Study Title

Analytical Study 2(N-Ethylperfluorooctane sulfonamido)-ethanol in Two Generation Rat Reproduction

Analytical Laboratory Report Title

Determination of the Presence and Concentration of PFOS, M556, PFOSAA, and PFOSA in the Liver and PFOS, M556, PFOSAA, PFOSA, and EtFOSE-OH in the Sera of Crl:CD®BR VAF/Plus® Rats Exposed to EtFOSE-OH

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

At signing

Performing Laboratories

Sera Analyses

3M Environmental Laboratory
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St. Paul, MN 55106

Liver Analyses

Battelle Memorial Institute
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Project Identification

3M Medical Department Study: T-6316.5
Argus In-Life Study: 418-009
Analytical Report: FACT TOX-013
3M Laboratory Request No. U2095

Total Number of Pages

135

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, M556, PFOSAA, and PFOSA in the Liver and PFOS, M556, PFOSAA, PFOSA, and EtFOSE-OH in the Sera of Crl:CD®BR VAF/Plus® Rats Exposed to EtFOSE-OH

Study Identification Number: T-6316.5, FACT TOX-013, LRN-U2095

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. All raw data, protocol, analytical report and samples for this study are retained in archives at the 3M Environmental Laboratory and will be retained for a period of at least ten years. The analytical phase completed at the 3M Environmental Laboratory was performed in accordance with 3M ET&SS Standard Operating Procedures.

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.
- Some changes made in the standard preparation logs obscured the original entry, did not document the reason for the change and/or were not initialed and dated by the person making the change.
- The samples that were analyzed on 3/16/00 utilized standards that had an expiration date of 2/00.
- Liver values generated at contract laboratories were corrected by 3M Environmental Laboratory to reflect the official purity values from the COA. Revised final reports will be solicited from the contract laboratory and will be added as a report amendment at a later date.
- Expiration dates on some reagents and solutions were missing.

Maura T Case — 9 October 2000

Study Director — Date

John Z. Butenhoff — October 9, 2000

Sponsor Representative — Date

GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, M556, PFOSAA, and PFOSA in the Liver and PFOS, M556, PFOSAA, PFOSA, and EtFOSE-OH in the Sera of Crl:CD®BR VAF/Plus® Rats Exposed to EtFOSE-OH

Study Identification Number: T-6316.5, FACT TOX-013, LRN-U2095

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
10/12/99	Extraction	10/26/99	10/26/99
6/5/00 – 6/14/00	Data	6/16/00	6/16/00
9/11/00 – 9/13/00	Draft report	9/14/00	9/14/00

Tanya K Rude — 9/29/00

QAU Representative — Date

Table of Contents

List of Tables

Study Personnel and Contributors

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Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test substance and analytical reference standard reserve samples, as well as the specimens pertaining to the analytical phase of this study, are archived at the 3M Environmental Laboratory. Control sera and liver will be maintained at the contract lab along with the test substance.

Introduction and Purpose

The purpose of the study is to determine the presence and concentration of PFOS, PFOSA, PFOSAA, and M556 in liver samples and PFOS, PFOSA, PFOSAA, EtFOSE-OH, and M556 in sera samples collected from rats exposed to EtFOSE-OH. This study was initiated on 1 October 1998.

Test System

Five groups of F0 generation male and female rats and 3 groups of F1 generation male and female rats were used as the test system. Table 1 outlines the rat population demographics and dosage levels for study 418-009.

On day 4 of lactation, litters were culled to four male and four female pups, where possible. On day 21 of lactation, 25 male and 25 female pups in Groups I, II, and III were selected for continued evaluation. F1 generation male and female rats were given appropriate dosages of the test article via gavage beginning on day 22 of lactation or postpartum through the day before sacrifice.

The test system species and strain selected was the Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat received from Charles River Laboratories, Inc., and assigned temporary numbers until assigned to the study. Rats were permanently identified using Monel® self-piercing ear tags when assigned to the study. F0 generation rats were identified with ear tags. Pups were not identified during lactation, as parameters were evaluated in terms of the litter. At weaning, each F1 generation rat selected for continued observation was identified with a Monel® self-piercing ear tag. F0 female rats were approximately 65 days of age and weighed approximately 179–229 g when received. F0 male rats were approximately 58–67 days of age and weighed approximately 223–331 g when received. Weight data are included in Argus Research Laboratories, Inc. final report (study number 418-009).

Table 1. Test System Population Demographics and Dosage Levels for Study (418-009)

Population	Number of F0 Generation Rats per Sex	Number of F1 Generation Rats per Sex	Dosage (mg/kg/day)
Dosage Group I (Control)	35	25	0 (vehicle)
Dosage Group II	35	25	1
Dosage Group III	35	25	5
Dosage Group IV	35	—	10
Dosage Group V	35	—	15

Specimen Collection and Analysis

Sample specimens were collected by Argus (study 418-009) and sent to the 3M Environmental Laboratory for analysis. Liver and sera specimens were collected from F0 male rats at the completion of the cohabitation period and F0 female rats on day 21 postpartum. Liver specimens were collected from F0 generation litters, and stomach content specimens were collected from the F0 and F2 generation litters. The analysis of the stomach contents were not part of the scope of analysis determined by the study director. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Study Groups I through V (through 11/30/98):
Serum Specimens—45 specimens
Liver Specimens—65 specimens

Blood specimens were centrifuged after collection. Serum was then harvested and immediately frozen on dry ice and maintained frozen at -70°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were frozen and retained at -70°C until shipped to the 3M Environmental Laboratory. Stomach content specimens were frozen at -20°C until shipped to the 3M Environmental Laboratory. Liver, sera, and stomach content specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice.

Sera and liver samples were extracted beginning on October 11, 1999 using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE) for the sera and ethyl acetate for the liver samples. Liver samples were homogenized prior to the extraction procedure. Sample extracts were analyzed using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple response monitoring mode. PFOS, PFOSA, PFOSAA, EtFOSE-OH, and M556 levels were quantitated by external calibration. PFOSEA was not analyzed due to inconsistent analysis and failed QC. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received from Argus, serum, liver and stomach content specimens collected at predetermined time points during and at the end of the*in-life* phase of Argus study 418-009 on 8-4-98, 10-1-98 and 1-29-99. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C. Specimens that were analyzed at Battelle were shipped frozen on dry ice.

Control matrices used in liver and sera analyses were obtained from commercial sources and are presented in Table 6 and 7. Samples analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

Chemical Characterization

EtFOSE-OH
CAS Number: 1691-99-2

Chemical Formula: $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$ Molecular Weight: 571.0

Chemical characterization information on the test article is presented in tabular form below. Chemical characterization information on the analytical reference materials used in this study is presented in tabular form in Appendix A (see Tables 8 and 9) and the interim Certificate of Analysis available in Appendix I.

Table 2. Characterization of the Test Article in Study FACT TOX-013

	Test Article
Chemical Name	**EtFOSE-OH** FM-3929 2(N-Ethylperfluorooctane sulfonamido)-ethanol
Source	3M
Expiration Date	05/2000
Storage Conditions	Ambient temperature
Chemical Lot #	30035, 30037, 30039
Physical Description	Waxy Solid
Purity	To be determined*

* The purity of the test article determined nominally by NMR analysis. Subsequent chemical characterization is occurring and this analytical report will be amended to indicate the purity when a certificate of analysis is issued.

Dose Confirmation Analyses

The dose confirmation data were collected according to a method that was not fully validated. Dose confirmation analyses were performed on test article samples taken at the start of dosage, at 6 weeks, and at the end of dosage during the in-life phase of the study.

Dose confirmation analyses were performed on 3 dose levels collected during the in-life phase of the study: the results are presented in Appendix A (see Tables 10 and 11).

Dose confirmation was performed by diluting the Tween dose samples with Milli-Q water into the linear range of the instrument. For each sample, a matrix spike was prepared (at approximately 50–100% of the expected dose level). In all cases, samples were analyzed versus an unextracted curve using HPLC-ES/MS/MS. The instrumental parameters and analytical conditions described in ETS-8-5.1 were used for dose solution analyses.

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 are located in Appendix C. The methods and analytical equipment settings used by Battelle are presented in the Battelle final report (see Appendix G).

3M Environmental Laboratory

PREPARATORY METHOD

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

  Sera 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 through a 3cc plastic syringe attached to a 0.2μm nylon filter into a glass autovial.

ANALYTICAL METHOD

- **ETS-8-5.1,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum 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-ESMSMS. For example, molecular ion 499, selected as the primary-ion for PFOS ($C_8F_{17}SO_3$-) analysis, was fragmented further to produce ion 99 (FSO_3-). The characteristic product-ion 99 was monitored for quantitative analysis.

ANALYTICAL EQUIPMENT

The following equipment and parameters are representative of those 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: 2mM aqueous ammonium acetate
Component B: methanol
Flow rate: 300 μL/min
Injection volume: 10 μL
Solvent Gradient: 10 minutes
Start at 40%B
Hold at 40%B for 1 minute
Increase to 95%B over 3.5 minutes

Hold at 95%B for 2 minutes
Return to 40%B over 0.5 minutes
Hold at 40%B for 3 minutes

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.2
Cone Voltage: 20–60 V
Collision Energy: 25–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Z-spray source
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499.0	99.0
PFOSA	498.0	78.0
PFOSAA	584.0	169.0
EtFOSE-OH	630.0	59.0
M556	556.0	78.0, 169.0
THPFOS	427.0	80.0

Deviations

Deviations from the original protocol and methods are documented in the table below:

Table 4. Deviation Summary for FACT TOX-013

Deviation	Date(s) of Occurrence	Impact on Study
Pipette was used instead Oxford dispenser	10/12/99	Standards and samples were prepared identically. No adverse impact on study.
0.2–1.0mL of sample was used for extraction instead of 1.0mL.	10/12/99	Current work indicates that volumes ≥0.5 mL provide results equivalent to 1 mL extraction volumes. Results of sample volumes <0.5 mL have not been validated and will be marked in the data table.
Milk curd samples were not analyzed.	Entire study	No milk curd data is available for the final report.

Data Quality Objectives and Data Integrity

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

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980
- **Limits of Quantitation (LOQ):** The LOQ for PFOS is 5.55 ppb, PFOSA is 4.79 ppb, PFOSAA is 20.5 ppb, EtFOSE-OH is 36.2 ppb, and M556 is 19.2 ppb.
- **Acceptable Spike Recoveries:** 70–130%

Data Summary, Analyses, and Results

With the exceptions noted in this report, data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory method ETS-8-5.1 (see Appendix C) and the Battelle final report (see Appendix G) were met. Although extraction and analysis were initiated in September 1998, the study was reprioritized and put on hold. Upon restarting the study, the decision was made to reextract and analyze the specimens. No data from the original analysis are included in this report. The data in this report reflect only that obtained from specimens extracted on, or after October 11, 1999.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curves were≥0.980.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis (1/x weighted) of 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 concentration range most appropriate to the data. All active curve points are accurate to within 70% of theoretical value. 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 outlier curve point may have been deactivated. Quantitation of each analyte was based on the response of one or more specific product ion(s) using the multiple response-monitoring mode of the instrument (see Appendix C).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest accepted standard in the calibration curve (defined as a standard with a concentration that is within ±30% of the theoretical value, and which has at least two times the analyte peak area detected in the extraction blanks).

Table 5. Determinations of the LOQ in the Analyses of Serum Extracts

Analyte	Method LOQ
PFOS	5.55 ppb
PFOSA	4.79 ppb
PFOSAA	20.5 ppb
EtFOSE-OH	36.2 ppb
M556	24.9 ppb

- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed rat sera, rabbit sera was selected as a suitable surrogate matrix for standard curves.
- **Precision:** Precision was determined by analysis of MS/MSD and was reproducible to within 10%.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs. With the exception of M556, all sera matrix spikes were within ±30% of the theoretical concentration. Both matrix spikes showed a recovery of 69% for the M556. These results were verified. Data quality objectives will be adjusted to reflect this recovery.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. The surrogate response of each analytical run was verified to determine that it did not vary more than ±50% from the mean within each analytical run.

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, sera data are quantitative to ±30% for all analysis but M556; M556 data is quantitated to 31%. The validity of this assumption has not been verified by other techniques.

Summary of Sample Results

- **Samples from Control Animals:** Low levels of PFOS, PFOSA, PFOSAA, EtFOSE-OH, and M556 were often detected in the sera and liver of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, PFOS, PFOSA, PFOSAA, EtFOSE-OH, and M556 levels found in the sera and liver of the test animals increased with dose group. 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-013.

Statement of Conclusion

Under the conditions of the present studies, PFOS, PFOSA, PFOSAA, EtFOSE-OH, and M556 were observed in the sera and liver of rats dosed with EtFOSE-OH during the in-life phase of the study.

Appendix A: Chemical Characterization, Control Matrices and Dose Confirmation Analyses

Table 6. Characterization of the Control Matrices Used for Sera Analyses in Study FACT TOX-013

Location	3M Lab	
Control Matrix	**Rat Serum (TN-A-2001)**	**Rabbit Serum (TN-A-2573)**
Source	Sigma	Sigma
Expiration Date	2010	2010
Storage Conditions	Ambient	Ambient
Chemical Lot #	17H9306	118H8418
Physical Description	Rat Serum	Rabbit Serum

N/R—not recorded

Table 7. Characterization of the Control Matrices Used for Liver Analyses in Study FACT TOX-013

Location	Battelle Memorial Institute
Control Matrix	**Rat Liver**
Source	Harlan
Expiration Date	N/R
Storage Conditions	N/R
Chemical Lot #	N/R
Physical Description	Rat Liver

N/R—not recorded

Table 8. Characterization of the Analytical Reference Materials Used for Sera Analyses in Study FACT TOX-013

Location	3M Lab					
Materials	**PFOS** $C_8F_{17}SO_3^-$	**PFOSA** $C_8F_{17}SO_2NH_2$	**PFOSAA** $C_8F_{17}SO_2N((CH_2CH_3)(CH_2COOH))$	**EtFOSE-OH** $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$	**M556** $C_8F_{17}SO_2N((H)(CH_2CH_3))$	**THPFOS*** $C_3H_6F_{13}NO_2S$
Source	3M Specialty Chemicals	N/R	N/R	3M ICP/PCP Division	3M	ICN Biomedicals
Expiration Date	08/31/01	01/01/2010	01/01/2010	01/01/2010	01/01/2010	01/2010
Storage Conditions	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature
Chemical Lot Number	171	L-15709	NB 112999-99	936	NB 113047-80	53406
Physical Description	White crystalline powder	Light yellow waxy solid	Tan waxy solid	Amber waxy solid	White powder	Brown waxy solid
Purity	86.4%	TBD	TBD	TBD	TBD	NA

*Surrogate standard— 1H,1H,2H,2H-Tetrahydroperfluorooctanesulfonic acid
N/R—not recorded
TBD—to be determined
NA—not applicable

Table 9. Characterization of the Analytical Reference Materials Used for Liver Analyses in Study FACT TOX-013

Location	Battelle Memorial Institute				
Materials	**PFOS**	**M556**	**PFOSAA**	**PFOSA**	**THPFOS***
Source	3M	3M	3M	3M	ICN
Expiration Date	08/31/01	01/01/2010	2010	01/01/2010	N/R
Storage Conditions	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature
Chemical Lot Number	171	NB 113047-80	617	L-15709	59909
Physical Description	White crystalline powder	White powder	N/R	Light yellow waxy solid	N/R
Purity	86.4%	TBD	TBD	TBD	NA

*Surrogate standard— 1H,1H,2H,2H-Tetrahydroperfluorooctanesulfonic acid
N/R—not recorded
TBD—to be determined
NA—not applicable

Table 10. Tween Dosing Confirmation for Study In-life #418-009

Group Dose	Sample Number	Expected Conc. EtFOSE (ng/mL)	Measured Conc. EtFOSE (ng/mL)	EtFOSE % Recovery Accuracy
Group 1—Control 0 mg/mL	B-418-009-A, 06/08/98	0.00	0.00	NA
	B-418-009-A, 07/15/98	NA	NA	NA
Group 2—0.2 mg/mL	B-418-009-B, 06/08/98	200000	NA	NA
	B-418-009-B, 07/15/98	200000	NA	NA
Group 3—1.0 mg/mL	B-418-009-C, 06/08/98	1000000	1020000	102
	B-418-009-C, 07/15/98	100000	942000	94
Group 4—2.0 mg/mL	B-418-009-D, 06/08/98	2000000	2190000	110
	B-418-009-D, 07/15/98	2000000	2750000	138
Group 5—3.0 mg/mL	B-418-009-E, 06/08/98	3000000	3060000	102
	B-418-009-E, 07/15/98	3000000	3640000	121
Homogeneity Samples—3.0 mg/mL	B-418-009-A, 05/08/98 1 of 6 T	3000000	3250000	108
	B-418-009-A, 06/08/98 3 of 6 M	3000000	3690000	123
	B-418-009-A, 06/08/98 5 of 6 B	3000000	3790000	126

NA = Not applicable

Table 11. Tween Dosing Confirmation—Matrix Spikes for Study In-life #418-009

Sample Number	Expected Conc. EtFOSE (ng/mL)	Measured Conc. EtFOSE (ng/mL)	EtFOSE % MS Recovery Accuracy
B-418-009-B, 06/08/98-MS	1200	NA	NA
B-418-009-B, 07/15/98-MS	1200	NA	NA
B-418-009-C, 06/08/98-MS	900	818	91
B-418-009-C, 07/15/98-MS	900	826	92
B-418-009-D, 06/08/98-MS	900	910	101
B-418-009-D, 07/15/98-MS	900	733	81
B-418-009-E, 06/08/98-MS	1100	973	88
B-418-009-E, 07/15/98-MS	1100	1089	99
B-418-009-A, 05/08/98 1 of 6 T-MS	1100	949	86
B-418-009-A, 06/08/98 3 of 6 M-MS	1100	1053	96
B-418-009-A, 06/08/98 5 of 6 B-MS	1100	944	86

NA = Not applicable

Appendix B: Protocol

3M ENVIRONMENTAL LABORATORY

PROTOCOL - ANALYTICAL STUDY
2(N-Ethylperfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

In-vivo study reference number: Argus 418-009
Study number: FACT 060998.1
Test substance: 2(N-Ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH)

Name and address of Sponsor:

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

Name and address of testing facility:

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

Experimental start date:
Expected termination date: December 31, 1998
Method numbers and revisions:

FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

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

FACT-M-3.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

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

Author: Lisa Clemen

Kris Hansen — 9/15/98
Kris Hansen — Date
Study Director

Marvin Case — 10 Oct 1998
Marvin Case — Date
Sponsor Representative

1.0 PURPOSE

The analytical portion of this dosing study is designed evaluate the levels of perfluorooctane sulfonate (PFOS), or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) designated by the study director, in the liver of the parent and subsequent generations of the test system, or in the serum as necessary.

