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

Study #: FACT-TOX-099

3M Environmental Lab -- Method Modification

Method:

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

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

Effective date of modifications: April 26, 1999

Section 10.3.2

Method reads:

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

Modify method to read:

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

Section 14.5.1

Method reads:

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

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

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

Study #: FACT-TOX-099

Section 14.3.3

Method reads:

NA

Modify method to read:

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

Section 14.3.4

Method reads:

NA

Modify method to read:

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

Section 14.3.5

Method reads:

NA

Modify method to read:

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

Section 14.3.6

Method reads:

NA

Modify method to read:

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

11/29/00

Signature of PAI and date

13 DEC 2000

Signature of Sponsor and date

13 Dec 2000

Signature of Study Director and date

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-7.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

[signature]	7/22/99
Laboratory Manager	Date
[signature]	7/14/99
Group Leader	Date
[signature]	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):

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

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. 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

Study #: FACT-TOX-099

3M Environmental Lab -- Method Modification

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

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

Effective date of modifications: July 22, 1999

Section 10.3.2

Method reads:

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

Modify method to read:

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

Section 14.5.1

Method reads:

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

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

Section 14.3.2

Method reads:

NA

Modify method to read:

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

Study #: FACT-TOX-099

Section 14.3.3

Method reads:

NA

Modify method to read:

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

Section 14.3.4

Method reads:

NA

Modify method to read:

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

Section 14.3.5

Method reads:

NA

Modify method to read:

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

Section 14.3.6

Method reads:

NA

Modify method to read:

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

Kristen Hebert 11/29/00
Signature of PAI and date

John Z. Butenhoff 13 DEC 2000
Signature of Sponsor and date

Marvin T Case 13 Dec 2000
Signature of Study Director and date

Appendix D: Data Summary Tables

Table 6. LOQ Values for PFOS Used in FACT TOX-099 Analyses by Method and Usage Dates

Method	Effective Date	LOQ	Usage Dates
Sera		**µg/mL**	
ETS-8-5.1	4/26/99	0.0214	10/19/00
		0.00426	10/24/00, 11/7/00
Liver		**µg/g**	
ETS-8-7.0	7/22/99	0.00529	11/1/00
		0.0529	12/21/00

NOTE: LOQ reported only for those data sets with values ≤ LOQ.

Table 7. Average Concentration of PFOS (µg/g) in Rabbit Liver by Dose Group

Dose Group	Mean PFOS in Liver
Group 1 (Control) 0 mg/kg/day	0.239
Group 2 0.1 mg/kg/day	13.1
Group 3 1.0 mg/kg/day	133
Group 4 2.5 mg/kg/day	317
Group 5 3.75 mg/kg/day	416

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, indicate that the data are quantitative to ±30% or greater.

Table 8. Average Concentration of PFOS (µg/mL) in Rabbit Serum by Dose Group

Dose Group	Mean PFOS in Serum
Group 1 (Control) 0 mg/kg/day	0.0690
Group 2 0.1 mg/kg/day	2.73
Group 3 1.0 mg/kg/day	23.8
Group 4 2.5 mg/kg/day	45.8
Group 5 3.75 mg/kg/day	88.9

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, indicate that the data are quantitative to ±30% or greater.

Table 9. PFOS (µg/g) in Rabbit Liver by Individual Animal

Group #	Animal #	Conc. PFOS µg/g
Group 1 0 mg/kg/day	8553F	0.287
	8554F	0.189
	8555F	0.240
Group 2 0.1 mg/kg/day	8556F	13.1
	8557F	13.0
	8558F	12.2
	8559F	11.2
	8560F	16.1
Group 3 1.0 mg/kg/day	8561F	98.6
	8562F	141
	8563F	158
Group 4 2.5 mg/kg/day	8564F	297
	8565F	360
	8566F	295
Group 5 3.75 mg/kg/day	8567F	432
	8568F	401
	8569F	436
	8570F	346
	8571F	466

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, indicate that the data are quantitative to ±30% or greater.

