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Document Processing Center (7407)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460
Attn: TSCA Section 8(e) Coordinator

Dear Section 8(e) Docket Coordinator:

Re: TSCA 8(e) Supplemental Notice on Sulfonate-based Fluorochemicals

With this letter, 3M is providing final reports and other supplemental information related to previous TSCA Section 8(e) notifications. Many of the enclosed items are analytical reports providing blood serum and liver levels of test materials for which the in-life report referring to administered doses has already been submitted to the 8(e) docket. In other cases where the 8(e) notification consisted of preliminary data, we are submitting a final study report.

All of the enclosed items are already in EPA's possession and available in TSCA Docket AR-226. We believe, however, that placing these items in the 8(e) docket may allow for more convenient access to information directly related to previous 8(e) notifications by 3M.

The table below lists the enclosed items and references the study or data which already has been the subject of an 8(e) notification by 3M:

Attached Submission	Related Study/Data Already Filed Under 8(e)
1. Amended Analytical Study, 2(N-Ethylperfluorooctane sulfonamido)-ethanol in Two Generation Rat Reproduction, 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 CrI:CDBR VAF/Plus Rats Exposed to EtFOSE-OH, 3M Reference No. T-6316.5, Analytical Report TOX-013, LRN-U2095, June 11, 2001.	Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of N-EtFOSE in Rats, 3M Reference No. T-6316.5, June 30, 1999, full report submitted February 15, 2000 to supplement earlier filing

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Attached Submission	Related Study/Data Already Filed Under 8(e)
<p>2. Analytical Laboratory Report, Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number: 2759-39-3) in the Serum and Liver of Sprague-Dawley® Rats Exposed to PFOS via Gavage, Laboratory Report No. U2006, Requestor Project No. 3M TOX 6295.9, October 27, 1999.</p> <p>3. Report Amendment 1, Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Argus Research Laboratories, Inc., Protocol 418-008, Sponsor's Study No. 6295.9, April 13, 2000.</p>	<p>Combined Oral (Gavage) Fertility, Developmental and Perinatal/Postnatal Reproduction Toxicity Study of PFOS in Rats, Argus Research Laboratories, Inc., Sponsor's Study No. 6295.9, June 10, 1999, full report submitted February 15, 2000 supplementing earlier filing</p>
<p>4. Analytical Report, Determination of the Presence and Concentration of Perfluorooctanesulfonate, Perfluorooctanesulfonylamide, M556, and M570 in the Liver and Sera Samples, 3M Environmental Laboratory Ref. No. U2636, TOX-028, February 23, 2001</p>	<p>13-Week Dietary Study of N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE) in Rats, 3M Ref. No. T-6314.1, Covance Study No. 6329-225, dated June 30, 2000, Section 8(e) filing July 24, 2000</p>
<p>5. Analytical Laboratory Report, Determination of the Concentration of PFOS, PFOSA, PFOSAA, and EtFOSE-OH in the Sera and Liver of CrI:CDBR VAF/Plus Rats Exposed to N-EtFOSE, 3M Environmental Laboratory Report No. TOX-098, Laboratory Request No. U2402, 3M Ref. No. T-6316.7, February 6, 2001.</p>	<p>Final Report, Oral (Gavage) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rats, 3M Reference No. T-6316.7, December 17, 1998, submitted to Section 8(e) docket per letter of August 21, 2000</p>
<p>6. Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (PFOS) or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE) in Liver and Serum Specimens, 3M Environmental Laboratory Report No. TOX-097, Laboratory Request No. U2452, 3M Ref. No. T-6316.8, February 8, 2001</p>	<p>Final Report, Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits, 3M Reference No. T-6316.8, January 11, 1999, submitted to Section 8(e) docket per letter of August 21, 2000</p>
<p>7. Final Report, Alexander, B., Mortality Studies of Workers Employed at the 3M Decatur Facility, University of Minnesota, April 26, 2001.</p>	<p>Preliminary data submitted to Section 8(e) docket in letter of December 15, 2000</p>