The in life portion of this study was conducted at Argus Research Laboratories.

2.0 REGULATORY COMPLIANCE

This study is conducted in compliance with the Food and Drug Administration Good Laboratory Practices regulation as stated in 21 CFR 58. Any exceptions will be noted in the final report.

3.0 TEST MATERIALS

3.1 Test, control, and reference substances and matrices

3.1.1 Analytical reference substance: Potassium perfluorooctanesulfonate (PFOS), lot # 217

3.1.2 Analytical reference substance matrix: Rat liver and serum

3.1.3 Analytical control substance: None

3.1.4 Analytical control substance matrix: Rat liver and serum

3.2 Source of materials

3.2.1 Analytical reference substance: 3M Specialty Chemical Division; traceability information will be included in the final report

3.2.2 Analytical reference substance matrix: Argus Research Laboratories; traceability information will be included in the final report

3.2.3 Analytical control matrix:

3.2.3.1 Rat liver – Argus Research Laboratories; traceability information will be included in the final report; or

Rabbit liver – Covance Laboratories; traceability information will be included in the final report

3.2.3.2 Rat serum - Sigma Chemical Company; traceability information will be included in the final report

3.3 Number of test and control samples. Liver samples for testing were received from 40 test animals and 10 control animals. Serum samples will be tested at the discretion of the Study Director.

3.4 Identification of test and control samples: The samples are identified using the Argus Research Laboratories identifiers, which consist of a letter followed by the Argus project number, the animal number, the group designation, and the draw date.

3.5 **Purity and strength of materials:** Characterization of the purity and identity of the reference material is the responsibility of the Sponsor.

3.6 **Stability of test material:** Characterization of the stability of the test material is the responsibility of the Sponsor.

3.7 **Storage conditions for test materials:** Test materials are stored at room temperature. Samples are stored at –20 ± 10 °C.

3.8 **Disposition of test and/or control substances:** Biological tissues and fluids are retained per GLP regulation.

3.9 **Safety precautions:** Refer to the material safety data sheets of chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

4.0 EXPERIMENTAL - Overview

Tissues from animals dosed as described in Argus Research Laboratories Protocol #418-009 are received for analysis of fluorine compounds. At the discretion of the Study Director, a series of analytical tests will be performed on select tissues.

Initially, all liver samples will be analyzed for PFOS by electrospray/mass spectrometry (ES/MS). On the basis of findings from these analyses, additional sample matrices may be evaluated or other metabolites may be targeted. If additional analysis is performed, a protocol amendment will be written.

5.0 EXPERIMENTAL - Analytical Methods

5.1 **FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.2 **FACT-M-2.0,** Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

5.3 **FACT-M-3.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.4 **FACT-M-4.0,** Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

6.0 DATA ANALYSIS

6.1 **Data transformations and analysis:** Data will be reported as the concentration (weight/weight) of fluoride per tissue or sample, or of PFOS per unit of tissue or fluid.

6.2 **Statistical analysis:** Statistics used may include regression analysis of the serum 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.

7.0 Maintenance of Raw Data and Records

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

7.1.1 Approved protocol and amendments

7.1.2 Study correspondence

7.1.3 Shipping records

7.1.4 Raw data

7.1.5 Electronic copies of data

7.2 Supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following:

7.2.1 Training records

7.2.2 Calibration records

7.2.3 Instrument maintenance logs

7.2.4 Standard Operating Procedures, Equipment Procedures, and Methods

7.2.5 Appropriate specimens.

8.0 References

8.1 3M Environmental Laboratory Quality System Chapters 1, 5 and 6

8.2 Other applicable 3M Environmental Laboratory Quality System Standard Operating Procedures

9.0 Attachments

9.1 **FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.2 **FACT-M-2.0,** Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

9.3 **FACT-M-3.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.4 **FACT-M-4.0,** Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

Study Title

Combined Oral (Gavage) Fertility Development and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:

July 28, 1999

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX-013
LIRN U2095

Protocol FACT-TOX-013
Amendment 1

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

1. ***PROTOCOL READS:*** The proposed study completion date is listed as 12/31/98.

   AMEND TO READ: The proposed study completion data is 6/30/00.

   REASON: The proposed completion date was changed to allow time for analyzing all matrices of interest.

Amendment Approval

Marvin T Case — 30 July 1999

Marvin Case Ph.D., Sponsor Representative — Date

Kris Hansen — 8/2/99

Kris J. Hansen Ph.D., Study Director — Date

3. ***PROTOCOL READS:***
Disposition of test and control substances: Biological tissues and fluids are retained per GLP regulation.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens 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.

Amendment Approval

Marvin T Case — 4 October 1999
Marv Case, D.V.M., Ph.D., Sponsor Representative — Date

James K Lundberg — 5 oct 1999
James K. Lundberg, Ph.D., Study Director — Date

Kristen Hansen — 10/5/99
Kristen J. Hansen, Ph.D., Previous Study Director — Date

[signature] — 10/15/99
Dale L. Bacon, ~~Ph.D.~~, 3M Environmental Laboratory Management — Date

Study Title

Analytical Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

PROTOCOL AMENDMENT NO. 4

Amendment Date:

20 January 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS LRN–U2095
FACT TOX-013
Argus Study: 418-009
3M Medical Department Study: T-6316.5

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 James K. Lundburg, 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, James K. Lundburg, 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.

Amendment Approval

John L. Butenhoff — February 10, 2000

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

February 21, 2000

James K. Lundberg, Ph.D., Outgoing Study Director — Date

Marvin T. Case — 10 February 2000

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

Study Title

Analytical Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

PROTOCOL AMENDMENT NO. 5

Amendment Date:

August 31, 2000

Performing Laboratory

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

Laboratory Project Identification

FACT-TOX-013
ET&SS LRN U2095
Argus Study: 418-009
3M Medical Department Study: T6316.5

Protocol FACT TOX-013
Amendment No. 5

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

1. ***PROTOCOL READS:*** The Principle Analytical Investigator for the present study was identified as James K. Lundberg, Ph.D.

2. ***AMEND TO READ:*** The role of Principle Analytical Investigator for the present study was reassigned to Kristen J. Hansen Ph.D.

   REASON: The role of Principle Analytical Investigator was reassigned due to availability of resources.

Protocol FACT TOX-013
Amendment No. 5

Amendment Approval

John L. Butenhoff 15 / Sept 2000

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

Marvin T Case 8 Sept 2000

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

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

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

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

Lisa A Clemen 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:

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

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

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

Appendix D: Data Summary Tables

Table 12. Reported Fluorochemical Levels in Sera Analyses in Study FACT TOX-013

Dosage Group	Specimen ID	PFOS (µg/mL)	PFOSA (µg/mL)	PFOSAA (µg/mL)	EtFOSE-OH (µg/mL)	M556 (µg/mL)
I	10097F	0.0394	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	10105F	0.0181	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	10106F	0.0258	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	10107F	0.0343	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	10108F	0.0253	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	9922M*	0.0115	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	9930M*	0.0134	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	9931M	0.00725	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	9932M	0.0162	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
I	9933M*	0.0156	<LOQ (4.79 ppb)	<LOQ (20.5 ppb)	<LOQ (36.2 ppb)	<LOQ (24.9 ppb)
II	10121F	9.62	0.0682	1.59	<LOQ (36.2 ppb)	1.86
II	10126F	19.8	0.112	4.55	<LOQ (36.2 ppb)	4.19
II	10136F	5.96	0.0663	1.18	<LOQ (36.2 ppb)	0.952
II	10140F	6.27	0.0507	0.690	<LOQ (36.2 ppb)	1.15
II	10142F	13.1	0.0665	2.09	<LOQ (36.2 ppb)	2.45
II	9961M*	34.8	0.0962	1.40	<LOQ (36.2 ppb)	4.69
II	9964M	30.4	0.188	5.86	<LOQ (36.2 ppb)	5.18
II	9965M*	74.9	0.114	1.86	<LOQ (36.2 ppb)	4.54
II	9967M	25.1	0.147	1.26	<LOQ (36.2 ppb)	3.44
II	9970M*	38.9	0.165	5.55	<LOQ (36.2 ppb)	6.11
III	10155F*	87.8	0.328	9.9	<LOQ (36.2 ppb)	43.3
III	10156F	76.1	0.352	6.91	<LOQ (36.2 ppb)	22.3
III	10164F	49.6	0.265	4.66	<LOQ (36.2 ppb)	18.0
III	10172F	68.4	0.325	8.17	<LOQ (36.2 ppb)	17.0
III	10177F	42.2	0.335	4.58	<LOQ (36.2 ppb)	17.9
III	9997M	108	0.574	11.8	<LOQ (36.2 ppb)	29.1
III	9999M*	178	0.579	18.7	<LOQ (36.2 ppb)	73.6
III	10001M	94.9	0.480	12.1	<LOQ (36.2 ppb)	25.1
III	10002M	113	0.393	10.4	<LOQ (36.2 ppb)	38.1
III	10004M	130	0.465	14.9	<LOQ (36.2 ppb)	37.8
IV	10187F	89.5	0.461	8.00	<LOQ (36.2 ppb)	39.0
IV	10194F	73.4	0.576	10.6	<LOQ (36.2 ppb)	25.6
IV	10203F	126	0.651	19.0	<LOQ (36.2 ppb)	39.6
IV	10211F	99.7	0.670	10.2	<LOQ (36.2 ppb)	28.8
IV	10214F	98.3	0.569	12.3	<LOQ (36.2 ppb)	33.8
IV	10019M	302	0.613	22.5	<LOQ (36.2 ppb)	71.3
IV	10024M*	477	0.553	40.5	<LOQ (36.2 ppb)	102
IV	10029M*	296	0.610	28.8	<LOQ (36.2 ppb)	94.9
IV	10033M	272	0.804	24.5	<LOQ (36.2 ppb)	90.9
IV	10034M	249	0.637	31.4	<LOQ (36.2 ppb)	56.7

* = Tentative values, initial volume was <0.5 mL

Table 12. Reported Fluorochemical Levels in Sera Analyses in Study FACT TOX-013 (continued)

Dosage Group	Specimen ID	PFOS (µg/mL)	PFOSA (µg/mL)	PFOSAA (µg/mL)	EtFOSE-OH (µg/mL)	M556 (µg/mL)
V	NR	NR	NR	NR	NR	NR
V	NR	NR	NR	NR	NR	NR
V	NR	NR	NR	NR	NR	NR
V	NR	NR	NR	NR	NR	NR
V	NR	NR	NR	NR	NR	NR
V	10042M	238	0.791	25.2	<LOQ (36.2 ppb)	62.4
V	10044M	235	0.972	20.7	<LOQ (36.2 ppb)	55.6
V	10045M	326	0.897	26.8	<LOQ (36.2 ppb)	93.8
V	10051M	162	0.574	14.0	<LOQ (36.2 ppb)	30.7
V	10054M	182	0.669	15.8	<LOQ (36.2 ppb)	55.5

NR = Sample not received or reported

* = Tentative values, initial volume was <0.5 mL

Table 13. Reported Fluorochemical Levels in Liver Analyses in Study FACT TOX-013

Dosage Group	Specimen ID	PFOS (µg/g)	PFOSA (µg/g)	PFOSAA (µg/g)	M556 (µg/g)
I	10097F	0.149	<LOQ	<LOQ	<LOQ
I	10105F	<LOQ	<LOQ	<LOQ	<LOQ
I	10106F	0.121	<LOQ	<LOQ	<LOQ
I	10107F	<LOQ	<LOQ	<LOQ	<LOQ
I	10108F	<LOQ	<LOQ	<LOQ	<LOQ
I	9922M	0.585	<LOQ	<LOQ	<LOQ
I	9930M	0.816	<LOQ	<LOQ	<LOQ
I	9931M	0.836	<LOQ	<LOQ	<LOQ
I	9932M	1.04	<LOQ	<LOQ	<LOQ
I	9933M	1.01	<LOQ	<LOQ	<LOQ
I	10097M	0.281	<LOQ	<LOQ	<LOQ
I	10105M	0.242	<LOQ	<LOQ	<LOQ
I	10106M	0.226	<LOQ	<LOQ	<LOQ
I	10107M	0.221	<LOQ	<LOQ	<LOQ
I	10108M	0.251	<LOQ	<LOQ	<LOQ
II	10121F	25.1	0.514	2.68	1.19
II	10126F	22.9	0.708	4.34	1.75
II	10136F	39.8	1.40	5.01	2.88
II	10140F	23.7	0.601	2.67	1.55
II	10142F	22.1	0.508	2.67	1.41
II	9961M	116	4.49	11.0	12.8
II	9964M	102	4.10	15.6	10.2
II	9965M	89.9	2.88	9.79	11.6
II	9967M	80.7	3.88	6.62	8.95
II	9970M	87.3	5.42	10.4	11.6
II	10121M	54.7	1.90	5.87	5.39
II	10126M	67.8	2.65	14.6	7.75
II	10136M	53.7	2.35	9.44	4.64
II	10140M	28.0	1.22	2.82	3.27
II	10142M	71.5	2.06	6.55	4.58

Table 13. Reported Fluorochemical Levels in Liver Analyses in Study FACT TOX-013 (continued)

Dosage Group	Specimen ID	PFOS (µg/g)	PFOSA (µg/g)	PFOSAA (µg/g)	M556 (µg/g)
III	10155F	102	2.22	15.6	7.17
III	10156F	130	2.24	20.2	7.64
III	10164F	179	1.94	22.7	8.41
III	10172F	119	2.17	11.9	5.87
III	10177F	105	2.88	20.0	8.16
III	9997M	415	10.8	73.5	63.5
III	9999M	234	9.41	28.6	39.3
III	10001M	498	8.67	85.2	66.4
III	10002M	257	8.29	34.2	38.0
III	10004M	386	8.41	64.9	54.6
III	10155M	89.0	5.11	12.0	11.5
III	10156M	219	6.14	27.3	33.3
III	10164M	203	6.26	29.4	43.1
III	10172M	188	6.20	33.7	29.9
III	10177M	153	6.91	17.1	31.4
IV	10187F	164	2.80	31.1	19.8
IV	10194F	240	4.06	51.5	26.8
IV	10203F	344	3.14	49.5	26.6
IV	10211F	255	3.39	46.6	27.5
IV	10214F	264	4.56	51.4	29.3
IV	10019M	831	12.8	122	84.5
IV	10024M	791	11.0	148	97.5
IV	10029M	556	11.2	86.2	78.2
IV	10033M	761	12.6	129	117
IV	10034M	556	11.0	135	82.2
IV	10187M	226	6.36	27.4	39.9
IV	10194M	277	9.96	45.5	39.5
IV	10203M	448	9.93	76.0	67.8
IV	10211M	457	11.4	56.2	65.4
IV	10214M	344	8.11	60.0	64.5
V	10042M	1218	16.4	188	128
V	10044M	1356	13.0	206	152
V	10045M	1132	10.9	150	133
V	10051M	1063	9.80	157	118
V	10054M	1054	10.8	165	161

Appendix E: Data Spreadsheets

FACT-TOX-013
Argus# 418-009

Study:	Argus 418-009, Two-Generation Reproductive Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSE-OH (T-6316.5)
Matrix:	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799, Soup 020199
Instrument Software/Version:	MassLynx 3.3
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/12/99 RWW
Dates of Analysis/Analyst:	10/14/99, 10/20/99, 10/22/99, 03/16/00 MMH/IAS
Date of Data Reduction/Analyst:	10/15/99, 10/21/99, 10/25/99, 03/17/00, 03/23/00 MMH/IAS

Sample Data

RAT SERA F0

Group Dose	Sample #	Initial Vol. mL	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10129-H2O Blk-1	1	0.00	<LOQ (24.8 ppb)		
	10129-H2O Blk-2	1	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS10129-Sera Blk-1	1	0.00	<LOQ (24.8 ppb)		
	RBS10129-Sera Blk-2	1	0.00	<LOQ	<LOQ	NA
QC-100 ppb	RBS10129-MS-1	1	231	93%		
	RBS10129-MSD-1	1	213	86%	90%	8%
Group 1 Control 0.0 mg/kg/day 0 mg/mL	10097F	0.70	34.4	0.0394		
	10105F	0.70	15.8	0.0181		
	10106F	0.50	16.1	0.0258		29.2
	10107F	0.60	25.7	0.0343		
	10108F	0.60	19.0	0.0253	0.0286	0.00834
	9922M**	0.40	5.74	0.0115		
	9930M**	0.40	6.71	0.0134		
	9931M	0.50	4.69	0.00752		27.4
	9932M	0.50	10.1	0.0162		
	9933M**	0.40	7.78	0.0156	0.0129	0.00352
Group 2 1 mg/kg/day 0.2 mg/mL	10121F	0.70	336	9.62		
	10126F	0.50	495	19.8		
	10136F	1.00	297	5.96		52.4
	10140F	1.00	313	6.27		
	10142F	0.60	394	13.1	10.9	5.74
	9961M**	0.40	347	34.8		
	9964M	0.50	379	30.4		
	9965M**	0.20	374	74.9		48.4
	9967M	0.50	313	25.1		
	9970M**	0.40	389	38.9	40.8	19.7
Group 3 5 mg/kg/day 1.0 mg/mL	10155F**	0.40	434	87.8		
	10156F	0.60	572	76.1		
	10164F	0.70	433	49.6		28.9
	10172F	0.60	515	68.4		
	10177F	0.70	369	42.2	64.8	18.8
	9997M	0.70	379	108		
	9999M**	0.30	259	178		
	10001M	0.70	331	94.9		25.8
	10002M	0.60	340	113		
	10004M	0.50	324	130	125	32.2
Group 4 10.0 mg/kg/day 2.0 mg/mL	10187F	0.60	686	89.5		
	10194F	0.80	760	73.4		
	10203F*	0.70	1100	126		19.6
	10211F*	0.80	995	99.7		
	10214F	0.70	842	98.3	97.4	19.1
	10019M	0.60	446	302		
	10024M**	0.30	360	477		
	10029M**	0.40	296	296		28.4
	10033M	0.50	340	272		
	10034M	0.50	311	249	319	90.6
Group 5 15.0 mg/kg/day 3.0 mg/mL	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR		
	NR	NR	NR	NR	NR	NR
	10042M	0.80	475	238		
	10044M	0.80	469	235		
	10045M	0.50	405	326		27.8
	10051M	1.00	401	162		
	10054M	0.90	404	182	229	63.7