Table 10. PFOS (µg/mL) in Rabbit Serum by Individual Animal

Group #	Animal #	Conc PFOS µg/mL
Group 1 0 mg/kg/day	8553F	0.0956
	8554F	0.0411
	8555F	0.0703
Group 2 0.1 mg/kg/day	8556F	2.78
	8557F	2.92
	8558F	2.86
	8559F	2.64
	8560F	2.44
Group 3 1.0 mg/kg/day	8561F	22.1
	8562F	25.0
	8563F	24.3
Group 4 2.5 mg/kg/day	8564F	46.5
	8565F	46.3
	8566F	44.5
Group 5 3.75 mg/kg/day	8567F	100
	8568F	92.1
	8569F	81.6
	8570F	88.1
	8571F	82.7

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, indicate that the data are quantitative to ±30% or greater.

Appendix E: Data Spreadsheets

FACT-TOX-099
Argus# 418-012

Study: 418-012, Oral Developmental Toxicity Study of PFOS in Rabbits: 4 matrices
Product Number(Test Substance): T-6295
Matrix: Rabbit Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Soup020199, Davey070799
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: 10/17/00 SAL
Dates of Analysis/Analyst: 10/19/00, 10/24/00, 11/07/00 KJH/MMH
Date of Data Reduction/Analyst: 10/23/00, 10/25/00, 11/08/00 KJH/MMH **Box #:** 00-96

Sample Data

RABBIT SERUM lot 171

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (optional)	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10170-H2O Blk-3	1	NA	0.8640	1	0.00	S001107020	<LOQ (0.00426 ug/mL)		
	10170-H2O Blk-4	1	NA	0.8640	1	0.00	D001024004	<LOQ (0.00426 ug/mL)	<LOQ (0.00426 ug/mL)	NA
Matrix Blk	RBS10170-Sera Blk-3	1	NA	0.8640	1	0.00	S001107021	<LOQ (0.00426 ug/mL)		
	RBS10170-Sera Blk-4	1	NA	0.8640	1	0.00	D001024005	<LOQ (0.00426 ug/mL)	<LOQ (0.00426 ug/mL)	NA
QC	RBS10170-MS-250 ppb-3	1	NA	NA	1	259	S001107022	104%		
	RBS10170-MSD-250 ppb-3	1	NA	NA	1	270	S001107023	109%	107%	4%
Group 1	8553F	1	NA	0.8640	1	111	S001019017	0.0956		
	8554F	1	NA	0.8640	1	47.6	S001019018	0.0411		39.5
	8555F	1	NA	0.8640	1	81.4	S001019019	0.0703	0.0690	0.0273
Group 2	8556F	1	NA	0.8640	20	161	D001024017	2.78		
	8557F	1	NA	0.8640	20	169	D001024018	2.92		
	8558F	1	NA	0.8640	20	166	D001024019	2.86		
	8559F	1	NA	0.8640	20	153	D001024020	2.64		7.10
	8560F	1	NA	0.8640	20	141	D001024021	2.44	2.73	0.194
Group 3	8561F	1	NA	0.8640	50	512	D001024024	22.1		
	8562F	1	NA	0.8640	50	578	D001024025	25.0		6.25
	8563F	1	NA	0.8640	50	563	D001024026	24.3	23.8	1.49
Group 4	8564F	1	NA	0.8640	100	538	D001024027	46.5		
	8565F	1	NA	0.8640	100	536	D001024028	46.3		2.40
	8566F	1	NA	0.8640	100	515	D001024031	44.5	45.8	1.10
Group 5	8567F	1	NA	0.8640	200	578	D001024032	100		
	8568F	1	NA	0.8640	200	533	D001024033	92.1		
	8569F	1	NA	0.8640	200	472	D001024034	81.6		
	8570F	1	NA	0.8640	200	510	D001024035	88.1		8.45
	8571F	1	NA	0.8640	200	478	D001024038	82.7	88.9	7.51