Attached Submission	Related Study/Data Already Filed Under 8(e)
<p>8. Final Report, Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0882AR0362, 3M Reference No. T-3290 (40 % K⁺PFOSAA in 3 % EtOH, 17 % IPA and 40 % H₂O, L-6778, F-6873, Lot 501), November 5, 1982 [Bibliography entry in Docket AR-226, final report was to be moved to TSCA 8(e) docket]</p>	<p>Acute Oral Toxicity Screen with T-3290CoC in Albino Rats, Safety Evaluation Laboratory, Riker Laboratories, Inc., Project No. 0882AR0362, 3M Reference No. T-3290 (40 % K⁺PFOSAA in 3 % EtOH, 17 % IPA and 40 % H₂O, L-6778, F-6873, Lot 501), November 5, 1982, submitted to Section 8(e) docket in August 21, 2000 self-audit letter (which erroneously refers to rabbits rather than rats)</p>
<p>9. Giesy, J.P., and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Fish Tissue, Michigan State University, June 20, 2001.</p> <p>10. Giesy, J.P., and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Mink and River Otters, Michigan State University, June 20, 2001.</p> <p>11. Giesy, J.P., and K. Kannan, Perfluorooctanesulfonate and Related Fluorochemicals in Oyster, <i>Crassostrea Virginica</i>, From the Gulf of Mexico and Chesapeake Bay, Michigan State University, June 20, 2001.</p> <p>12. Giesy, J.P. and K. Kannan, Perfluorooctanesulfonate and Related Fluorochemicals in Fish-Eating Water Birds, Michigan State University, June 20, 2001.</p> <p>13. Giesy, J.P. and K. Kannan, Accumulation of Perfluorooctanesulfonate and Related Fluorochemicals in Marine Mammals, Michigan State University, June 20, 2001.</p>	<p>Preliminary data submitted to Section 8(e) docket May 26, 1999</p>

If you have any questions about this submission, please contact me at (651)737-4795.

Sincerely,



Georjean Adams
Manager, 3M Corporate Product Responsibility

Enclosures

Study Title

Oral (Stomach Tube) Developmental Toxicity Study of
2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rabbits

Analytical Laboratory Report Title

Determination of the Presence and Concentration of Potassium
Perfluorooctanesulfonate (PFOS) or another metabolite of
2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE)
in Liver and Serum Specimens

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

At signing

Performing Laboratory

Liver and Serum, Extraction and Analyses

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

Project Identification

3M Medical Department Study: T-6316.8
Argus In-Life Study: 418-010
Analytical Report: FACT TOX-097
3M Laboratory Request No. U2452

Total Number of Pages

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GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (PFOS) or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE) in Liver and Serum Samples
Study Identification Numbers: T-6316.8, FACT TOX-097, LRN-U2452

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

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase was considered to end after teratological examination 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.
- Stability not determined for test and control articles under all conditions of administration.
- Characterizations of all analytical reference materials have not been completed at this time. An amendment to this report will be issued when characterizations have been completed for all reference materials with the exception of PFOSEA. A characterization of PFOSEA will not be completed as all the material was consumed during the analytical phase of the study.
- There were no expiration dates on the reagent/solutions bottle per 21 CFR Part 58.83.
- The electronic data systems have not been validated and there is not an electronic audit trail of corrections currently available (21 CFR Part 58.130 (e)). Hardcopies of chromatograms will be considered as the original raw data.

(See next page for Signatures)

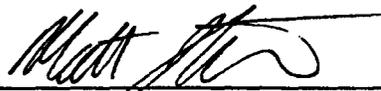
GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (PFOS) or another metabolite of 2(N-ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE) in Liver and Serum Samples

Study Identification Numbers: T-6316.8, FACT TOX-097, and LRN-U2452

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
09/23/98	Sample receipt	11/21/98	11/21/98
10/30/00 – 11/01/00	Data	11/02/00	11/02/00
01/02/01 - 01/05/01	Draft report (1)	01/05/01	01/05/01
01/22/01 – 01/23/01	Draft report (2)	01/23/01	01/23/01


QAU Representative

02/07/01
Date

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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 for a minimum of ten years.

Introduction and Purpose

The purpose of the analytical study is to provide semi-quantitative or qualitative determination of PFOS, PFOSA, PFOSAA, PFOSEA and N-EtFOSE in sera samples and liver samples collected from pregnant rabbits exposed orally to N-EtFOSE. The study was initiated on 18 September 1998.

Test System

The test system were timed-pregnant female New Zealand White [Hra: (NZW)SPF] Rabbits received from Covance Research Products Inc. The individual body weights of the female rabbits ranged from 2.9 to 4.2 kg; the rabbits were approximately five to six months of age at the time of study assignment (Argus Study #418-010).