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, PFOSAA = 20.5 ppb, EtFOSE = 36.2 ppb, PFOSEA = 18.2 ppb, M556 = 19.2 ppb

Correction factors not applicable for MS/MSD QC data

* Tentative value, PFOS concentration was not within the range of the curve, it's approximately 15% above the highest standard. LAC 03/24/00

Date Entered/By: 03/11/00, 03/23/00, 03/24/00 LAC

Date Verified/ By:

Purity Entered/Verified: 09/13/00 LAC, boj 9/13/00

** Tentative values, initial volume below 0.3 mL. LAC 08/31/00

FACT-TOX-013
Argus# 418-009

Study:	Argus 418-009, Two-Generation Reproduction Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSB-OH (T-6316.5)
Matrix:	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799, Soup 020199
Instrument Software/Version:	MassLynx 3.3
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/12/99 RWW
Dates of Analysis/Analyst:	10/14/99, 10/20/99, 10/22/99, 03/16/00 MMH/IAS
Date of Data Reduction/Analyst:	10/15/99, 10/21/99, 10/25/99, 03/17/00, 03/23/00 MMH/IAS

Sample Data

RAT SERA F0

Group Dose	Sample #	Initial Vol. mL	PFOSA Conc. ng/mL	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10129-H2O Blk-1	1	0.00	<LOQ		
	10129-H2O Blk-2	1	1.57	<LOQ	<LOQ	NA
Matrix Blk	RBS10129-Sera Blk-1	1	0.00	<LOQ		
	RBS10129-Sera Blk-2	1	1.37	<LOQ	<LOQ	NA
QC-100 ppb	RBS10129-MS-1	1	220	89%		
	RBS10129-MSD-1	1	215	87%	88%	3%
Group 1 Control 0.0 mg/kg/day 0 mg/mL	10097F	0.70	1.46	<LOQ		
	10105F	0.70	1.28	<LOQ		
	10106F	0.50	1.15	<LOQ		
	10107F	0.60	1.44	<LOQ		NA
	10108F	0.60	1.14	<LOQ	<LOQ	NA
	9912M**	0.40	2.41	<LOQ		
	9930M**	0.40	1.89	<LOQ		
	9931M	0.50	1.44	<LOQ		
	9932M	0.50	1.41	<LOQ		NA
	9933M**	0.40	1.33	<LOQ	<LOQ	NA
Group 2 1 mg/kg/day 0.2 mg/mL	10121F	0.70	47.7	0.0682		
	10126F	0.50	56.0	0.112		
	10136F	1.00	66.3	0.0663		
	10140F	1.00	50.7	0.0507		31.7
	10142F	0.60	39.9	0.0665	0.0727	0.0231
	9961M**	0.40	38.5	0.0962		
	9964M	0.50	94.2	0.188		
	9965M**	0.20	22.7	0.114		
	9967M	0.50	73.6	0.147		26.4
	9970M**	0.40	66.2	0.165	0.142	0.0376
Group 3 5 mg/kg/day 1.0 mg/mL	10155F**	0.40	131	0.328		
	10156F	0.60	211	0.352		
	10164F	0.70	185	0.265		
	10172F	0.60	195	0.325		10.3
	10177F	0.70	235	0.335	0.321	0.0331
	9997M	0.70	402	0.574		
	9999M**	0.30	174	0.579		
	10001M	0.70	336	0.480		
	10002M	0.60	236	0.393		15.8
	10004M	0.50	233	0.465	0.498	0.0786
Group 4 10.0 mg/kg/day 2.0 mg/mL	10187F	0.60	276	0.461		
	10194F	0.80	461	0.576		
	10203F	0.70	456	0.651		
	10211F	0.80	536	0.670		14.1
	10214F	0.70	398	0.569	0.585	0.0826
	10019M	0.60	368	0.613		
	10024M**	0.30	166	0.553		
	10029M**	0.40	244	0.610		
	10033M	0.50	402	0.804		14.8
	10034M	0.50	318	0.637	0.643	0.0951
Group 5 15.0 mg/kg/day 3.0 mg/mL	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR	NR	NR
	10042M	0.80	633	0.791		
	10044M	0.80	778	0.972		
	10045M	0.50	448	0.897		
	10051M	1.00	574	0.574		20.8
	10054M	0.90	602	0.669	0.780	0.163

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, PFOSAA = 20.5 ppb, EtFOSE = 36.2 ppb, PNR = Sample not received nor reported.
Correction factors not applicable for MS/MSD QC data
* Tentative value, concentration was not within the range of the curve, it's approximately 15% above the highest standard. LAC 03/24/00
Date Entered/By: 03/11/00, 03/13/00, 03/14/00 LAC
Date Verified/ By:

** Tentative values, initial volume below 0.5 mL. LAC 08/31/00

FACT-TOX-013
Argus# 418-009

Study:	Argus 418-009, Two-Generation Reproduction Study of EtPOSE-OH in Rats
Product Number(Test Substance):	EtPOSB-OH (T-6316.5)
Matrix:	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799, Soup 020199
Instrument Software/Version:	MassLynx 3.3
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/12/99 RWW
Dates of Analysis/Analyst:	10/14/99, 10/20/99, 10/22/99, 03/16/00 MMH/IAS
Date of Data Reduction/Analyst:	10/15/99, 10/21/99, 10/25/99, 03/17/00, 03/23/00 MMH/IAS

Sample Data

RAT SERA F0

Group Dose	Sample #	Initial Vol. mL	PFOSAA Conc. ng/mL	Concentration of PFOSAA ng/mL or % Rec	Mean PFOSAA ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10129-H2O Blk-1	1	0.00	<LOQ (34.8)		
	10129-H2O Blk-2	1	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS10129-Sera Blk-1	1	0.00	<LOQ (34.8)		
	RBS10129-Sera Blk-2	1	0.00	<LOQ	<LOQ	NA
QC-100 ppb	RBS10129-MS-1	1	212	86%		
	RBS10129-MSD-1	1	198	80%	83%	7%
Group 1 Control 0.0 mg/kg/day 0 mg/mL	10097F	0.70	16.2	<LOQ		
	10105F	0.70	0.00	<LOQ		
	10106F	0.50	0.00	<LOQ		
	10107F	0.60	13.6	<LOQ		NA
	10108F	0.60	0.00	<LOQ	<LOQ	NA
	9922M**	0.40	0.00	<LOQ		
	9930M**	0.40	0.00	<LOQ		
	9931M	0.50	0.00	<LOQ		
	9932M	0.50	5.42	<LOQ		NA
	9933M**	0.40	2.94	<LOQ	<LOQ	NA
Group 2 1 mg/kg/day 0.2 mg/mL	10121F	0.70	111	1.59		
	10126F	0.50	227	4.55		
	10136F	1.00	118	1.18		
	10140F	1.00	690	0.690		74.6
	10142F	0.60	126	2.09	2.02	1.50
	9961M**	0.40	561	1.40		
	9964M	0.50	117	5.86		
	9965M**	0.20	372	1.86		
	9967M	0.50	629	1.26		72.6
	9970M**	0.40	88.8	5.55	3.19	2.31
Group 3 5 mg/kg/day 1.0 mg/mL	10155F**	0.40	394	9.85		
	10156F	0.60	417	6.91		
	10164F	0.70	326	4.66		
	10172F	0.60	493	8.17		33.3
	10177F	0.70	321	4.58	6.84	2.27
	9997M	0.70	81.2	11.8		
	9999M**	0.30	55.6	18.7		
	10001M	0.70	83.0	12.1		
	10002M	0.60	63.7	10.4		24.3
	10004M	0.50	74.5	14.9	13.6	3.30
Group 4 10.0 mg/kg/day 2.0 mg/mL	10187F	0.60	49.2	8.00		
	10194F	0.80	88.1	10.6		
	10203F	0.70	130	19.0		
	10211F	0.80	84.9	10.2		34.8
	10214F	0.70	84.3	12.3	12.0	4.19
	10019M	0.60	54.5	22.5		
	10024M**	0.30	47.2	40.5		
	10029M**	0.40	46.1	28.8		
	10033M	0.50	49.1	24.5		23.8
	10034M	0.50	62.9	31.4	29.5	7.03
Group 5 15.0 mg/kg/day 3.0 mg/mL	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR	NR	NR
	10042M	0.80	80.3	25.2		
	10044M	0.80	66.0	20.7		
	10045M	0.50	53.6	26.8		
	10051M	1.00	55.6	14.0		27.5
	10054M	0.90	56.1	15.8	20.5	5.63

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, PFOSAA = 20.5 ppb, EtFOSE = 34.2 ppb, PFOSEA = 18.2 ppb, M556 = 19.2 pp

Correction factors not applicable for MS/MSD QC data

* Tentative value, concentration was not within the range of the curve, it's approximately 15% above the highest standard. LAC 03/24/00

Data Entered/By: 03/11/00, 03/23/00, 03/24/00 LAC

Data Verified/ By:

** Tentative values, initial volume below 0.5 mL. LAC 08/31/00

FACT-TOX-013
Argus# 418-009

Study:	Argus 418-009, Two-Generation Reproduction Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSE-OH (T-6316.5)
Matrix:	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799, Soup 020199
Instrument Software/Version:	MassLynx 3.3
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/12/99 RWW
Dates of Analysis/Analyst:	10/14/99, 10/20/99, 10/22/99, 03/16/00 MJH/JAS
Date of Data Reduction/Analyst:	10/15/99, 10/21/99, 10/25/99, 03/17/00, 03/23/00 MJH/JAS

Sample Data

RAT SERA F0

Group Dose	Sample #	Initial Vol. mL	EtFOSE Conc. ng/mL	Concentration of EtFOSE ng/mL or % Rec	Mean EtFOSE ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10129-H2O Blk-1	1	1.45	<LOQ		
	10129-H2O Blk-2	1	1.33	<LOQ	<LOQ	NA
Matrix Blk	RBS10129-Sera Blk-1	1	0.00	<LOQ		
	RBS10129-Sera Blk-2	1	0.00	<LOQ	<LOQ	NA
QC-100 ppb	RBS10129-MS-1	1	259	104%		
	RBS10129-MSD-1	1	247	99%	102%	5%
Group 1 Control 0.0 mg/kg/day 0 mg/mL	10097F	0.70	0.00	<LOQ		
	10105F	0.70	0.00	<LOQ		
	10106F	0.50	1.30	<LOQ		
	10107F	0.60	0.00	<LOQ		NA
	10108F	0.60	0.00	<LOQ	<LOQ	NA
	9922M**	0.40	2.12	<LOQ		
	9930M**	0.40	0.00	<LOQ		
	9931M	0.50	1.76	<LOQ		
	9932M	0.50	1.65	<LOQ		NA
	9933M**	0.40	1.45	<LOQ	<LOQ	NA
Group 2 1 mg/kg/day 0.2 mg/mL	10121F	0.70	0.00	<LOQ		
	10126F	0.50	0.00	<LOQ		
	10136F	1.00	0.00	<LOQ		
	10140F	1.00	0.00	<LOQ		NA
	10142F	0.60	0.00	<LOQ	<LOQ	NA
	9961M**	0.40	0.00	<LOQ		
	9964M	0.50	0.270	<LOQ		
	9965M**	0.20	0.00	<LOQ		
	9967M	0.50	0.740	<LOQ		NA
	9970M**	0.40	0.00	<LOQ	<LOQ	NA
Group 3 5 mg/kg/day 1.0 mg/mL	10155F**	0.40	3.29	<LOQ		
	10156F	0.60	5.20	<LOQ		
	10164F	0.70	2.06	<LOQ		
	10172F	0.60	0.00	<LOQ		NA
	10177F	0.70	2.96	<LOQ	<LOQ	NA
	9997M	0.70	10.1	<LOQ		
	9999M**	0.30	4.15	<LOQ		
	10001M	0.70	8.41	<LOQ		
	10002M	0.60	12.5	<LOQ		NA
	10004M	0.50	10.6	<LOQ	<LOQ	NA
Group 4 10.0 mg/kg/day 2.0 mg/mL	10187F	0.60	9.35	<LOQ		
	10194F	0.80	14.6	<LOQ		
	10203F	0.70	11.6	<LOQ		
	10211F	0.80	13.6	<LOQ		NA
	10214F	0.70	6.26	<LOQ	<LOQ	NA
	10019M	0.60	8.01	<LOQ		
	10024M**	0.30	9.20	<LOQ		
	10029M**	0.40	11.5	<LOQ		
	10033M	0.50	15.3	<LOQ		NA
	10034M	0.50	22.2	<LOQ	<LOQ	NA
Group 5 15.0 mg/kg/day 3.0 mg/mL	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR	NR	NR
	10042M	0.80	18.7	<LOQ		
	10044M	0.80	15.9	<LOQ		
	10045M	0.50	10.4	<LOQ		
	10051M	1.00	15.0	<LOQ		NA
	10054M	0.90	17.3	<LOQ	<LOQ	NA

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, PFOSAA = 20.5 ppb, EtFOSE = 36.2 ppb, PFOSEA = 18.2 ppb, M356 =

Correction factors not applicable for MS/MSD QC data

* Tentative value, concentration was not within the range of the curve, it's approximately 15% above the highest standard. LAC 03/24/00

NR = Sample not received nor reported

Date Entered/By: 03/11/00, 03/23/00, 03/24/00 LAC

Date Verified/ By:

** Tentative values, initial volume below 0.5 mL. LAC 08/31/00

FACT-TOX-013
Argus# 418-009

Study:	Argus 418-009, Two-Generation Reproduction Study of EtFOSE-OH in Rats
Product Number(Test Substance):	EtFOSE-OH (T-6316.5)
Matrix:	Rat Serum
Method/Revision:	ETS-8 4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799, Sonp 030199
Instrument Software/Version:	MassLynx 3.3
Filename:	See listing to the right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	10/12/99 RWW
Dates of Analysis/Analyst:	10/14/99, 10/20/99, 10/22/99, 03/16/00 MMH/IAS
Date of Data Reduction/Analyst:	10/15/99, 10/21/99, 10/25/99, 03/17/00, 03/23/00 MMH/IAS

Sample Data

RAT SERA F0

Group Dose	Sample #	Initial Vol. mL	M556 Conc. ng/mL	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	10129-H2O Blk-1	1	8.70	<LOQ (49.5 ppb)		
	10129-H2O Blk-2	1	2.90	<LOQ (24.9 ppb)	<LOQ	NA
Matrix Blk	RBS10129-Sera Blk-1	1	0.00	<LOQ (49.5 ppb)		
	RBS10129-Sera Blk-2	1	0.00	<LOQ (24.9 ppb)	<LOQ	NA
QC-100 ppb	RBS10129-MS-1	1	170	69%		
	RBS10129-MSD-1	1	171	69%	69%	0%
Group 1 Control 0.0 mg/kg/day 0 mg/mL	10097F	0.70	0.00	<LOQ (24.9 ppb)		
	10105F	0.70	0.00	<LOQ (24.9 ppb)		
	10106F	0.50	0.00	<LOQ (24.9 ppb)		
	10107F	0.60	0.00	<LOQ (24.9 ppb)		NA
	10108F	0.60	0.00	<LOQ (24.9 ppb)	<LOQ	NA
	9922M**	0.40	9.05	<LOQ (24.9 ppb)		
	9930M**	0.40	9.11	<LOQ (24.9 ppb)		
	9931M	0.50	0.00	<LOQ (24.9 ppb)		
	9932M	0.50	8.67	<LOQ (24.9 ppb)		NA
	9933M**	0.40	0.00	<LOQ (24.9 ppb)	<LOQ	NA
Group 2 1 mg/kg/day 0.2 mg/mL	10121F	0.70	130	1.86		
	10126F	0.50	210	4.19		
	10136F	1.00	95.2	0.952		
	10140F	1.00	115	1.15		61.4
	10142F	0.60	148	2.45	2.12	1.30
	9961M**	0.40	938	4.69		
	9964M	0.50	1296	5.18		
	9965M**	0.20	454	4.54		
	9967M	0.50	859	3.44		20.3
	9970M**	0.40	1222	6.11	4.79	0.975
Group 3 5 mg/kg/day 1.0 mg/mL	10155F**	0.40	173	43.3		
	10156F	0.60	134	22.3		
	10164F	0.70	126	18.0		
	10172F	0.60	102	17.0		47.0
	10177F	0.70	125	17.9	23.7	11.1
	9997M	0.70	204	29.1		
	9999M**	0.30	221	73.6		
	10001M	0.70	176	25.1		
	10002M	0.60	228	38.1		47.2
	10004M	0.50	189	37.8	40.7	19.2
Group 4 10.0 mg/kg/day 2.0 mg/mL	10187F	0.60	234	39.0		
	10194F	0.80	205	25.6		
	10203F	0.70	277	39.6		
	10211F	0.80	230	28.8		18.5
	10214F	0.70	236	33.8	33.3	6.16
	10019M	0.60	171	71.3		
	10024M**	0.30	123	102		
	10029M**	0.40	152	94.9		
	10033M	0.50	182	90.9		22.5
	10034M	0.50	113	56.7	83.2	18.7
Group 5 15.0 mg/kg/day 3.0 mg/mL	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		
	NR	NR	NR	NR		NR
	NR	NR	NR	NR	NR	NR
	10042M	0.80	200	62.4		
	10044M	0.80	178	55.6		
	10045M	0.50	188	93.8		
	10051M	1.00	123	30.7		38.0
	10054M	0.90	200	55.5	59.6	22.6

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 pg

Correction factors not applicable for MS/MSD QC data

* Tentative value, concentration was not within the range of the curve

Date Entered/By: 03/13/00, 03/23/00, 03/24/00 LAC

Date Verified/ By:

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctanesulfonamide

PFOSAA = Perfluorooctanesulfonamidoacetate

EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

PFOSEA = Perfluorooctane sulfonyl ethylamide

M556 = Perfluorooctanesulfonylazamidoacetate $C_8F_{17}SO_2N((H)CH_2COO)$

** Tentative values, initial volume below 0.5 mL. LAC 08/31/00

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-013

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

Calculation Used for Group 4, Animal ID 10033M

$$340\ \text{ng/mL} \times 500 \times 0.9275 \times \frac{1\ \text{mL}}{0.5\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} \times 0.864 = \textbf{272 µg/mL}$$

AR—Analytical result from MassLynx summary
DF—Dilution factor
SC—PFOS salt correction constant (0.9275)
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted
PC—PFOS purity correction factor (86.4%)

Appendix G: Contract Lab Report

This appendix includes the following contract laboratory report:

Battelle Memorial Institute, Study Number: N003604-D,
2 (N-Ethylfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction,
(59 pages)

Battelle
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BIOLOGICAL SAMPLE ANALYSIS

Battelle Study Number: N003604-D

3M Environmental Laboratory Study Number: FACT 060998.1

FINAL REPORT

2 (N-Ethylfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

SPONSOR

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

Study Initiation: September 15, 1998

Testing Facility

Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

Prepared By

Patrick L. South, B.S.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

FINAL REPORT

2 (N-Ethylfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

Jon C. Andre, Ph.D.
Battelle Principal Investigator

Date

Richard W. Slauter, Ph.D., D.A.B.T.
Battelle Senior Program Director

Date

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

2 (N-Ethylfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

EXECUTIVE SUMMARY

Rat liver samples sent to Battelle by 3M Environmental Technology and Services were analyzed by the previously validated method "Method for Analysis of Potassium Perfluorooctanesulfonate (PFOS) in Rat Liver by LC/MS/MS". Samples were extracted and analyzed by High-Performance Liquid Chromatography Mass Spectroscopy (LC/MS/MS) for PFOS, M-556, PFOSAA, and PFOSA content only. Related fluorochemicals mentioned in the analytical method were not investigated.