Original PFOS LOQ (24.8 ng/mL, 4.93 ng/mL) updated to reflect purity information on 01/30/01. LAC 01/30/01
LOQ = Limit of Quantitation
PFOS = Perfluorooctanesulfonate
Date Entered/By: 10/26/00, 10/27/00, 11/08/00 LAC
Date Verified/ By: 11/08/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

Printed 1/30/01 R. Dick

FACT-TOX-099
Argus #418-012

Study:	418-012, Oral Developmental Toxicity Study of PFOS in Rabbits		
Product Number(Test Substance):	T-6295		
Matrix:	Rabbit Liver	Filename:	See Below
Method/Revision:	ETS-8-6.0 & ETS-8-7.0	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Soup020199, Davey070799	Slope:	See Attachments
Instrument Software/Version:	Masslynx 3.4	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	10/16/00 SAL/KJK	Box #: 00-095	
Date of Analysis/Analyst:	10/25/00, 11/01/00, 12/21/00 MMH		
Date of Data Reduction/Analyst:	10/26/00, 11/02/00, 12/22/00 MMH/KJH		

Sample Data

RABBIT LIVER lot 171

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Purity Correction Factor	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	10160-H2O Blk-3	NA	1.0000	NA	0.8640	0.00	1	0.00	D001221016	<LOQ (0.0529 ug/g) **		
	10160-H2O Blk-4	NA	1.0000	NA	0.8640	0.00	1	0.00	D001101005	<LOQ (0.00529 ug/g)	<LOQ	NA
Matrix Blk	RBL10160-Liver Blk-3	NA	1.0000	NA	0.8640	0.00	1	0.00	D001221017	<LOQ (0.0529 ug/g) *		
	RBL10160-Liver Blk-4	NA	1.0000	NA	0.8640	0.00	1	0.00	D001101006	<LOQ (0.00529 ug/g) **	<LOQ	NA
QC	8553F-MS-250 ppb-3	NA	1.0074	NA	NA	804	1	465	D001221029	156% **		
Analyzed 12/21/00	8553F-MSD-250 ppb-3	NA	1.0074	NA	NA	698	1	360	D001221030	120%	138%	26%
QC	8553F-MS-250 ppb-3	NA	1.0074	NA	NA	608	1	271	D001101042	91% *		
Analyzed 11/01/00	8553F-MSD-250 ppb-3	NA	1.0074	NA	NA	584	1	247	D001101043	83% *	87%	9%
Group 1	8553F	NA	1.0074	NA	0.8640	335	1	287	D001221031	0.287 **		
	8554F	NA	1.0474	NA	0.8640	229	1	189	D001221032	0.189 **		20.5
	8555F	NA	0.9981	NA	0.8640	278	1	240	D001221033	0.240 **	0.239	0.0490
Group 2	8556F	NA	1.0151	NA	0.8640	308	50	13100	D001101018	13.1		
	8557F	NA	0.9931	NA	0.8640	299	50	13003	D001101019	13.0		
	8558F	NA	1.0386	NA	0.8640	293	50	12193	D001101020	12.2		
	8559F	NA	1.0152	NA	0.8640	264	50	11246	D001101021	11.2		13.8
	8560F	NA	1.0263	NA	0.8640	382	50	16069	D001101022	16.1	13.1	1.81
Group 3	8561F	NA	1.0586	NA	0.8640	242	500	98561	D001101025	98.6		
	8562F	NA	1.0014	NA	0.8640	328	500	141282	D001101026	141		23.2
	8563F	NA	1.0398	NA	0.8640	381	500	158417	D001101027	158	133	30.8
Group 4	8564F	NA	1.0057	NA	0.8640	345	1000	296674	D001101028	297		
	8565F	NA	1.0223	NA	0.8640	426	1000	360449	D001101029	360		11.8
	8566F	NA	0.9907	NA	0.8640	338	1000	294686	D001101032	295	317	37.4
Group 5	8567F	NA	1.0137	NA	0.8640	506	1000	431642	D001101033	432		
	8568F	NA	1.0494	NA	0.8640	487	1000	400623	D001101034	401		
	8569F	NA	1.0268	NA	0.8640	519	1000	436300	D001101035	436		
	8570F	NA	1.0328	NA	0.8640	413	1000	345684	D001101036	346		10.9
	8571F	NA	0.9820	NA	0.8640	529	1000	465531	D001101039	466	416	45.5