Table 1 summarizes the number of female rabbits in each group. Group I consisted of control female rabbits that were administered 0.0 mg/kg/day in 2% Tween[®] 80 (vehicle). Group II through Group V female rabbits were administered 0.1, 1.0, 2.5, or 3.75 mg of N-EtFOSE per kg of body weight/day in 2% Tween[®] 80. Serum and liver samples were collected on day 21 of presumed gestation. Additionally, female rabbits were Caesarean-sectioned and pooled fetal tissue(s) taken from the rabbits. Details of the in-life phase of the study are presented in the Argus Laboratory final report, "Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits, #418-010."

Table 1. Dosage Levels, Concentration and Volumes for Argus Study #418-010

Dosage Group	Number of Rabbits	Dosage* (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Assigned Rabbit Numbers
I	3	0 (Vehicle)	0	5	8682F – 8684F
II	5	0.1	0.02	5	8685F – 8689F
III	3	1.0	0.2	5	8690F – 8692F
IV	3	2.5	0.5	5	8693F – 8695F
V	5	3.75	0.75	5	8696F – 8700F

*The test article will be considered 100% pure for the purpose of dosage calculations.

Specimen Collection and Analysis

In the analytical study reported here, 19 liver specimens, 19 sera specimens and 19 pooled fetal tissues were collected from 19 presumed pregnant female rabbits at the end of the in-life phase of Argus Study #418-010 and sent to the 3M Environmental Laboratory to be extracted and analyzed for perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA), perfluorooctanesulfonamido(ethyl)acetate (PFOSAA), N-ethyl perfluorooctanesulfonamido ethyl alcohol (N-EtFOSE), and perfluorooctanesulfonylethylamide (PFOSEA). Only non-quantitative screening data is provided for PFOSAA and N-EtFOSE determination in liver and sera samples.

Blood specimens were centrifuged within one hour of collection. Serum was then harvested and stored in a freezer set to maintain specimens at -70° until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were flash frozen in liquid nitrogen then stored in a freezer set to maintain specimens at -70°C until shipped to the 3M Environmental Laboratory. Pooled fetal tissues (per litter, fetuses and placenta) were collected then stored in a freezer set to maintain specimens at -70°C until shipped to the 3M Environmental Laboratory. (Note: Although fetal and placenta tissues were collected at the same time as liver and serum, results from these analyses will not be included in this report. A separate report may be issued for fetal tissue data.)

Sera and liver samples were extracted beginning on 16 October 1998. Liver samples were homogenized prior to the extraction procedure. Sample extracts were analyzed beginning 17 October 1998 using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple reaction monitoring. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received serum, liver, fetuses and placenta specimens for the *in-life* phase of this study FACT TOX-097 on 23 September 1998 from Argus Research Laboratories. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-097 were obtained from commercial sources and are presented in Appendix A (see Table 5). Samples analyzed at the 3M Environmental Laboratory will be maintained for a maximum period of 10 years and will be stored at the laboratory at -20°C ±10°C.

Chemical Characterization of Analytical Reference Material/Substance

**Table 2. Characterization of the Analytical Reference Materials/Substances in Study
FACT-TOX-097**

Substance	KPFOS ^{a,c}	PFOSA	PFOSAA	N-EtFOSE	PFOSEA ^c
Source	3M	3M Specialty Chemicals	3MICP/PCP Division	3MICP/PCP Division	3M
Formula	C ₈ F ₁₇ SO ₃ -K ⁺	C ₈ F ₁₇ SO ₂ NH ₂	C ₈ F ₁₇ SO ₂ N (CH ₂ CH ₃) CH ₂ COO-H	C ₈ F ₁₇ SO ₂ N (C ₂ H ₅) CH ₂ CH ₂ OH	C ₈ F ₁₇ SO ₂ NHC ₂ H ₅
Expiration Date	01/01/2010	01/01/2010	01/01/2010	01/01/2010	TBD
Storage Conditions	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature	Ambient temperature
Chemical Lot Number	193	L2353	617	936	TN-A-1885
Physical Description	White powder	Amber to brown waxy solid	Yellow to amber liquid	Amber waxy solid	Amber waxy solid
Purity	TBD ^b	TBD ^b	TBD ^b	88.9%	NA

TBD – To be determined

^a The target analyte is PFOS (Perfluorooctanesulfonate), C₈F₁₇SO₃⁻

^b The purity of the substance listed above was based only on NMR analyses. Subsequent chemical characteristics are occurring and this analytical report will be amended to indicate the purity of these substances when a certificate of analysis is issued.