The results for the concentration determinations in the liver samples from this study are attached as appendices to this report. Concentrations are reported as mass of analyte (μg) per gram of liver tissue extracted.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the task leader, study director, and associated management as follows:

Phase Inspected	Inspection Date	Date Reported to Battelle Task Leader/ Battelle Management	Date Reported to Offsite Study Director/ Management
Sample weights	10/12/1999		
Sample homogenization	10/12/1999		
Extraction	10/13/1999		
Sample analysis	10/13/1999		

Quality Assurance Unit
Battelle Memorial Institute

Date

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

Study Title: **2 (N-Ethylfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction**

This study was conducted in compliance with the Food and Drug Administration's Good Laboratory Practice Regulations (21 CFR 58), with the exception that the mass spectrometry data for the liver samples was collected and processed with the MassLynx software system (version 3.1), which was not fully validated. The study was listed on Battelle's Master List of regulated studies.

Jon C. Andre, Ph.D.
Battelle Principal Investigator

Date

Kris Hansen, Ph.D.
Study Director

Date

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Table of Contents

List of Tables

Appendix A (Results)

Appendix B (Daily Acceptance Criteria Summary)

Appendix C (Sample Inventory List)

Appendix D (Chromatograms)

Appendix E (Protocol, Amendments, and Deviations)

Appendix F (PFOS Purity Report)

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

1.0 Introduction

This report presents a description of the method used to analyze PFOS, M-556, PFOSAA, and PFOSA in rat liver samples from 3M Study Number FACT 060998.1 (TOX-013) and the results from this analysis. See Appendix E for a copy of the study protocol, amendments, and protocol deviation reports (signed deviation reports were not available for inclusion here).

2.0 Reference Substances

The analytical reference substances for this study were supplied by 3M. The following lot number or tracking number designations apply: PFOS (lot 171), M-556 (TN-A-2203), PFOSAA (TN-A-1283) and PFOSA (L-15709). Note that based on information supplied to Battelle from 3M, PFOS has two equivalent names. The name appearing on the Material Safety Data Sheet and bottle label is potassium perfluoroalkyl sulfonate. The name more commonly used by 3M in analytical methods and correspondence is potassium perfluorooctanesulfonate. The latter name will be used in this report. See Appendix F for purity data supplied by 3M to Battelle. The reference substances were stored at room temperature.

The surrogate standard was 1H,1H,2H,2H-Perfluorooctane sulfonic acid, lot number 59909, supplied by ICN. The surrogate standard was stored at room temperature.

3.0 Receipt of Samples

Samples were received frozen and intact at Battelle, from 3M Environmental Technology and Services, in one batch on October 6, 1999. Samples were generated by Argus Research under protocol number 418-009. See Appendix C for a copy of the inventory list. The samples were stored at approximately –20°C.

4.0 Analysis of Samples

4.1 Summary of Method

Samples were analyzed by a previously validated method (Battelle study number N003604-A). The current version of the method is attached to this report in Appendix E. Samples were analyzed by LC/MS/MS, and an example of the instrument parameters is listed in Table 1. Note that only PFOS, M-556, PFOSAA, and PFOSA (and the surrogate) were quantitated. The other related fluorochemicals, although present in the stock solutions, were not monitored. Quadratic regressions weighted 1/x were used to construct the calibration curves.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Table 1. Example of Instrument Parameters Used to Analyze Samples

LC/MS/MS System	
Autosampler	Make: Gilson Model: 234
HPLC pumps	Make: Gilson Models: 305 and 306
Mass spectrometer	Make: Micromass Model: Quattro LC with Z-spray source
Analytical column	Keystone Betasil C18, 5µm, 2 x 50 mm, Part No. 055-701-2
Mobile phase components	Component A: Ammonium acetate(2mM):methanol, 60:40, v:v Component B: Ammonium acetate(2mM):methanol, 5:95, v:v
Gradient profile	Time, min / %B / Flow, mL/min 0 / 0 / 0.3 1 / 0 / 0.3 4.5 / 100 / 0.3 6 / 100 / 0.3 6.1 / 100 / 0.6 8.5 / 100 / 0.6 9 / 0 / 0.3 11 / 0 / 0.3
Injection volume	10 µL
Flow	LC column flow at start split to 20 µL/min into the MS
Column temp	Ambient
HPLC pressure	Approximately 840 psi at gradient start
MS source	Electrospray, Negative Ion
Desolvation gas	Nitrogen at ~575 L/hr
Nebulizer gas	Nitrogen at ~80 L/hr
Source block temp	140°C
Desolvation temp	250°C
Cone voltage	70 V for SS, PFOS 20 V for M-556, PFOSAA, PFOSA
Collision energy	40 eV
Collision gas	Argon, gas cell, at ~2.5×10^{-3} mb
Multiplier	650 V
Resolution	12.0 for MS1; 10.0 for MS2
Ions monitored	427>81 MRM transition for SS 499>99 MRM transition for PFOS 556>78 MRM transition for M-556 584>169 MRM transition for PFOSAA 498>78 MRM transition for PFOSA
Total run time	11 minutes
Approximate retention times:	SS: 4 min PFOS: 4.2 min M-556: 4.4 PFOSAA: 4.5 PFOSA: 5

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

4.2 Results

4.2.1 Quality Control

System suitability acceptance criteria were established during the method validation and are included in Appendix C, Section IX *Acceptance Criteria*. Relevant statistics from each sample set are provided in Appendix B. Representative chromatograms are given in Appendix D.

4.2.2 Sample Results

The results of the sample analyses as well as a method detection limit determination are presented in Appendix A All liver samples were initially extracted as undiluted homogenates. After the data were reviewed, dilutions of the homogenates were performed in order to bring analyte concentrations within the calibration range. The first analysis that provided acceptable data for an analyte was used in reporting. Four extraction sets were required to provide data for each analyte.

The limit of quantitation is defined as the concentration of the lowest standard which meets acceptance criteria for accuracy (25% RE). The notation BLOQ denotes "Below Limit of Quantitation" for samples that had concentrations lower than the theoretical concentration for the 0.13 µg/g calibration standard. The notation ALOQ denotes "Above Limit of Quantitation" for samples that had concentrations higher than the theoretical concentration for the 13 µg/g calibration standard. Samples that were initially ALOQ were diluted with blank liver homogenate and re-extracted. Samples that were expected to be ALOQ were first diluted with blank liver homogenate before extraction. The "Corrected PFOS Conc" presented in the results tables is the concentration found for the diluted sample multiplied by its dilution factor (final volume + sample homogenate volume).

The method detection limit (MDL) of PFOS was calculated to be 0.0173 µg/g from the analysis of 7 replicate preparations of 0.13 µg/g calibration standard. The MDL was calculated by multiplying the standard deviation of the found concentrations of the 7 reps by 3.143; the Signal-to-Noise (S/N) ratio was calculated by dividing the mean found concentration of the 7 reps by their standard deviation. The method of MDL determination was provided by the Sponsor.

5.0 Conclusions

All analyses met acceptance criteria unless otherwise noted.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

6.0 Acknowledgements

Acknowledgement of principal contributors participating in the performance of this study at Battelle is presented in the following list.

Participant	Title
Jon C. Andre, Ph.D.	Battelle Principal Investigator
Richard W. Slauter, Ph.D., D.A.B.T.	Senior Program Director
Patrick L. South, B.S.	Mass spectroscopist
Gerke H. van der Zwaag, M.S.	Sample preparation chemist
Bobby C. Braswell, B.A.	Quality assurance auditor
Cynthia J. Ryan, B.S.	Lead quality assurance auditor

7.0 Specimen Storage and Record Archives

All original paper data, as well as electronic copies of data, will be forwarded to 3M for archival. Copies of both the final report and all original paper data generated in conjunction with this study will be maintained by Battelle. All residual liver samples, extracts, and unused test article will be disposed of or returned to the Sponsor as directed by the Sponsor.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

APPENDIX A -RESULTS

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

PFOS, M-556, PFOSAA, PFOSA IN RAT LIVER
BATTELLE STUDY: N003604-D
SPREADSHEET SOFTWARE: EXCEL 97
DATA ENTERED: MANUALLY

Sample	Dose Group	Animal Number	Sample Type	PFOS Conc, µg/g	M-556 Conc, µg/g	PFOSAA Conc, µg/g	PFOSA Conc, µg/g
1	1	9922	Maternal	6.77E-01	BLOQ	BLOQ	BLOQ
2	1	9930	Maternal	9.44E-01	BLOQ	BLOQ	BLOQ
3	1	9931	Maternal	9.66E-01	BLOQ	BLOQ	BLOQ
4	1	9932	Maternal	1.20E+00	BLOQ	BLOQ	BLOQ
5	1	9933	Maternal	1.17E+00	BLOQ	BLOQ	BLOQ
6	2	9961	Maternal	1.34E+02	1.28E+01	1.10E+01	4.49E+00
7	2	9964	Maternal	1.18E+02	1.02E+01	1.54E+01	4.10E+00
8	2	9965	Maternal	1.04E+02	1.16E+01	9.79E+00	2.88E+00
9	2	9967	Maternal	9.34E+01	8.96E+00	8.62E+00	3.86E+00
10	2	9970	Maternal	1.01E+02	1.16E+01	1.04E+01	5.42E+00
11	3	9997	Maternal	4.80E+02	6.36E+01	7.36E+01	1.08E+01
12	3	9999	Maternal	2.71E+02	3.93E+01	2.86E+01	8.41E+00
13	3	10001	Maternal	5.76E+02	6.64E+01	8.52E+01	8.67E+00
14	3	10002	Maternal	2.97E+02	3.80E+01	3.42E+01	8.29E+00
15	3	10004	Maternal	4.47E+02	5.46E+01	6.49E+01	8.41E+00
16	4	10019	Maternal	9.62E+02	8.45E+01	1.22E+02	1.28E+01
17	4	10024	Maternal	9.15E+02	8.75E+01	1.48E+02	1.10E+01
18	4	10029	Maternal	6.43E+02	7.82E+01	8.82E+01	1.12E+01
19	4	10033	Maternal	9.04E+02	1.17E+02	1.29E+02	1.28E+01
20	4	10034	Maternal	6.43E+02	8.22E+01	1.35E+02	1.10E+01
21	5	10042	Maternal	1.41E+03	1.28E+02	1.88E+02	1.84E+01
22	5	10044	Maternal	1.57E+03	1.52E+02	2.06E+02	1.30E+01
23	5	10045	Maternal	1.31E+03	1.33E+02	1.50E+02	1.09E+01
24	5	10051	Maternal	1.23E+03	1.18E+02	1.57E+02	9.80E+00
25	5	10054	Maternal	1.22E+03	1.61E+02	1.65E+02	1.09E+01
26	1	10097	Fetal	1.72E-01	BLOQ	BLOQ	BLOQ
27	1	10105	Fetal	BLOQ	BLOQ	BLOQ	BLOQ
28	1	10106	Fetal	1.40E-01	BLOQ	BLOQ	BLOQ
29	1	10107	Fetal	BLOQ	BLOQ	BLOQ	BLOQ
30	1	10108	Fetal	BLOQ	BLOQ	BLOQ	BLOQ
31	2	10136	Fetal	4.61E+01	2.86E+00	5.01E+00	1.40E+00
32	2	10140	Fetal	2.74E+01	1.55E+00	2.97E+00	9.01E-01
33	2	10142	Fetal	2.58E+01	1.41E+00	2.97E+00	9.08E-01
34	2	10121	Fetal	2.90E+01	1.19E+00	2.88E+00	8.14E-01
35	2	10126	Fetal	2.65E+01	1.75E+00	4.34E+00	7.08E-01
36	3	10177	Fetal	1.21E+02	8.16E+00	2.00E+01	2.88E+00
37	3	10155	Fetal	1.18E+02	7.17E+00	1.58E+01	2.22E+00
38	3	10156	Fetal	1.50E+02	7.84E+00	2.02E+01	2.24E+00
39	3	10164	Fetal	2.07E+02	9.41E+00	2.27E+01	1.84E+00
40	3	10172	Fetal	1.38E+02	5.87E+00	1.19E+01	2.17E+00
41	4	10187	Fetal	1.90E+02	1.98E+01	3.11E+01	2.80E+00
42	4	10194	Fetal	2.78E+02	2.68E+01	5.15E+01	4.09E+00
43	4	10203	Fetal	3.98E+02	2.68E+01	4.95E+01	3.14E+00
44	4	10211	Fetal	2.95E+02	2.75E+01	4.66E+01	3.39E+00
45	4	10214	Fetal	3.08E+02	2.93E+01	5.14E+01	4.56E+00
46	1	10097	Maternal	3.26E-01	BLOQ	BLOQ	BLOQ
47	1	10105	Maternal	2.80E-01	BLOQ	BLOQ	BLOQ
48	1	10106	Maternal	2.81E-01	BLOQ	BLOQ	BLOQ
49	1	10107	Maternal	2.56E-01	BLOQ	BLOQ	BLOQ
50	1	10108	Maternal	2.90E-01	BLOQ	BLOQ	BLOQ
51	2	10136	Maternal	6.21E+01	4.84E+00	8.44E+00	2.36E+00
52	2	10140	Maternal	3.24E+01	3.27E+00	2.62E+00	1.22E+00
53	2	10142	Maternal	8.28E+01	4.56E+00	6.56E+00	2.06E+00
54	2	10121	Maternal	8.33E+01	5.39E+00	6.87E+00	1.90E+00
55	2	10126	Maternal	7.86E+01	7.75E+00	1.44E+01	2.05E+00
56	3	10177	Maternal	1.77E+02	3.14E+01	1.71E+01	6.91E+00
57	3	10155	Maternal	1.03E+02	1.15E+01	1.20E+01	5.11E+00
58	3	10156	Maternal	2.53E+02	3.33E+01	2.73E+01	8.14E+00
59	3	10164	Maternal	2.36E+02	4.31E+01	2.94E+01	8.28E+00
60	3	10172	Maternal	2.18E+02	2.39E+01	3.37E+01	8.20E+00
61	4	10187	Maternal	2.62E+02	3.99E+01	2.74E+01	6.36E+00
62	4	10194	Maternal	3.21E+02	3.96E+01	4.56E+01	9.86E+00
63	4	10203	Maternal	9.19E+02	6.78E+01	7.90E+01	9.83E+00
64	4	10211	Maternal	9.29E+02	6.54E+01	5.62E+01	1.14E+01
65	4	10214	Maternal	3.98E+02	8.45E+01	8.00E+01	8.11E+00

BLOQ = BELOW LIMIT OF QUANTITATION

Analysis data key:

Normal font = October 13, 1999	All samples undiluted
Underline = October 16, 1999	All samples undiluted
Bold = October 18, 1999	All samples diluted
Bold Underline = October 20, 1999	All samples diluted

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD DETECTION LIMIT (MDL) RESULTS
STUDY: N003604-D
ANALYSIS DATE AND INSTRUMENT ID: **13Oct99; 9053**
DATA ENTERED: **Electronically and manually**
SPREADSHEET SOFTWARE: **Excel 97**

All concs in µg/g

	M-556	PFOSAA	PFOSA
Calculated Concentration of Replicate 1	0.1269	0.1484	0.1167
Calculated Concentration of Replicate 2	0.1220	0.1281	0.1325
Calculated Concentration of Replicate 3	0.1433	0.1484	0.1521
Calculated Concentration of Replicate 4	0.1368	0.1605	0.1441
Calculated Concentration of Replicate 5	0.1670	0.1661	0.1490
Calculated Concentration of Replicate 6	0.1225	0.1513	0.1382
Calculated Concentration of Replicate 7	0.1190	0.1385	0.1413
Mean Concentration	0.1339	0.1488	0.1391
Std. Dev.	0.0170	0.0128	0.0119
Spike Level	0.1331	0.1334	0.1315
MDL determined	0.05345	0.04011	0.03725
S/N	7.88	11.66	11.74
Valid	Yes	Yes	Yes
LOQ (det. from 10 x std.dev. "noise")	0.17005	0.12763	0.11853
LOQ (det. from cal curve low std.)	0.1331	0.1334	0.1315
Curve Coeff of Determination	0.9978	0.9937	0.9891
Date analyzed	13Oct99	13Oct99	13Oct99
Method	LC/MS/MS	LC/MS/MS	LC/MS/MS

Key
1 - Spike Level too high; Spike Level must be < 10x MDL
2 - Spike Level too low; Spike Level must be > MDL
3 - S/N too low; S/N must be > 5
4 - Coeff of Det of calibration curve unacceptable

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

APPENDIX B-DAILY ACCEPTANCE CRITERIA SUMMARY

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

PFOS et al IN RAT LIVER
STUDY NUMBER: N003604-D
DATA ENTERED: MANUALLY
SOFTWARE: EXCEL 97

REGRESSION PARAMETERS

Analysis Date	Analyte	X^2 Coeff	X Coeff	Intercept	Coeff of Determination	Comments/ Deviations
October 13, 1999	PFOS	-0.0164	1.86	-0.0772	0.964	
	M-556	-0.00846	0.627	-0.0213	0.998	One rep of Cal pt 1 excluded
	PFOSAA	0.00232	0.0970	-0.00398	0.994	
	PFOSA	-155	1.50E+04	-872	0.989	One rep of Cal pt 1 excluded
October 18, 1999	PFOS	-0.00389	1.82	-0.00920	0.999	One rep of cal pt 7 excluded
	M-556	8.85E-06	0.578	-0.00807	0.996	One rep of cal pt 5 excluded
	PFOSAA	0.00360	0.0931	-0.00321	0.998	
	PFOSA	-250	1.73E+04	-966	0.990	
October 18, 1999	PFOS	0.00393	2.17	-0.0905	0.985	One rep of cal pt 7 excluded
	M-556	0.0221	0.312	-0.00269	0.984	One rep of pts 1, 3, 5 excluded
	PFOSAA	0.00370	0.0853	-0.000603	0.993	One rep of pts 4, 7 excluded
	PFOSA	-71.9	1.23E+04	-722	0.996	
October 20, 1999	PFOS	0.00615	2.07	-0.0361	0.997	
	PFOSA	-153	1.68E+04	-1.02E+03	0.999	