Original PFOS LOQ (30.6 n/g, 6.12 ng/g, 61.2 ng/g) updated to reflect purity information on 01/30/01. LAC 01/30/01

LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate

* QC analyzed 11/01/00 are in support of dilutions for Group 2 - 5 data and were within 30% criteria.

** QC analyzed 12/21/00 are in support of Group 1 data. The 8553-MS-250 ppb-3 spike was above the 30% criteria, therefore, the group 1 data may be biased high. LAC 01/02/01

Date Entered/Analyst: 10/27/00, 11/03/00, 12/28/00 LAC
Date Verified/Analyst: 11/06/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

Printed 1/30/01
R. Dick

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-099

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

Calculation Used for Group 3, Animal ID 8561F

$$512\ \text{ng/mL} \times 50 \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu g}{1000\ ng} \times 0.864 = \textbf{22.1 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted
PC—PFOS purity correction factor (86.4%)

Formula Used for Liver Analyses in Study FACT TOX-099

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

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

Calculation Used for Group 3, Animal ID 8561F

$$241.52\ \text{ng/g} \times \frac{1\ \text{g/}\ 5\ \text{mL}}{1.0586\ \text{g/}\ 5\ \text{mL}} \times 500 \times \frac{1.0\ \mu g}{1000\ ng} \times 0.864 = \textbf{98.6 µg/g}$$

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
PC—PFOS purity correction factor (86.4%)

Appendix G: Interim Certificate of Analysis

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018B
3M Product: PFOS, Lot 171
Reference #: SD-009
Purity: 86.4%

Test Name	Specifications	Result
Purity[1]		**86.4%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.017 wt./wt.% 2. 0.007 wt./wt.% 3. 1.355 wt./wt.% 4. 6.552 wt./wt.% 5. 0.003 wt./wt.% 6. 0.004 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.00 wt./wt.%
Total % Impurity (LC/MS)		10.60 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.30 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		 1. <0.015 wt./wt.% 2. 0.27 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.82 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. <0.1 wt./wt.% 4. <0.25 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. 12.08 wt./wt.% 2. 0.794 wt./wt.% 3. 1.61 wt./wt.% 4. 10.1 wt./wt.% 5. 50.4 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.39% +LC/MS impurities, 10.60%+Inorganic Fluoride, 0.27%+NMR impurities, 1.00%+ POAA, 0.30%)
Total impurity from all tests = 13.56%
Purity = 100% - 13.56% = 86.4%

[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	Nonofluoropentanoic 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

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.03
C5	1.56
C6	6.38
C7	1.63
Total	**10.60**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ 9/10/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 9/11/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

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INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-018A
3M Product: PFOS, Lot 217
Reference #: SD-018
Purity: 86.9%

Test Name	Specifications	Result
Purity[1]		**86.9%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 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)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 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

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.93%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.09%
Purity = 100% - 13.09% = 86.9%

[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	Nonofluoropentanoic 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

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	1.22
C5	1.33
C6	4.72
C7	1.14
Total	**8.41**

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ 9/10/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 9/11/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Appendix H: Report Signature Page

Marvin T. Case, D.V.M., Ph.D., *Study Director* — 9 Feb 2001 — Date

John L. Butenhoff, Ph.D., *Sponsor Representative* — 02/08/01 — Date

Bill Reagen, Ph.D., *Laboratory Manager* — 02/05/01 — Date

Kris J. Hansen, Ph.D., *Principal Analytical Investigator* — 02/05/01 — Date