^c Purity will not be determined due to lack of material. See compliance statement.

Note: All reference materials that were used during the course of the study were stored at ambient temperature and were frozen when moved to storage.

Method Summaries

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

As the present study progressed, more advanced methods evolved and earlier methods listed in the protocol were not used. It was determined that applying a 1/X weighting to the curve improved the method accuracy at the low end of the curve. The original data sets were reworked utilizing the improved practice. These changes only improved the effectiveness of the method. Amendments to the protocol were written to cover method changes. A copy of protocol amendments and method deviations are presented in Appendix B (Table 6) of this report.

3M Environmental Laboratory

PREPARATORY METHODS

- **FACT-M-1.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry".
- **FACT-M-3.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Compounds from Serum or Other Fluids for Analysis Using HPLC-Electrospray/Mass Spectrometry".

An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into ethyl acetate. A portion of the ethyl acetate was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, and then filtered through a 3 mL disposable plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

- **ETS-8-4.1**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry,"
- **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into methyl-*tert*-butyl-ether. The extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter, using a 3 mL disposable plastic syringe into glass autosampler vials.

ANALYTICAL METHODS

- **FACT-M-2.0**, "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **FACT-M-4.0**, "Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-5.1**, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

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

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC/ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS (C₈F₁₇SO₃⁻) analysis, was fragmented to produce ion 99 (FSO₃⁻). The characteristic ion 99 was monitored for quantitative analysis (Table 3).

ANALYTICAL EQUIPMENT

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

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

Analytical column: Keystone® Betasil™ C₁₈ 2x100 mm (5 µm)

Column temperature: Ambient

Mobile phase components:

Component A: 2mM ammonium acetate

Component B: methanol

Flow rate: 300 µL/min

Injection volume: 10 µL

Solvent Gradient: 13.5 minutes

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

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

Software: Mass Lynx™ 3.4

Cone Voltage: 20–60 V

Collision Gas Energy: 25–45 eV

Mode: Electrospray Negative

Source Block Temperature: 150°C ±10°C

Electrode: Z-spray

Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Negative Ions Monitored

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS**	499.0	80.0, 99.0, 130.0
PFOSA	498.0	78.0
PFOSAA	584.0	83.0, 169.0
N-EtFOSE	630.0	59.0
PFOSEA	526.0	65.0, 119.0
THPFOS*	427.0	80.0

*Surrogate

** One or more product ions were used in the determination of PFOS.

Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Earlier methods were used with deviations until amendments to the protocol were written. Deviations from the original protocol and methods are documented in the Appendix B.

Data collected prior to November 1999 was reworked in 2000 to accommodate improvements in data reduction methods. Both the original and "reworked" data are archived; reworked data is presented in the final results. The improved methods are documented in the form of method modifications.

Data Quality Objectives and Data Integrity

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

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Acceptable Precision:** Precision is better than 30% for the method.
- **Acceptable Spike Recoveries:** 70–130%
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and daughter ion characterization.

Data Summary, Analyses, and Results

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the extracted standard curve was ≥ 0.980 except for the determination of PFOSAA. Acceptable curves could not be derived from the analysis of some data sets. All PFOSAA data should be regarded as non-qualitative screening data only.
- **Calibration Standards:** Quantitation of the compounds was based on linear regression analysis (1/x weighted) of a single or 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 curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of the compounds was based on the response of one specific product ion using the multiple reaction monitoring mode of the instrument (see Appendix C, Analytical Methods).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within $\pm 30\%$ of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks.
- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest, except as noted in Appendix B (see table 6).
- **Precision:** Precision was not specifically determined within this study, but has been characterized to be better than $\pm 30\%$ for this method.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs at the 3M Environmental Laboratory. Rabbit sera and liver from control animals were spiked prior to extraction. All target analytes were spiked approximately 250 ng/mL or 250 ng/g.

Sera spikes for PFOS, PFOSA, PFOSAA, and PFOSEA were within $\pm 30\%$ of expected values. Spike recoveries studies for N-EtFOSE were dramatically higher than expected, with an average recovery of 196%. The N-EtFOSE data shall be regarded as non-qualitative screening data only.

Liver spikes for PFOS, PFOSA, PFOSAA, and PFOSEA were within $\pm 30\%$ of expected values. Spike recoveries studies for N-EtFOSE were within $\pm 50\%$. The N-EtFOSE data shall be regarded as non-qualitative screening data only.

- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards prior to extraction. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. The surrogate response of each analytical run was monitored to determine that it did not vary more than $\pm 50\%$ from the mean within each analytical run. No problems were observed with these data.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicates the data are quantitative to $\pm 30\%$ or greater with the exception of PFOSAA and N-EtFOSE. The PFOSAA and N-EtFOSE data shall be regarded as non-qualitative screening data only.

Summary of Sample Results

Samples from Dosed Animals: In general, PFOS, PFOSA, PFOSAA and N-EtFOSE levels found in the sera and liver of the test animals increased with dose group. PFOSEA was not detected in sera or liver samples of dosed animals. 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-097.

Statement of Conclusion

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

References

Argus In-life Final Report #418-010, "Oral (Stomach Tube) Developmental Toxicity Study of N-EtFOSE in Rabbits"

Appendix A: Chemical Characterization of Test Material, Control Matrices

Chemical Characterization of 2(N-Ethylperfluorooctanesulfonamido)-ethanol

CAS Number: 1691-99-2 Chemical Formula: C₈F₁₇SO₂N (C₂H₅) CH₂CH₂OH

Molecular Weight: 571.0

Table 4. Characterization of Test Substance in Study FACT-TOX-097

Test Substance	
Chemical Name	N-EtFOSE 2(N-Ethylperfluorooctanesulfonamido)-ethanol
Source	From Sponsor
Expiration Date	5/01/2000
Storage Conditions	Ambient temperature
Chemical Lot #	FM-3929 (Mixture of Lots 30035, 30037, 30039)
Physical Description	Waxy solid
Purity	97.4%

* The purity of the substances listed above was based only on NMR analyses. Subsequent chemical characterization is occurring and this analytical report will be amended to indicate the purity of these substances when a certificate of analysis is issued.
This information is from the in-life protocol.

Characterization of Control Matrices

Table 5. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT-TOX-097

Control Matrix	Rabbit Liver	Rabbit Sera
Source	Coming Hazelton Wisconsin	Sigma
Expiration Date	01/01/2010	01/01/2010
Storage Conditions	-20°C ±10°C	-20°C ±10°C
Chemical Lot #	F00013	96H4639
Physical Description	Rabbit Liver	Rabbit Sera

Appendix B: Protocol, Amendments and Deviations

3M ENVIRONMENTAL LABORATORY

PROTOCOL - ANALYTICAL STUDY Oral (Stomach Tube) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rabbits

In-vivo study reference number: Argus #418-010
Study number: FACT-TOX-97
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

Exact Copy of Original

LAC 9/21/98
Initial **Date**

The original protocol could not be located. This copy will be considered the original.

Name and address of testing facility:

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

LAC 10/15/00

Experimental start date:
Expected termination date:
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 9/18/98
Kris Hansen Date
Study Director

Marvin Case 15 Dec 2000
Marvin Case Date
Sponsor Representative

As the time of Kris Hansen signature, I was Sponsor Representative for the study, and approved the protocol as written since then I have become Study Director for the study. Marvin Case 15 Dec 2000

FACT-TOX-97, U2452
Argus #418-010
Page 1 of 5

1.0 PURPOSE

The analytical portion of this study is designed to evaluate levels of potassium perfluorooctanesulfonate (PFOS), or another metabolite of 2(N-ethylperfluorooctane-sulfonamido)-ethanol (N-EtFOSE-OH) determined by the Study Director, in liver and serum samples of the test system when it is administered directly through a stomach tube.

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: Rabbit liver and serum

3.1.3 Analytical control substance: None

3.1.4 Analytical control substance matrix: Rabbit 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 Rabbit 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 16 test and 3 control animals for the toxicokinetic portion of the study. Liver samples for testing were received from 88 test and 22 control animals for the developmental portion of the study. 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 #418-010 are received for analysis of fluorine compounds. Mated female rabbits were dosed on Day 7 of presumed gestation, with administration continuing through Day 20. At Day 21, serum and liver samples, as well as fetuses and placenta, were taken from rabbits in the toxicokinetic portion of the study. At Day 29 for the rabbits remaining in the study, samples of serum and liver were taken, as well as fetuses and placenta.

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 samples may be evaluated. If additional analysis is performed, a protocol amendment will be written to add the matrices and methods to the protocol.