PFOS et al IN RAT LIVER
STUDY NUMBER: N003604-D
DATA ENTERED: MANUALLY
SOFTWARE: EXCEL 97

QC Results

Analysis Date	Analyte	QC Level, ng/mL	%RSD	%RE
October 13, 1999	PFOS	10	8.6	-4.0
		3.3	3.8	-4.8
		0.7	7.9	-6.4
		0.16	3.1	-12.2
	M-556	10	4.7	-2.4
		3.3	11.7	2.5
		0.7	3.0	-6.6
		0.16	11.5	-5.1
	PFOSAA	10	6.5	-1.7
		3.3	5.1	-10.4
		0.7	8.7	-13.0
		0.16	14.5	8.2
	PFOSA	10	16.9	-4.3
		3.3	9.2	-2.1
		0.7	5.8	1.3
		0.16	4.6	3.9
October 16, 1999	PFOS	10	5.5	14.8
		3.3	4.5	15.5
		0.7	8.3	8.8
		0.16	9.7	-2.7
	M-556	10	3.4	-2.8
		3.3	2.7	5.7
		0.7	8.0	-3.7
		0.16	17.7	4.5
	PFOSAA	10	4.3	-0.8
		3.3	2.1	-2.9
		0.7	7.4	-7.0
		0.16	17.7	15.8
	PFOSA	10	11.8	-3.9
		3.3	6.1	5.4
		0.7	8.0	-4.4
		0.16	8.6	13.7
October 18, 1999	PFOS	10	8.7	-4.5
		3.3	11.7	-1.7
		0.7	7.0	-20.3
		0.16	15.1	-8.1
	M-556	10	14.7	-2.9
		3.3	17.0	23.4
		0.7	21.2	11.6
		0.16	28.8	15.4
	PFOSAA	10	14.7	0.8
		3.3	11.4	-1.8
		0.7	15.1	-20.6
		0.16	21.3	-18.5
	PFOSA	10	16.7	0.0
		3.3	11.5	13.7
		0.7	12.6	0.0
		0.16	6.4	16.0
October 20, 1999	PFOS	10	2.4	-8.0
		3.3	2.0	-12.0
		0.7	3.7	-21.7
		0.16	5.3	-16.0
	PFOSA	10	2.3	-1.6
		3.3	4.0	6.5
		0.7	2.3	1.9
		0.16	3.4	11.5

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

APPENDIX C-SAMPLE INVENTORY LIST

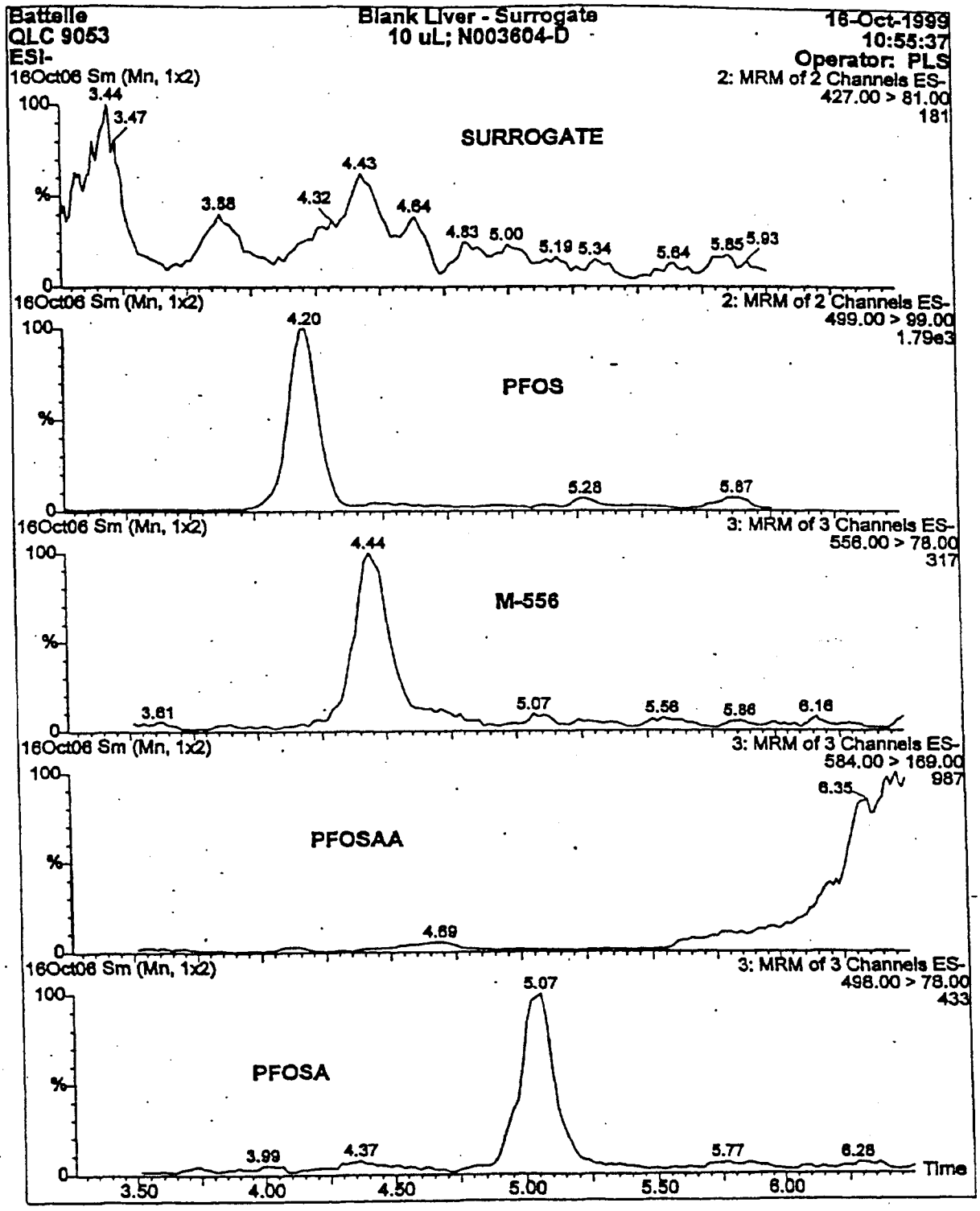
Study TOX-013, Argus 418-009. Sample Information for shipment to Battelle

Sample	Sample Description	Sample Type
1	F0-9922-grpI-M	Maternal Rat Liver
2	F0-9930-grpI-M	Maternal Rat Liver
3	F0-9931-grpI-M	Maternal Rat Liver
4	F0-9932-grpI-M	Maternal Rat Liver
5	F0-9933-grpI-M	Maternal Rat Liver
6	F0-9961-grpII-M	Maternal Rat Liver
7	F0-9964-grpII-M	Maternal Rat Liver
8	F0-9965-grpII-M	Maternal Rat Liver
9	F0-9967-grpII-M	Maternal Rat Liver
10	F0-9970-grpII-M	Maternal Rat Liver
11	F0-9997-grpIII-M	Maternal Rat Liver
12	F0-9999-grpIII-M	Maternal Rat Liver
13	F0-10001-grpIII-M	Maternal Rat Liver
14	F0-10002-grpIII-M	Maternal Rat Liver
15	F0-10004-grpIII-M	Maternal Rat Liver
16	F0-10019-grpIV-M	Maternal Rat Liver
17	F0-10024-grpIV-M	Maternal Rat Liver
18	F0-10029-grpIV-M	Maternal Rat Liver
19	F0-10033-grpIV-M	Maternal Rat Liver
20	F0-10034-grpIV-M	Maternal Rat Liver
21	F0-10042-grpV-M	Maternal Rat Liver
22	F0-10044-grpV-M	Maternal Rat Liver
23	F0-10045-grpV-M	Maternal Rat Liver
24	F0-10051-grpV-M	Maternal Rat Liver
25	F0-10054-grpV-M	Maternal Rat Liver
26	F0-10097-grpI-F	Fetal Liver
27	F0-10105-grpI-F	Fetal Liver
28	F0-10106-grpI-F	Fetal Liver
29	F0-10107-grpI-F	Fetal Liver
30	F0-10108-grpI-F	Fetal Liver
31	F0-10136-grpII-F	Fetal Liver
32	F0-10140-grpII-F	Fetal Liver
33	F0-10142-grpII-F	Fetal Liver
34	F0-10121-grpII-F	Fetal Liver
35	F0-10126-grpII-F	Fetal Liver
36	F0-10177-grpIII-F	Fetal Liver
37	F0-10155-grpIII-F	Fetal Liver
38	F0-10156-grpIII-F	Fetal Liver
39	F0-10164-grpIII-F	Fetal Liver
40	F0-10172-grpIII-F	Fetal Liver
41	F0-10187-grpIV-F	Fetal Liver
42	F0-10194-grpIV-F	Fetal Liver
43	F0-10203-grpIV-F	Fetal Liver
44	F0-10211-grpIV-F	Fetal Liver
45	F0-10214-grpIV-F	Fetal Liver
46	F0-10097-grpI-F	Maternal Rat Liver
47	F0-10105-grpI-F	Maternal Rat Liver
48	F0-10106-grpI-F	Maternal Rat Liver
49	F0-10107-grpI-F	Maternal Rat Liver
50	F0-10108-grpI-F	Maternal Rat Liver
51	F0-10136-grpII-F	Maternal Rat Liver
52	F0-10140-grpII-F	Maternal Rat Liver
53	F0-10142-grpII-F	Maternal Rat Liver
54	F0-10121-grpII-F	Maternal Rat Liver
55	F0-10126-grpII-F	Maternal Rat Liver
56	F0-10177-grpIII-F	Maternal Rat Liver
57	F0-10155-grpIII-F	Maternal Rat Liver
58	F0-10156-grpIII-F	Maternal Rat Liver
59	F0-10164-grpIII-F	Maternal Rat Liver
60	F0-10172-grpIII-F	Maternal Rat Liver
61	F0-10187-grpIV-F	Maternal Rat Liver
62	F0-10194-grpIV-F	Maternal Rat Liver
63	F0-10203-grpIV-F	Maternal Rat Liver
64	F0-10211-grpIV-F	Maternal Rat Liver
65	F0-10214-grpIV-F	Maternal Rat Liver

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

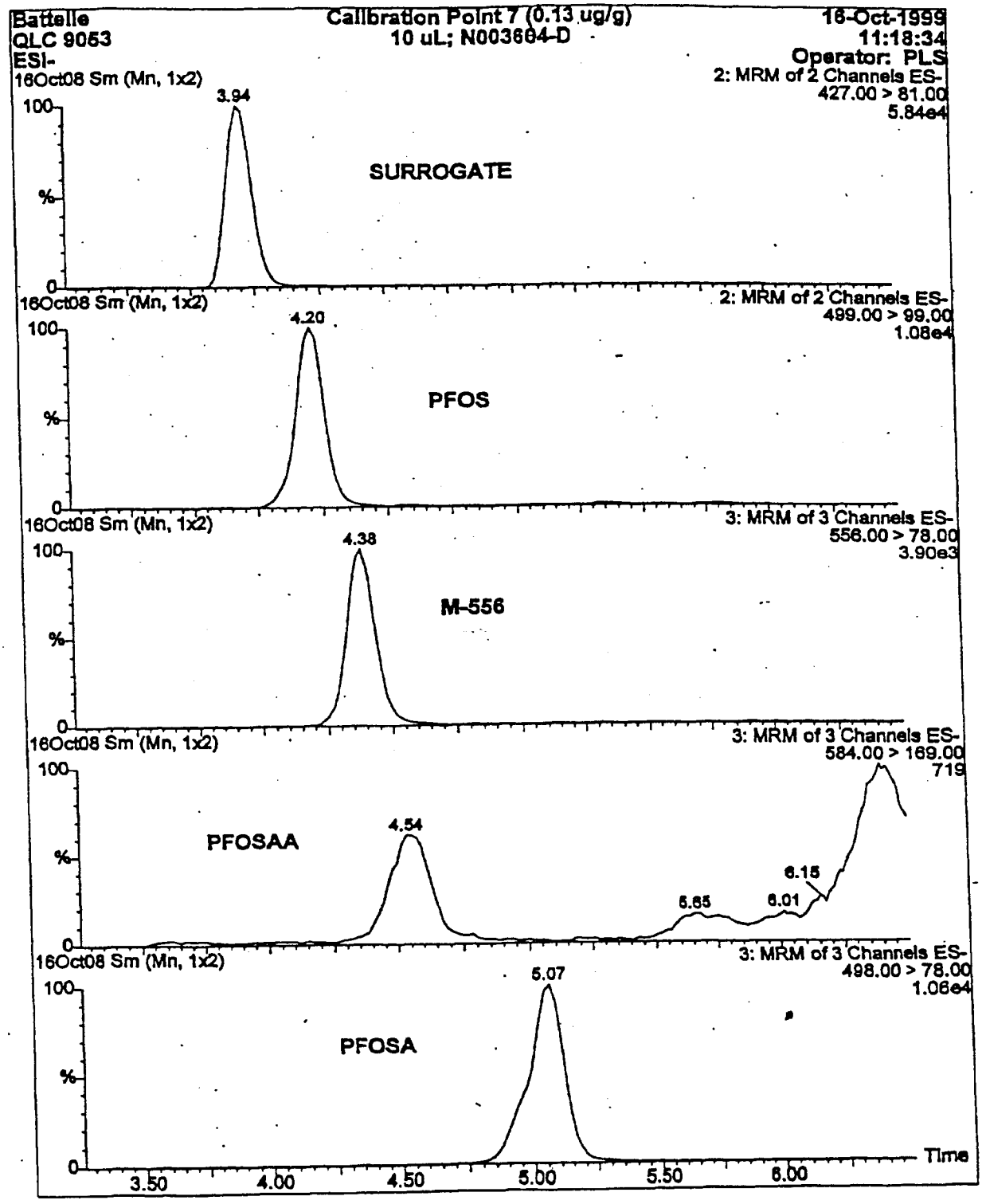
APPENDIX D-REPRESENTATIVE CHROMATOGRAMS

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1



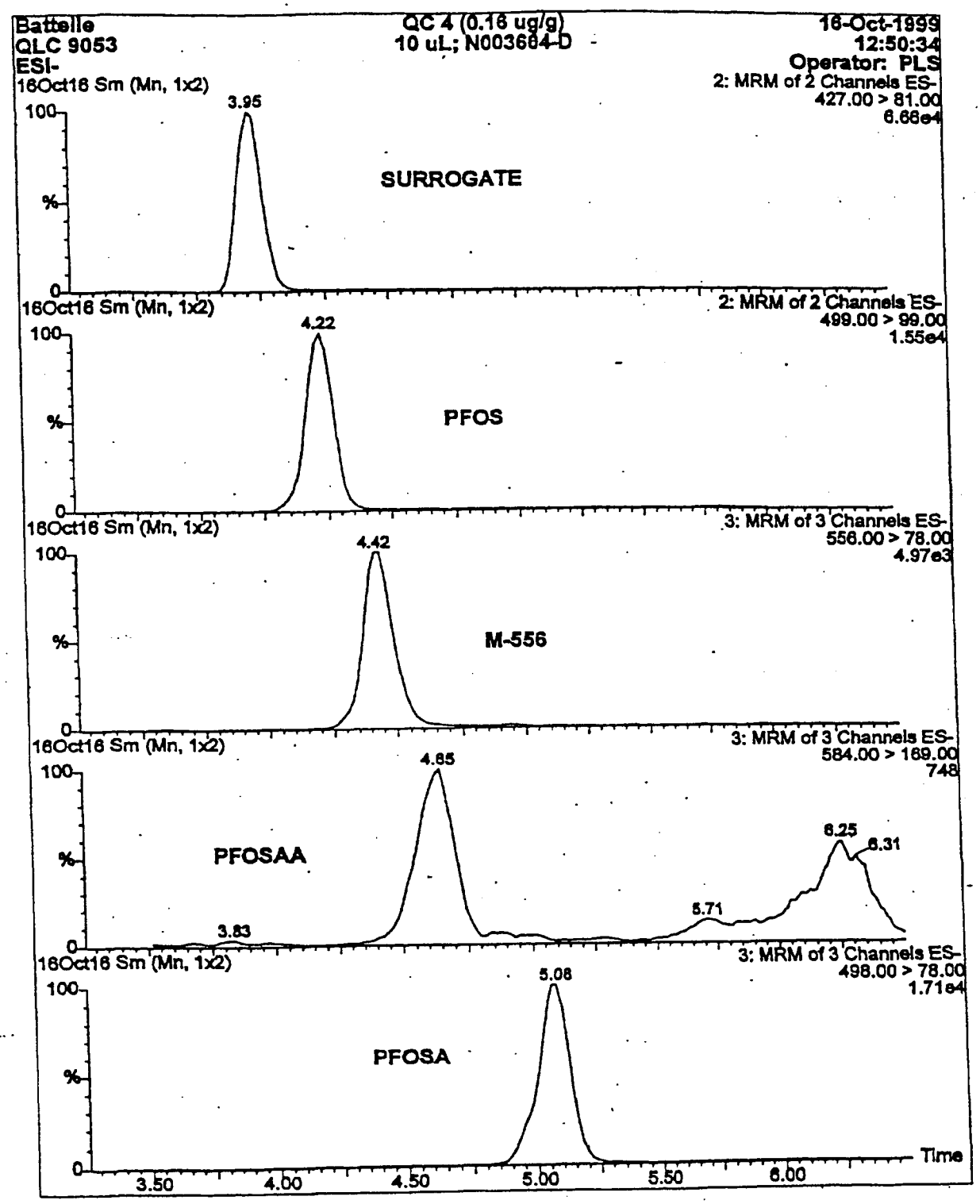
D-1

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1



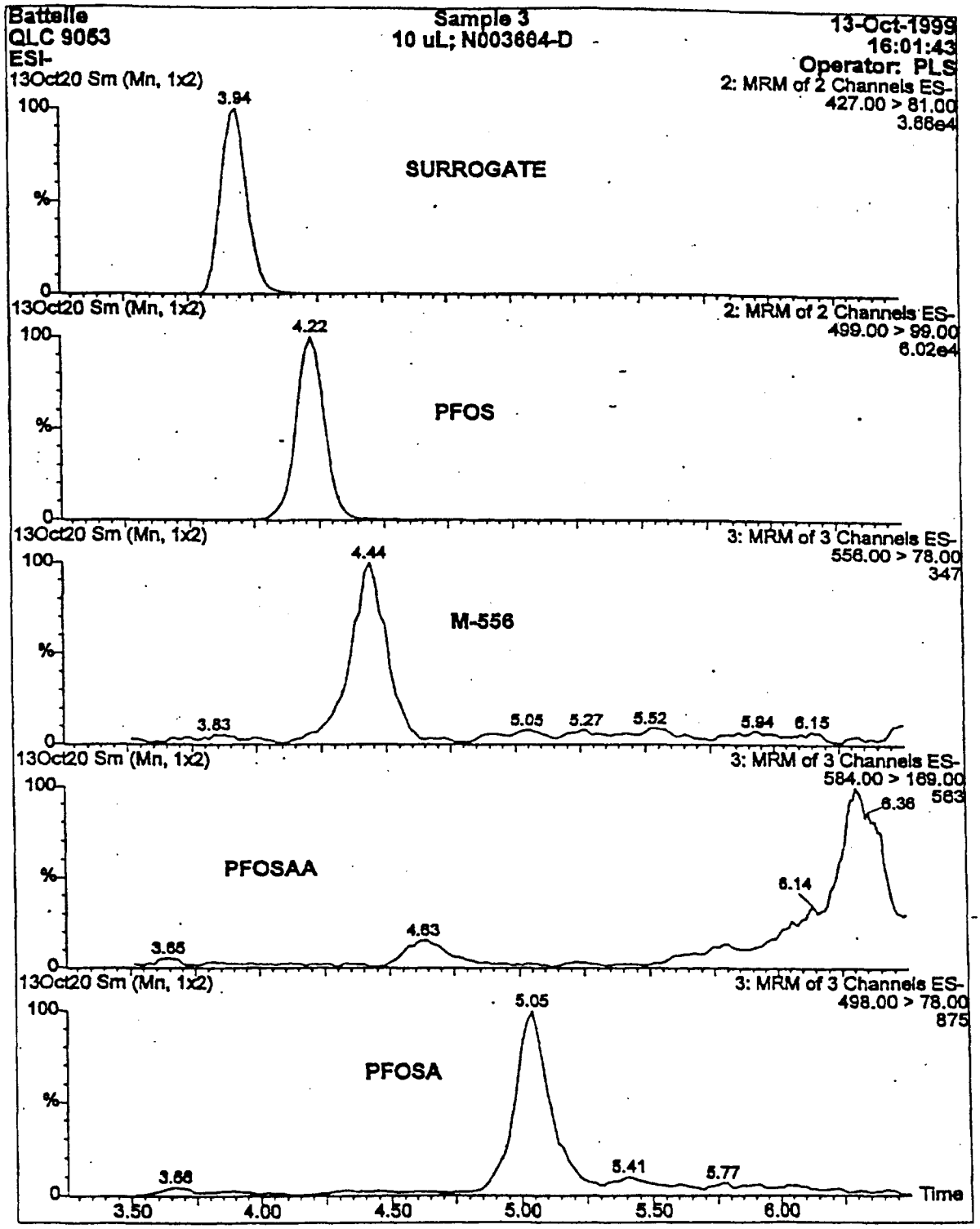
D-2

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1



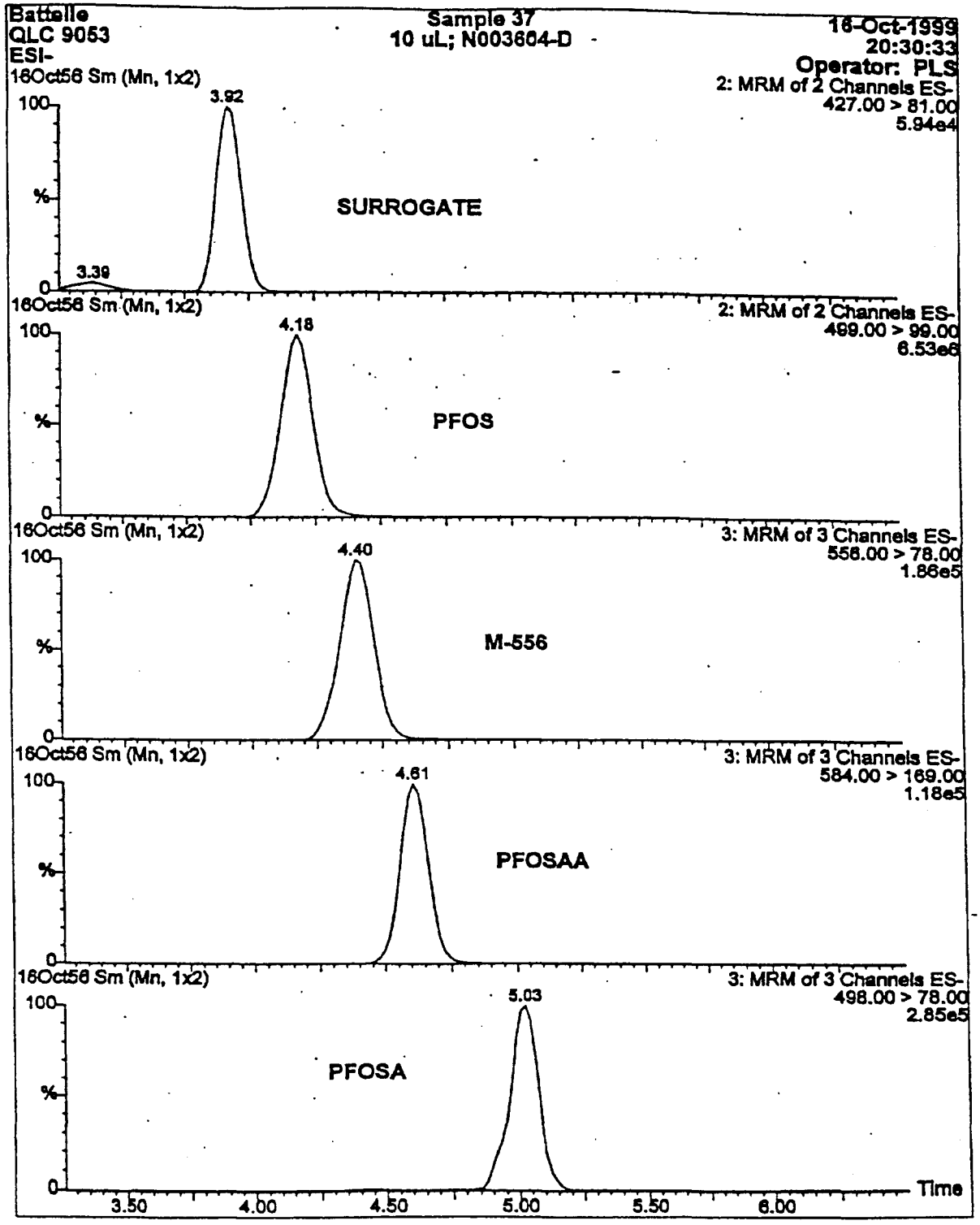
D-3

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1



D-4

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1



Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

APPENDIX E-PROTOCOL, AMENDMENTS, AND DEVIATIONS

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

FACT - TOX - 013
U2095

3M ENVIRONMENTAL LABORATORY

PROTOCOL - ANALYTICAL STUDY
2(N-Ethylperfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

In-vivo study reference number: Argus 418-009
Study number: FACT 060998.1
Test substance: 2(N-Ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH)

Name and address of Sponsor:

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

Name and address of testing facility:

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

Experimental start date:
Expected termination date: December 31, 1998
Method numbers and revisions:

FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

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

FACT-M-3.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

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

Author: Lisa Clemen

Kristen Hansen 9/15/98
Kris Hansen — Date
Study Director

Marvin Case 10 Sept 1998
Marvin Case — Date
Sponsor Representative

1

1.0 PURPOSE

The analytical portion of this dosing study is designed evaluate the levels of perfluorooctane sulfonate (PFOS), or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) designated by the study director, in the liver of the parent and subsequent generations of the test system, or in the serum as necessary.

The in life portion of this study was conducted at Argus Research Laboratories.

2.0 REGULATORY COMPLIANCE

This study is conducted in compliance with the Food and Drug Administration Good Laboratory Practices regulation as stated in 21 CFR 58. Any exceptions will be noted in the final report.

3.0 TEST MATERIALS

3.1 **Test, control, and reference substances and matrices**

- 3.1.1 **Analytical reference substance:** Potassium perfluorooctanesulfonate (PFOS), lot # 217
- 3.1.2 **Analytical reference substance matrix:** Rat liver and serum
- 3.1.3 **Analytical control substance:** None
- 3.1.4 **Analytical control substance matrix:** Rat liver and serum

3.2 **Source of materials**

- 3.2.1 **Analytical reference substance:** 3M Specialty Chemical Division; traceability information will be included in the final report
- 3.2.2 **Analytical reference substance matrix:** Argus Research Laboratories; traceability information will be included in the final report
- 3.2.3 **Analytical control matrix:**
  - 3.2.3.1 Rat liver – Argus Research Laboratories; traceability information will be included in the final report; or

    Rabbit liver – Covance Laboratories; traceability information will be included in the final report
  - 3.2.3.2 Rat serum - Sigma Chemical Company; traceability information will be included in the final report

3.3 **Number of test and control samples.** Liver samples for testing were received from 40 test animals and 10 control animals. Serum samples will be tested at the discretion of the Study Director.

3.4 **Identification of test and control samples:** The samples are identified using the Argus Research Laboratories identifiers, which consist of a letter followed by the Argus project number, the animal number, the group designation, and the draw date.

3.5 **Purity and strength of materials:** Characterization of the purity and identity of the reference material is the responsibility of the Sponsor.

3.6 **Stability of test material:** Characterization of the stability of the test material is the responsibility of the Sponsor.

3.7 **Storage conditions for test materials:** Test materials are stored at room temperature. Samples are stored at –20 ± 10 °C.

3.8 **Disposition of test and/or control substances:** Biological tissues and fluids are retained per GLP regulation.

3.9 **Safety precautions:** Refer to the material safety data sheets of chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

4.0 EXPERIMENTAL - Overview

Tissues from animals dosed as described in Argus Research Laboratories Protocol #418-009 are received for analysis of fluorine compounds. At the discretion of the Study Director, a series of analytical tests will be performed on select tissues.

Initially, all liver samples will be analyzed for PFOS by electrospray/mass spectrometry (ES/MS). On the basis of findings from these analyses, additional sample matrices may be evaluated or other metabolites may be targeted. If additional analysis is performed, a protocol amendment will be written.

5.0 EXPERIMENTAL - Analytical Methods

5.1 FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.2 FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

5.3 FACT-M-3.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

5.4 FACT-M-4.0, Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

6.0 DATA ANALYSIS

6.1 **Data transformations and analysis:** Data will be reported as the concentration (weight/weight) of fluoride per tissue or sample, or of PFOS per unit of tissue or fluid.

6.2 **Statistical analysis:** Statistics used may include regression analysis of the serum 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.

7.0 Maintenance of Raw Data and Records

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

- 7.1.1 Approved protocol and amendments
- 7.1.2 Study correspondence
- 7.1.3 Shipping records
- 7.1.4 Raw data
- 7.1.5 Electronic copies of data

7.2 Supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following:

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

8.0 References

8.1 3M Environmental Laboratory Quality System Chapters 1, 5 and 6

8.2 Other applicable 3M Environmental Laboratory Quality System Standard Operating Procedures

9.0 Attachments

9.1 FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.2 FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

9.3 FACT-M-3.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

9.4 FACT-M-4.0, Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Revisions to the method

Date of Revision	Revision	Version No.

Written by: _____ Date: 18 Aug 99
Patrick L. South

Approved by: _____ Date: August 23, 1999
Jon C. Andre, Ph.D.
Manager, Bioanalytical Chemistry

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____
Analyst/Date: _____

I. SUMMARY

The extraction and analysis of potassium perfluorooctanesulfonate and related fluorochemicals in rat liver is performed. Calibration standards are prepared by spiking blank liver homogenate with solvent standards from two independently-prepared stocks. The calibration standards are fortified with surrogate standard, buffered, and extracted with ethyl acetate. The organic phases are evaporated to dryness and reconstituted in methanol for analysis by LC/MS/MS.

II. PURPOSE

To extract and analyze potassium perfluorooctanesulfonate and related fluorochemical compounds found in Sprague-Dawley rat liver.

III. SAMPLES

See Chain of Custody records if applicable.

IV. GENERAL INSTRUCTIONS

- Calibrate all required balances according to the SOP on balance usage.
- Make equivalent dilutions when the volume needed varies from the volume stated in the method.
- Label all standard and reagent solutions as specified in the appropriate SOP. If you intend to reuse a solution for future tasks, be sure the label includes the preparation date and study number for which the solution was initially prepared.
- Sign on the final page of this method to signify that you have followed the method as written, all materials and reagents are current, and all equipment has been properly calibrated. If you deviate from the method, document the change, and obtain the approval of the unit manager, study director, or task leader as soon as possible.
- Initial and date all data entries on the page on which they were made. If only one person enters all data on a single day, the documentation may be made in a single location on that page. If multiple staff make entries, the additional entries must be initialed and dated by the person making the entry.
- Line-outs or NA denotes "Not Applicable".
- The method is written in general chronological order, but the sequence of steps may be altered if the analyst deems it appropriate, unless the order for certain activities is specified.
- Stocks will be used for the duration of the study unless consumed or unless stability is considered suspect.
- No correction will be made for purity or salt content of any test article but PFOSAA.
- Use glass volumetric, Eppendorf repeater, or positive-displacement pipets for dispensing methanolic solutions.
- Contact with Teflon by the test article should be minimized.

V. MATERIALS

See Table 1 for all required chemicals, reagents, and solvents. Use Table 1 for documentation. Check all labels carefully to ensure that all materials are not expired and that they are the proper purity or grade.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____
Analyst/Date: _____

Table 1. Materials

Materials	Use	Supplier	Grade or Purity	Storage Temp	Lot or ID
Potassium Perfluorooctane-sulfonate (PFOS)	Analytical Standard	3M		Room Temp	
1H,1H,2H,2H-Perfluorooctane Sulphonic Acid	Surrogate Standard	ICN		Room Temp	
M-536	Analytical Standard	3M		Room Temp	
M570	Analytical Standard	3M		Room Temp	
PFOSAA	Analytical Standard	3M		Room Temp	
PFOSA	Analytical Standard	3M		Room Temp	
PFOSEA	Analytical Standard	3M		Room Temp	
Rat Liver	Matrix	Harlan	Sprague-Dawley	−20°C	
Ammonium Acetate, $NH_4C_2H_3O_2$	Mobile Phase			RT	
Sodium Hydroxide, NaOH	Reagent Prep			RT	
Tetrabutylammonium Hydrogensulfate (TBA), $[CH_3(CH_2)_3]_4N(HSO_4)$	Extract Prep			RT	
Sodium Carbonate, Na_2CO_3	Extract Prep			RT	
Sodium Bicarbonate, $NaHCO_3$	Extract Prep			RT	
Ethyl Acetate	Extract Prep			RT	
Methanol	Mobile Phase, Stocks, WS			RT	
Milli-Q Water	Reagent Prep, Mobile Phase	Millipore	ASTM Type I	RT	
pH 7 Buffer	pH meter calibration			RT	
pH 10 Buffer	pH meter calibration			RT	

RT means Room Temperature.

VI. EQUIPMENT

See Table 2 for all required major pieces of equipment. Use the table to document the actual piece (e.g. make, model) of equipment. Check calibration of all equipment requiring calibration (e.g. balances) to ensure it is current.

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Table 2. Equipment

Equipment	Use	Manufacturer	Model	Y or S/N
Analytical Balance	Weigh Standards or Reagents			
Weight Set	Calibrate Balance			
Pipettor	Pipet Samples			
Pipettor	Pipet Samples		-	
Pipettor	Pipet EtOAc extraction phase			
Pipettor	Pipet Reagents, WIS	Eppendorf	Repeater	
Vortexer	Mix Samples			
Freezer (-20°C)	Store QCs, Blank Liver			
Refrigerator (1-9°C)	Store Buffer, Stocks			
Centrifuge	Phase separation			
Test Tubes	Liver sample homogenization	Stockwell Scientific	Polypropylene, 15 mL	SW8599
Centrifuge Tubes	Extract Samples	Blue Falcon	Polypropylene, 15 mL	2096
Test Tubes	Evaporate Extracts	Blue Falcon	Polypropylene, 12 x 75 mm	2002
Transport tubes	Store QCs	Elkay	5 mL polypropylene	127-T160-56P
Magnetic stirrer	Stir matrix			
Orbital Shaker	Extract Samples			
Evaporator	Evaporate Extracts	Zymark	Turbovap LV	
Syringe Filters	Filter Extract			
Homogenizer	Grind liver			
pH meter	Determine Buffer pH			
Electrode	Determine Buffer pH			
Volumetric Flasks, Class A	Make Volumetric Dilutions	NA	NA	NA
Volumetric Pipets, Class A	Make Volumetric Dilutions	NA	NA	NA
Transfer Pipets, Plastic	Transfer Extracts to Centrifuge Filters and LC Inserts	Samco		

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____
Analyst/Date: _____

VII. PROCEDURE

A. Preparation of 2 mM Ammonium Acetate

Weigh 0.1500 ± 0.0020 g of ammonium acetate and transfer to a 1000-mL volumetric flask. Dissolve the solid in water and dilute to volume with water. Solution may be used for one month stored at room temperature.

Actual mass of ammonium acetate: _____
Actual final volume: _____
Date of preparation: _____
Study No: _____

B. Preparation of ~29% Sodium Hydroxide Solution

Weigh 200 ± 2 g of sodium hydroxide into a beaker. Add 500 mL of Milli-Q water and mix to dissolve. Cool and transfer to a polypropylene bottle for storage. Solution may be stored for 6 months at room temperature.

Actual mass of sodium hydroxide: _____
Actual volume Milli-Q water: _____
Date of preparation: _____
Study No: _____

C. Preparation of ~2.9% Sodium Hydroxide Solution

Add 10 mL of ~29% Sodium Hydroxide Solution to a 100-mL volumetric flask and dilute to volume with Milli-Q water. Transfer to a polypropylene bottle for storage. Solution may be stored for 6 months at room temperature.

Actual volume of ~29% NaOH solution: _____
Actual final volume: _____
Date of preparation: _____
Study No: _____

D. Preparation of Tetrabutylammonium Hydrogensulfate (TBA) Solution, 0.5 M, (pH 10)

pH Meter Calibration

pH buffer: 7 pH reading: _____
pH buffer: 10 pH reading: _____

Add 169 ± 1 g of TBA to ~500 mL of Milli-Q water in a beaker. Adjust the pH to 10.00 ± 0.02 using ~55-60 mL of 29% Sodium Hydroxide Solution, dilute to 1000 mL with Milli-Q water, and mix. Adjust the pH to 10.00 ± 0.02 using ~2.9% NaOH and mix. Transfer to a polypropylene bottle for storage. Solution may be used for one month stored at room temperature, but the pH must be checked prior to each use. Adjust to pH 10.0 ± 0.02 with 2.9% Sodium Hydroxide Solution as necessary.

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____
Analyst/Date: _____

Actual mass of TBA: _____
Actual final volume: _____
Actual final pH: _____
Date of preparation: _____
Study No: _____
pH after rechecking and/or readjusting: _____

E. Preparation of 0.25 M Carbonate Buffer

Weigh 26.5 ± 0.1 g of sodium carbonate and 21.0 ± 0.1 g of sodium bicarbonate and transfer to the same 1000-mL volumetric flask. Dissolve the materials in Milli-Q water, dilute to volume with Milli-Q water, mix, and transfer to a polypropylene bottle for storage. Solution may be used for 1 month when stored refrigerated.

Actual mass of sodium carbonate: _____
Actual mass of sodium bicarbonate: _____
Actual final volume: _____
Date of preparation: _____
Study No: _____

F. Preparation of Mobile Phase

Component A: Mix together 600 mL of 2 mM ammonium acetate and 400 mL of methanol. Solution may be used for 1 month when stored at room temperature.

Actual volume of 2 mM ammonium acetate: _____ mL
Actual volume of methanol: _____ mL
Date of preparation: _____
Study No: _____

Component B: Mix together 50 mL of 2 mM ammonium acetate and 950 mL of methanol. Solution may be used for 1 month when stored at room temperature.

Actual volume of 2 mM ammonium acetate: _____ mL
Actual volume of methanol: _____ mL
Date of preparation: _____
Study No: _____

G. Preparation of Stock Surrogate Standard and Working Surrogate Standard (WSS)

1. Stock Surrogate Standard (250,000 ng/mL):

Weigh 25 ± 2 mg of 1H, 1H, 2H, 2H,-perfluorooctane sulphonic acid and transfer to a 100-mL volumetric flask. Dissolve in methanol, dilute to volume with methanol, and mix. Store refrigerated, protected from UV light.

Actual Weight: _____
Actual Dilution Volume: _____
Date of Preparation: _____
Study No: _____

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS
Version 1.0

Study No.: _____
Analyst/Date: _____

2. WSS (1000 ng/mL):

Dilute 100 µL of stock surrogate standard to 25 mL with methanol and mix.

Actual Volume of Stock Internal Standard: _____
Actual Dilution Volume: _____
Date of Preparation: _____

H. Preparation of Calibration Solvent Stocks and Working Standards

1. Solvent Stocks:

For each analyte weigh the specified amount of standard (independently weighed as A and B replicates) listed in Table 3 and transfer into separate volumetric flasks. Dissolve in methanol, dilute to volume with methanol, and mix well. Store refrigerated, protected from UV light.

2. Mixed Solvent Stocks:

Pipet the specified amount of each analytical standard Replicate A as listed in Table 3 and transfer into a single volumetric flask. Dissolve in methanol, dilute to volume with methanol, and mix well. Store refrigerated, protected from UV light. Repeat the process with Replicate B stocks. *The mixed solvent stocks are used to prepare the working standards.*

Date of preparation: _____
Study No: _____

3. Working Standards (WS):

Dilute the mixed stocks and working standards with methanol as specified in Table 3 and mix well.

Date of preparation: _____

Table 3. Calibration Solvent Stocks and Working Standards

Standard	Source	Target Amount	Actual Amount Analytical Std. Stock, or WS	Target Final Vol (mL)	Actual Final Vol. (mL)	Nominal Conc. ng/mL
Stock 1A	PFOS	50 ± 1 mg		10		5,000,000
Stock 1B	PFOS	25 ± 0.5 mg		10		2,500,000
Stock 2A	M-356	50 ± 1 mg		10		5,000,000
Stock 2B	M-356	25 ± 0.5 mg		10		2,500,000
Stock 3A	M570	50 ± 1 mg		10		5,000,000
Stock 3B	M570	25 ± 0.5 mg		10		2,500,000
Stock 4A	PFOSAA	93 ± 1 mg		10		5,000,000
Stock 4B	PFOSAA	46 ± 0.5 mg		10		2,500,000
Stock 5A	PFOSA	50 ± 1 mg		10		5,000,000
Stock 5B	PFOSA	25 ± 0.5 mg		10		2,500,000
Stock 6A	PFOSEA	50 ± 1 mg		10		5,000,000

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Stock 6B	PFOSEA	25 ± 0.5 mg	[redacted]	10	[redacted]	2,500,000
Mixed Stock A	Stocks 1 thru 6 Rep A	5 mL each**	[redacted]	50	[redacted]	500,000
Mixed Stock B	Stocks 1 thru 6 Rep B	5 mL each**	[redacted]	50	[redacted]	250,000
WS 1	Mixed Stock A	1 mL **	[redacted]	25	[redacted]	20,000
WS 2	Mixed Stock B	1 mL **	[redacted]	25	[redacted]	10,000
WS 3	WS 1	2 mL**	[redacted]	10	[redacted]	4000
WS 4	WS 2	2 mL**	[redacted]	10	[redacted]	2000
WS 5	WS 3	2.5 mL**	[redacted]	10	[redacted]	1000
WS 6	WS 4	2.5 mL**	[redacted]	10	[redacted]	500
WS 7	WS 5	2 mL**	[redacted]	10	[redacted]	200

* Weigh all analytical standards to at least the nearest 0.01 mg.
** Use volumetric or positive-displacement pipet(s).

I. Preparation of Calibration Standards and Blanks

1. Liver homogenate

Prepare blank liver homogenate in bulk by weighing approximately 40 g of blank liver into a 500 mL Nalgene bottle containing 200 mL of Milli-Q water. Grind to a homogeneous suspension. Aliquot into approx 30 mL portions for frozen (approx –20°C) storage.

Actual Mass of Liver:_____
Actual volume of water:_____
Date of prep: _____ Study: _____

Determine density of calibration/QC matrix:

MIX HOMOGENATE THOROUGHLY and determine the mass in milligrams of 10 replicate weighings of 1 mL portions of the THOROUGHLY MIXED homogenate. MIX HOMOGENATE IMMEDIATELY PRIOR TO EACH ALIQUOT REMOVAL.

Table 4. Calibration Stds/QCs Matrix Density

Replicate #	[redacted]									
Mass (mg)										

2. Liver Calibration Standards

Prepare each liver calibration standard by adding 0.45 mL of undiluted liver homogenate (STIR HOMOGENATE WHILE ALIQUOTING) into a 15 mL extraction tube and adding 50 µL of WS or MeOH. Prepare triplicate cal standards and 6 blanks. See Table 5 for volumes. The diluted liver density is assumed to be approximately 150 mg/mL. Mix well.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Table 5. Calibration Standards and Blanks

Cal Std/Blank	Source	Target Vol (µL)	Actual Vol (µL)	Target Final Vol (mL)	Actual Final Vol (mL)	Nominal Conc (ng/mL)	Nominal Conc (µg/g)
1	WS 1	50		0.5		2000	13
2	WS 2	50		0.5		1000	6.6
3	WS 3	50		0.5		400	2.6
4	WS 4	50		0.5		200	1.3
5	WS 5	50		0.5		100	0.66
6	WS 6	50		0.5		50	0.33
7	WS 7	50		0.5		20	0.13
Blank	MeOH	50		0.5		0	0

Date of preparation of cal stds/blank: _____

J. Preparation of Quality Control Liver Samples (QCs)

1. Quality Control Working Standards

Dilute the following source volumes methanol in volumetric flasks and mix well. Prepare fresh when used. Actual volumes are in parentheses.

Table 6. QC WS Preparation

Soln ID	Source	Vol Source, mL	Final Vol, mL	Conc, ng/mL
QC WS 1	Mixed Stock A			15,000
QC WS 2	Mixed Stock B			5000
QC WS 3	QC WS 1			1125
QC WS 4	QC WS 2			250

2. Preparation of Quality Control Liver Samples

Prepare each QC in bulk by filling the volumetric flask approximately half full with undiluted liver homogenate (STIR HOMOGENATE WHILE ALIQUOTING), adding the appropriate QC WS, mixing, and diluting to volume with undiluted liver homogenate (STIR HOMOGENATE WHILE ALIQUOTING). MIX THOROUGHLY and dispense 2.5-mL aliquots into polypropylene tubes and store at approximately -20°C.

Table 7. QC Preparation

QC	Source	Vol Source, mL	Final Vol, mL	Conc, ng/mL	Conc, µg/g
1	QC WS 1			1500	10
2	QC WS 2			500	3.3
3	QC WS 3			112.5	0.7
4	QC WS 4			25	0.16

Date of QC prep: _____ Study: _____

K. Preparation of MS Check Standard for System Suitability

Pipet 250 µL of WS 2 at ~10,000 ng/mL and 2.5 mL of WSS at ~1000 ng/mL in methanol into the same 50-mL volumetric flask. Dilute to volume with MeOH and mix.

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

L. Preparation of homogenizer recovery liver samples

To determine the recovery from the homogenization process, unhomogenized blank liver will be fortified in duplicate at 3 concentration levels and homogenized as follows. This needs to be done every day that homogenization of study samples is performed.

1. Place approximately 0.5 g of unhomogenized blank liver into each of 6, 15 mL polypropylene centrifuge tubes. Record weights of liver.
2. Add 100 µL of WS 1, 3, and 4 (one WS per duplicate tubes) to prepare fortifications at approximately 4, 0.8, and 0.4 µg/g.
3. Multiply the mass of liver in g by 2.5 and add this many mL of water.
4. Homogenize each liver sample, and rinse homogenizer probe with another volume of water used in step 3, adding rinse to homogenized sample.
5. Clean homogenizer with MeOH between samples.
6. Cap and vortex homogenate for use in extraction.

M. Preparation of Dilution Check Sample

1. Place 2.95 mL of undiluted liver homogenate (STIR HOMOGENATE WHILE ALIQUOTING) into a 15 mL extraction tube and add 50 µL of Mixed Stock A.
2. Dilute 50 µL of step 1 solution (VORTEX SOLUTION WHILE ALIQUOTING) with 0.45 mL of undiluted liver homogenate (STIR HOMOGENATE WHILE ALIQUOTING) in 3, 15 mL extraction tubes.
3. This sample should be prepared for extraction only on days when study samples will be diluted and extracted.

N. Homogenization of study samples

1. Place approximately 0.5 g of unhomogenized study sample liver into a 15 mL polypropylene tube. Record weights of liver.
2. Multiply the mass of liver in g by 2.5 and add this many mL of water.
3. Homogenize each liver sample, and rinse homogenizer probe with another volume of water used in step 2, adding rinse to homogenized sample.
4. Clean homogenizer with MeOH between samples.
5. Cap and vortex homogenate for use in extraction.

O. Analysis Standards, Blanks, QCs, and Samples

1. MIX LIVER HOMOGENATES THOROUGHLY BEFORE ALIQUOTING and pipet 500 µL of each QC (4 replicates per level), and other samples being extracted into 15-mL polypropylene extraction tubes. The cal stds and blanks are already aliquoted.
2. To the Blanks – IS (3 reps), add 100 µL of MeOH and vortex.
3. To the Blanks + IS (3 reps) and to the remaining samples, add 100 µL WSS and vortex.
4. Add 0.5 mL of 0.5 M TBA (pH 10) to all tubes and vortex briefly.
5. Add 1 mL of 0.25 M carbonate buffer and vortex briefly.
6. Add 2.5 mL of ethyl acetate. Place the tubes sideways on the orbital shaker at a setting of 300 for ~20 minutes.
7. Centrifuge tubes at a setting of 3500 rpm for ~20 minutes to separate layers.
8. Transfer 2 mL of the top organic layer to a clean polypropylene tube.

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

9. Evaporate to dryness under nitrogen at a setting of 30°C for ~60 minutes.
10. Reconstitute the residues in 500 µL of methanol with vortexing.
11. Syringe-filter extracts into autosampler vials for analysis. Store vials refrigerated (up to 1 month) if LC/MS/MS will not be performed the same day. Since 3-day room temperature extract stability was demonstrated during validation, the extracts of the cal stds, blanks, and QCs may be reused for up to 3 days after their initial preparation if held at room temperature (recap the vials if reusing).

Date of cal std/blank extract prep: _____
Date of QC extract prep: _____

P. LC/MS/MS Analysis

1. Use the system conditions specified in Table 8. The conditions which are designated may be modified by the analyst to produce acceptable peak shape.

Table 8 - LC/MS/MS Conditions

LC/MS/MS System	
Autosampler	Make: _____ Model: _____ ID: _____
HPLC Pumps	Make: _____ Model: _____ ID: _____
Mass Spectrometer	Make: Model: ID:
Analytical column	Keystone Betasil C18, 5µ, 2 x 50 mm, Part No. 055-701-2, S/N: ; Lot:
Mobile Phase Components	Component A: Ammonium acetate:methanol, 60:40, v:v Component B: Ammonium acetate:methanol, 5:95, v:v
Gradient profile	Time, min / %B / Flow, mL/min 0 / 0 / 0.3 1 / 0 / 0.3 4.5 / 100 / 0.3 6 / 100 / 0.3 6.1 / 100 / 0.6 8.5 / 100 / 0.6 9 / 0 / 0.3 11 / 0 / 0.3
Injection volume	10 µL (µL)
Flow split	LC column flow split to *30 µL/min (µL/min) into the MS at run start
Column Temp	Ambient
HPLC Pressure	1000 psi at gradient start (psi)
MS Source	Electrospray, Negative Ion
Desolvation gas	*Nitrogen at 575 L/hr (L/hr)
Nebulizer gas	*Nitrogen at 80 L/hr (L/hr)
Source Block Temp	*140°C (°C)
Desolvation Temp	*250°C (°C)
Cone voltage	*70 V (V) PFOS, 18 *20 V (V) PFOSA, PFOSAA, PFOSEA, M-356, M570
Collision energy	*40 eV (eV) PFOS, 18, PFOSA, PFOSAA, M-356, M570 *30 eV (eV) PFOSEA
Collision gas	Argon at *2.5 x 10^{-3} mb gas cell (mb)
Multiplier	*650 V (V)
Resolution	*12.0 for MS1 ( ); *10.0 for MS2 ( )

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Ions monitored	427>81 MRM transition for Surrogate Standard (SS) 499>99 MRM transition for PFOS 556>78 MRM transition for M-556 570>169 MRM transition for M570 584>169 MRM transition for PFOSAA 498>78 MRM transition for PFOSA 526>169 MRM transition for PFOSEA
Total run time:	*11 minutes (_____ min)
Approximate retention times:	SS: 4 min (_____ min) PFOS: 4.3 min (_____ min) M-556: 4.5 min (_____ min) M570: 4.6 min (_____ min) PFOSAA: 4.7 min (_____ min) PFOSA: 5.1 min (_____ min) PFOSEA: 5.7 min (_____ min)

* Parameters that may be changed by the analyst. Actual values in ( ).

2. The above conditions should be suitable for the Micromass Quattro LC (S/N 9053). Modifications may be necessary if another Micromass Quattro Series spectrometer is used. Split the flow post-column via a Keystone BIO-tee or similar device.
3. Calibrate the mass spectrometer using a suitable reference compound, or verify that the calibration is suitable by visual inspection (on the tune page) that a suitable mobile phase ion is still accurately determined. Resolution may need to be higher than that used for analyzing samples.
4. To check the proper performance of the instrument, inject the instrument check standard. The results should be comparable to a recent injection if available.
5. Use an automated chromatography integration software system to collect the output from the analysis.
6. Loading Order: See the loading report from the automated chromatography integration software system.
7. Make single injections of each cal standard, QC, study sample, or blank. Make at least 4 injections of the instrument check standard.
8. Run set sizes should typically not exceed 80 injections due to instrument response roll-off considerations. Longer runs may be performed, but they pose a risk of yielding unacceptable curve results.

VIII. CALCULATIONS

1. Spreadsheet Software: _____ Version _____
2. MS Analysis Software: _____ Version _____
3. Calculate the average density of the liver homogenate (10 reps) in mg/mL.
4. Using the average density of the homogenate, calculate its liver density (mg of liver per mL of diluted homogenate):

   Undiluted liver density (mg/mL) = (g of liver x average density of homogenate)/(g of liver + g of water)

   where g of liver and g of water are masses used to prepare bulk homogenate; density of water is assumed to be 1 g/mL.

   Diluted liver density (mg/mL) = Undiluted density ÷ Dilu Factor

METHOD FOR ANALYSIS OF POTASSIUM
PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS
Version 1.0

Study No.: _____
Analyst/Date: _____

Where dilu factor = 2/1.8 = 1.1111 to account for 10% dilu of liver homogenate in cal std and QC matrices.

5. Calculate the actual concentration (ng/mL) of PFOS and other fluorochemicals in the suspensions of calibration standards and QCs by using the mass of analytes and dilution factors only (no liver density correction). Use purity correction for PFOSAA only.

6. Calculate the actual concentration of PFOS and other fluorochemicals in liver for the calibration standards and QCs as follows:

$$\text{Conc}(\mu g/g) = \text{Conc (ng/mL)} \div \text{Diluted Liver density (mg/mL)} \times 1000\ mg/g \times 10^{-3}\ \mu g/ng$$

7. Assure that the integrations of the peak areas of the test article and surrogate standard are correct. Flag manual integrations where performed. Calculate the exact concentration of each liver standard.

8. Calculate the regression equation relating the peak response ratio (test article/SS) of each calibration standard (y-axis) to test article concentration in liver (x-axis) for PFOS, M-356, M570, and PFOSAA. Calculate the regression equation relating the peak area of each calibration standard to test article concentration in liver for PFOSA and PFOSEA. PFOS, M-556, M570, and PFOSAA are quantitated by using the surrogate standard as an internal standard; PFOSA and PFOSEA are quantitated without reference to the surrogate (external standard calibration curve). Use a quadratic regression weighted 1/x , origin excluded, for all analytes.

9. Calculate a determined concentration for each injection of calibration standard, QC, and sample using the regression parameters and the peak response ratios or areas.

10. Calculate the relative error, average relative error, standard deviation, and relative standard deviation for all QCs. Calculate the relative error for each injection of calibration standard.

11. Calculate the average recovery for the homogenizer recovery fortifications.

12. Calculate the relative standard deviation for the PFOS to SS peak area ratio of the replicate injections of the check standard.

IX. Acceptance Criteria

A. MS Check Standard (System Suitability)

At least 3 injections of the MS Check Standard must provide a %RSD of 10% or less for the PFOS to SS peak area ratio.

B. Calibration Standards

The percent relative errors for the concentration-level averages of the calibration standards should meet the following limits:

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.0

Study No.: _____

Analyst/Date: _____

Table 9. Calibration Standard Acceptance limits

ANALYTE	[illegible]
PFOS	20 (2.5% at LOQ)
M-556	20 (2.5% at LOQ)
M570	20 (2.5% at LOQ)
PFOSAA	20 (2.5% at LOQ)
PFOSA	20 (2.5% at LOQ)
PFOSEA	25 (30% at LOQ)

Up to 5 calibration standard injections may be excluded from the curve, provided that one injection remains per level. Removal of an entire level may be done if approval is obtained. If an entire level is removed, the samples bracketed by the remaining calibration range will be considered acceptable. The calibration curve should have a coefficient of determination of 0.97 or better.

C. QCs

The concentration-level average percent relative errors and percent relative standard deviations of the QCs should meet the following limits:

Table 10. QC Acceptance limits

ANALYTE	[illegible]
PFOS	20
M-556	20
M570	20
PFOSAA	20
PFOSA	20
PFOSEA	25

Removal of individual values from the QC calculations may be done if accompanied by a reasonable explanation (e.g., instrument malfunction or Dixon's Q test results).

If the average determined concentration for any QC level exceeds the acceptance limit, the task leader or study director should be notified. The run may be repeated or a portion of the run may be considered acceptable. For example, if the low QC fails the stated requirements, samples may be accepted that have concentrations bracketed by the highest calibration standard and a mid-level QC concentration.

D. Homogenizer Recovery and Dilution Check Samples

The average recovery across the 3 levels of homogenizer recovery samples as well as that of the dilution check samples should fall within the range of 70-130% inclusive. Removal of individual outliers from the calculations may be done if accompanied by a reasonable explanation.

E. Sensitivity (LOQs)

The validated limits of quantitation are nominally 0.13 µg/g each for PFOS, M-556, M570, and PFOSAA.

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS

Version 1.8

Study No.: _____

Analyst/Date: _____

For PFOSA and PFOSEA, the validated LOQs are nominally 0.33 µg/g each. Due to the nature of the preparation of the calibration standards, lower concentrations of PFOSA and PFOSEA will be carried through the extraction. These lower concentration values will be evaluated with each run set, and may be included in the regressions if they meet acceptance criteria. If they are included, study samples which are quantitated to have concentrations below the validated level (nominally 0.33 µg/g) will be appropriately flagged.

F. Specificity

The method suffers from endogeneous matrix interferences at levels sometimes exceeding 20% of LOQ. The intercept of the calibration curve appears to offer some correction for any effect on quantitations. Acceptabe performance (error) of the lowest used standard, therefore, will be considered sufficient evidence that bracketed study samples are quantified properly.

G. General

The above acceptance criteria indicate that this method is capable of producing occasional errors outside the normal acceptance criteria of a validated method (15% normally). Where indicated, replicate analyses lessen the impact of these occasional outliers.

X. RESULTS

See attached hard copy of spreadsheet or see file on network drive.

XI. COMMENTS

METHOD FOR ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE (PFOS) IN RAT LIVER BY LC/MS/MS
Version 1.0

Study No.: _____
Analyst/Date: _____

XII. CONCLUSIONS

_____

XIII. SIGNATURES

Analysts

_____ Date: _____
_____ Date: _____
_____ Date: _____
_____ Date: _____

Technical Review

_____ Date: _____
_____ Date: _____

QC Review

_____ Date: _____
_____ Date: _____

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Study Title

Combined Oral (Gavage) Fertility Development and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:

July 28, 1999

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX-013
LIRN U2095

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Protocol FACT-TOX-013
Amendment 1

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

1. ***PROTOCOL READS:*** The proposed study completion date is listed as 12/31/98.

   AMEND TO READ: The proposed study completion data is 6/30/00.

   REASON: The proposed completion date was changed to allow time for analyzing all matrices of interest.

Amendment Approval

Marvin Case Ph.D., Sponsor Representative — Date: 30 July 1999

Kris J. Hansen Ph.D., Study Director — Date: 8/2/99

3M Environmental Laboratory

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Study Title

Combined Oral (Gavage) Fertility Development and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:

September 10, 1999

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX-013
LIRN U2095

3M Environmental Laboratory

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Protocol FACT-TOX-013
Amendment 2

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

1. ***PROTOCOL READS:*** The protocol states that liver will be extracted and analyzed at the 3M Environmental Laboratory.

   AMEND TO READ: The liver specimens will be extracted and analyzed at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

   REASON: The liver specimens will be sent to Battelle Memorial Institute for extraction and analysis due to time constraints in the 3M Environmental Laboratory.

2. ***PROTOCOL READS:*** The protocol states that serum specimens will be extracted and analyzed following methods:

   FACT-M-3.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
   FACT-M-4.0, "Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

   AMEND TO READ: The serum specimens will be extracted and analyzed following methods:

   ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray Mass Spectrometry"
   ETS-8-5.1, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts HPLC-Electrospray Mass Spectrometry"

   REASON: The extraction and analytical methods FACT-M-3.0 and FACT-M-4.0, respectively, were updated on 04/27/99 to ETS-8-4.1 and ETS-8-5.1.

3. ***PROTOCOL READS:*** The protocol states that liver specimens will be extracted and analyzed following methods:

   FACT-M-1.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic surfactants from Liver for analysis Using HPLC-Electrospray/Mas Spectrometry"
   FACT-M-2.0, "Analysis of Friuorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

   AMEND TO READ: The liver specimens will be extracted and analyzed following method:

   Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat liver by LC/MS/MS, Version 1.0

   REASON: Since the liver extraction and analysis was sub-contracted to Battelle Memorial Institute, this amendment was written to include their liver methods and titles.

Amendment Approval

Marvin T Case — 28 Sept 1999

Marvin Case Ph.D., Sponsor Representative — Date

Kristen Hansen — 9/29/99

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

3M Environmental Laboratory

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Study Title
Analytical Study 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Two Generation Rat Reproduction

PROTOCOL AMENDMENT NO. 3

Amendment Date:
October 4, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX-013
LIRN U2095

3M Environmental Laboratory

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Protocol FACT Tox-013
Amendment Number 3

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

1. ***PROTOCOL READS:***
Kristen J. Hansen, Ph.D. is the Study Director.

AMEND TO READ:
James K. Lundberg, Ph.D. is the Study Director.

REASON:
Original study design has changed due to availability of resources and James K. Lundberg will begin serving as the study director for FACT-TOX-013 as of 4 October 1999.

2. ***PROTOCOL READS:***
Section 7.1 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; and electronic copies of data. Additionally, Section 7.2 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 7 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

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

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

Protocol FACT Tox-013
Amendment Number 3

3. ***PROTOCOL READS:***
Disposition of test and control substances: Biological tissues and fluids are retained per GLP regulation.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens 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.

Amendment Approval

Marvin T Case — 4 October 1999
Marv Case, D.V.M., Ph.D., Sponsor Representative — Date

James K Lundberg — 5 oct 1999
James K. Lundberg, Ph.D., Study Director — Date

Kristen Hansen — 10/5/99
Kristen J. Hansen, Ph.D., Previous Study Director — Date

D L Bacon — 10/15/99
Dale L. Bacon, ~~Ph.D.~~, 3M Environmental Laboratory Management — Date

3M Environmental Laboratory

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

DEVIATION REPORT

Battelle Study Number: N003604-D
3M Environmental Technology and Services Study Number: FACT 060998.1

2 (N-Ethylperfluorooctanesulfonamido)-ethanol in
Two Generation Rat Reproduction

TYPE OF DEVIATIONS: PROTOCOL

DATES OF DEVIATIONS: October 18, 1999

NATURE OF DEVIATIONS: Some of the analytical method acceptance criteria were not met for the LC/MS/MS analysis conducted 18Oct99 at Battelle. These deviations relate to protocol amendment 2. See below for summary.

Analyte	Acceptance criterion not met
PFOS	QC3 exceeded 20% error (-20.3% actual)
M-556	QC2 exceeded 20% error (23.4% actual)
M-556	QC3 exceeded 20% RSD (21.2% actual)
M-556	QC4 exceeded 20% RSD (28.8% actual)
M-556	Dilution recovery exceeded 130% (131.5% actual with 21.6% RSD)
PFOSAA	QC3 exceeded 20% error (-20.6% actual)
PFOSAA	QC4 exceeded 20% RSD (21.3% actual)

CAUSE OF DEVIATIONS: Sample preparation and/or LC/MS/MS variabilities over the course of the sample set may have contributed to the deviations.

IMPACT OF DEVIATIONS ON THE STUDY: The errant QC values were bracketed by acceptable QC concentration levels which demonstrates that the calibration curves generally provided good accuracy over the tested range. The dilution recovery for M-556 was not considered to be exceedingly high enough, at only approximately 1.5% above the normal acceptance level, to have significantly impacted the data.

CORRECTIVE ACTION: This protocol deviation summary was prepared for inclusion in the final report.

APPROVED BY:

_____ _____
Jon C. Andre, Ph.D. Date
Battelle Principal Investigator

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

N003604-D Protocol Deviation 1, Page 1 of 1

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

DEVIATION REPORT

Battelle Study Number: N003604-D
3M Environmental Technology and Services Study Number: FACT 060998.1

2 (N-Ethylperfluorooctanesulfonamido)-ethanol in
Two Generation Rat Reproduction

TYPE OF DEVIATIONS: PROTOCOL

DATE OF DEVIATIONS: October 20, 1999

NATURE OF DEVIATIONS: PFOS QC3 exceeded the 20% RE method requirement (actual -21.7%). The dilution recovery check standard did not meet the 70-130% recovery requirement (actuals 5.0% for PFOS and 4.6% for PFOSA). These method deviations relate to amendment 2 of the study protocol.

CAUSE OF DEVIATIONS: Sample preparation and/or LC/MS/MS variabilities over the course of the sample set may have contributed to the QC deviation. Sample preparation error appears to have been the cause for the dilution recovery results.

IMPACT OF DEVIATIONS ON THE STUDY: The errant QC value level was bracketed by acceptable QC concentration levels which demonstrates that the calibration curves generally provided good accuracy for study samples over the tested range.

A comparison of the results obtained for the diluted study samples from 20Oct99 and previous results that were slightly ALOQ (13Oct99 and 18Oct99) demonstrated good agreement between the 2 determinations. This would indicate that the dilution of the study samples was performed correctly 20Oct99 so that no impact on the quantitations occurred.

CORRECTIVE ACTION: This protocol deviation summary was prepared for inclusion in the final report.

APPROVED BY:

Jon C. Andre, Ph.D.
Battelle Principal Investigator

Date

Kristen J. Hansen, Ph.D.
Study Director

Date

N003604-D Protocol Deviation 2, Page 1 of 1

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

DEVIATION REPORT

Battelle Study Number: N003604-D
3M Environmental Technology and Services Study Number: FACT 060998.1

2 (N-Ethylperfluorooctanesulfonamido)-ethanol in
Two Generation Rat Reproduction

TYPE OF DEVIATIONS: PROTOCOL

DATES OF DEVIATIONS: October 12, 1999 through October 20,1999

NATURE OF DEVIATIONS: The lot number of PFOS used was not 217 as per section 3.1.1 of the protocol. The source of reference substance matrix was not Argus Research Laboratories as specified in section 3.2.2 of the protocol. Initial analyses of liver did not exclusively target PFOS as per section 4.0 of protocol.

CAUSE OF DEVIATIONS: Only PFOS lot number 171 was available at Battelle. Harlan was the supplier of control rat livers used to prepare blanks, standards, and QCs for the analytical portion of the study. All 4 analytes of interest (PFOS, M-556, PFOSAA, and PFOSA) were monitored during each analysis.

IMPACT OF DEVIATIONS ON THE STUDY: PFOS lot number 171 and Harlan-supplied liver were both used for Battelle's validation of the analytical method (Battelle study number N003604-A). These materials allowed achievement of the reported method acceptance criteria so that there is no impact on the study. The concurrent analysis of PFOS and metabolites was an efficiency improvement.

CORRECTIVE ACTION: This protocol deviation report was prepared.

APPROVED BY:

_____ _____

Jon C. Andre, Ph.D. Date
Battelle Principal Investigator

_____ _____

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

N003604-D Protocol Deviation 3, Page 1 of 1

Battelle Study Number: N003604-D
3M Environmental Laboratory Study Number: FACT 060998.1

APPENDIX F - PFOS PURITY REPORT

3M SPECIALTY ADHESIVES & CHEMICALS ANALYTICAL LABORATORY

Request # 57830

To: Lisa Clemen - (8-5568) – ET&SS- 2-03-09

From: Tom Kestner - (3-3633) - SA&C Analytical Lab - 236-2B-11

Subject: *Chemical Characterization of POSF-Based Fluorochemicals by ^{1}H-NMR & ^{19}F-NMR Spectroscopy*

Date: March 24, 1999: Preliminary report for FC-95 (PFOS), lot 171

SAMPLE DESCRIPTION:

- FC-95, lot 171 (PFOS), TN-A-0834; Nominal product = $C_8F_{17}SO_3(-)$ K(+) (white powder)

INTRODUCTION:

This sample was subjected to ^{1}H-NMR and ^{19}F-NMR spectral analyses to determine the purity of the nominal product and to characterize as many impurity components as possible.

EXPERIMENTAL:

A portion of the sample was accurately weighed, spiked with a known amount of 1,4-bis(trifluoromethyl)benzene (p-HFX), and then totally dissolved in DMSO-d_6 for subsequent analysis by NMR. A 400 MHz ^{1}H-NMR spectrum (# h57830.401) and a 376 MHz ^{19}F-NMR spectrum (# f57830.401) were acquired using a Varian UNITYplus 400 FT-NMR spectrometer. Use of the p-HFX internal standard was intended to permit the determination of the absolute weight percent concentrations of the assigned components without necessarily needing to identify or quantify all the components in the sample mixture.

RESULTS:

The combined NMR spectral data were used to assign all of the major and most of the minor components in this sample as received. The qualitative and quantitative compositional results that were derived from the single trial NMR internal standardization analyses are summarized in TABLE-1 on the following page. I have reported both relative and absolute weight percent concentrations. One possible reason that the absolute wt.% values add up to more than 100% may be due to the fact that I assumed all of the components contained 8 carbons. If there were any shorter chain homologs present (i.e., 7, 6, 5, etc. carbons), then the average compound molecular weights would have been somewhat less than those used in the calculations. In general, the ^{19}F-NMR technique is not particularly well suited for identifying or quantifying small amounts of various fluorochemical homolog impurity components unless the chains are very short. A more complete characterization of any other fluorochemical homologs would require analysis by electrospray MS or a similar technique.

Additional work would be required in an effort to positively verify the tentatively assigned components listed in TABLE-1 (denoted by possible). Small amounts of other unidentified impurities are also detected in the NMR spectra, but additional work would be required in an effort to identify or quantify these other materials.

Copies of the NMR spectra will be provided for you at a later date. If you have any questions about the results in this initial report for FC-95, lot 171, please let me know. I apologize for the delay in completing this initial work.

Tom Kestner

c: Rick Paytor - SA&C Analytical Lab – 236-2B-11

File Reference: LCS7830.DOC/61

March 24, 1999

SA&C Analytical Lab Request # 57830
Initial Report for FC-95, lot 171

TABLE-1

Sample: PC-95, lot 171 (PFOS), TN-A-0834
Overall Quantitative Compositional Results by $^1H/^{19}F$-NMR Internal Standardization Analyses

Structural Assignments	NMR Absolute Weight% Concentrations (single trial measurement)	NMR Relative Weight% Concentrations (single trial measurement)
$CF_3(CF_2)_x$-$SO_3(-)$ K(+) (Normal chain; assume x =7 for calculation purposes)	70.3%	68.6%
$CF_3(CF_2)_x$-$CF(CF_3)$-$(CF_2)_y$-$SO_3(-)$ K(+) (Internal monomethyl branch; assume x+y = 5, x ≠ 0, & y ≠ 0, for calculation purposes)	17.7%	17.3%
$(CF_3)_2CF$-$(CF_2)_x$-$SO_3(-)$ K(+) (Isopropyl branch; assume x =5 for calculation purposes)	10.5%	10.2%
C_xF_{2x+1}-$CF(CF_3)$-$SO_3(-)$ K(+) (Alpha branch; assume x = 6 for calculation purposes)	3.3%	3.2%
Possible F-SF_4-C_xF_{2x}-$SO_3(-)$ K(+) (assume x = 8 for calculation purposes)	0.37%	0.36%
CF_3-$(CF_2)_x$-$C(CF_3)_2$-$(CF_2)_y$-$SO_3(-)$ K(+) (Internal gem-dimethyl branch; assume x+y = 4 and x ≠ 0 for calculation purposes)	0.16%	0.16%
Possible CF_3-SF_4-C_xF_{2x}-$SO_3(-)$ K(+) (assume x = 7 for calculation purposes)	0.11%	0.10%
Probable C_xH_{2x+1}-$SO_3(-)$ K(+) (Hydrocarbon sulfonate salt; assume x = 8 for calculation purposes)	0.031%	0.030%
$(CF_3)_3C$-$(CF_2)_x$-$SO_3(-)$ K(+) (t-butyl branch; assume x = 4 for calculation purposes)	0.027%	0.026%

Appendix H: Interim Certificate of Analysis

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.%

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).

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

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

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

Prepared By: _____ _____
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ _____
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Appendix I: Report Signature Page

Marvin T Case — 9 October 2000

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

John L. Butenhoff — October 9, 2000

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

10/3/00

Dale L. Bacon, *Laboratory Manager* — Date

Kris Hansen 9/29/00

Kris Hansen, PAI