For analysis performed by the 3M Environmental Laboratory, the methods listed in the analytical methods section will be used. At the discretion of the Study Director, select analysis may be performed by a contract laboratory where competence has been demonstrated, using validated analytical methods. If a contract laboratory is used, the methods and data provided to the Study Director will be identified in the final report.

5.0 EXPERIMENTAL - Analytical Methods

- 5.1 For analysis performed by the 3M Environmental Laboratory, the following methods will be used:
 - 5.1.1 **FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
 - 5.1.2 **FACT-M-2.0,** Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

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

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

5.2 If analysis is performed at a contract analytical laboratory, copies of the validated methods will be included in the data packet provided to the Study Director.

6.0 DATA ANALYSIS

6.1 Data Reporting: For analysis performed by a contract laboratory, the contract laboratory will provide all data to the analytical phase Study Director, and copies of the methods will be attached to the data. The contract laboratory and the data it provides will be identified in the data packet provided by the analytical phase Study Director to the Sponsor.

6.2 Data transformations and analysis: Data will be reported as the concentration (weight/weight) of target analyte per tissue or sample, or of target analyte per unit of tissue or fluid.

6.3 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** Copies of the following validated 3M Environmental Laboratory methods are attached for information purposes:
 - 9.1.1** **FACT-M-1.0**, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
 - 9.1.2** **FACT-M-2.0**, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
 - 9.1.3** **FACT-M-3.0**, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Surfactants from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry
 - 9.1.4** **FACT-M-4.0**, Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry
- 9.2** If a contract analytical laboratory performs analysis, copies of the validated methods performed will be attached to the data packet provided to the Study Director.

Study Title

Analytical Study Oral (Stomach Tube) Development Toxicity Study of
2(N-Ethylperfluorooctanesulfonamide)-ethanol in Rabbits

PROTOCOL AMENDMENT NO. 1

Amendment Date:

18 February 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS LRN-U2452
FACT TOX-097
Argus Study: 418-010
3M Medical Department Study: T-6316.8

3M Environmental Laboratory

*Protocol LRN-U2452
Amendment Number 1*

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

1. PROTOCOL READS:

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

AMEND TO READ:

The role of study director for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of the signing of this amendment.

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 18 February 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.

3M Environmental Laboratory

*Protocol LRN-U2452
Amendment Number 1*

3. PROTOCOL READS:

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

AMEND TO READ:

Method numbers and revisions:

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

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

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:

New methodologies were implemented following the approval of the original protocol for FACT Tox-097.

*Protocol LRN-U2452
Amendment Number 1*

4. PROTOCOL READS:

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

AMEND TO READ:

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

REASON:

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

5. PROTOCOL READS:

3.1 Test, control, and reference substances and matrices

- 3.1.2 Analytical reference substance matrix: Rabbit liver and serum
- 3.1.4 Analytical control substance matrix: Rabbit liver and serum

AMEND TO READ:

3.1 Test, control, and reference substances and matrices

- 3.1.2 Analytical reference substance matrix: Rabbit liver, serum, and pooled fetal tissue(s)
- 3.1.4 Analytical control substance matrix: Rabbit liver, serum, and pooled fetal tissue(s)

REASON:

Analysis of fetal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3M Environmental Laboratory

*Protocol LRN-U2452
Amendment Number 1*

6. PROTOCOL READS:

The analytical portion of this study is designed to evaluate levels of potassium perfluoro-octanesulfonate (PFOS), or another metabolite of 2(N-Ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) determined by the Study Director, in liver and serum samples of the test system when it is administered directly through a stomach tube.

AMEND TO READ:

The analytical portion of this study is designed to evaluate levels of potassium perfluoro-octanesulfonate (PFOS), or another metabolite of 2(N-Ethylperfluorooctanesulfonamido)-ethanol (N-EtFOSE-OH) determined by the Study Director, in liver, serum, and fetal tissue(s) samples of the test system when it is administered directly through a stomach tube.

REASON:

Analysis of fetal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

7. PROTOCOL READS:

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

AMEND TO READ:

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

3M Environmental Laboratory

*Protocol LRN-U2452
Amendment Number 1*

8. PROTOCOL READS:

3.2.3 Analytical control matrix

- 3.2.3.1 Rabbit 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

AMEND TO READ:

3.2.3 Analytical control matrix

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

REASON:

Argus Research Laboratories will be conducting the in life portion of the study.

9. PROTOCOL READS:

3.2.3 Analytical control matrix

- 3.2.3.1 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

AMEND TO READ:

3.2.3 Analytical control matrix

- 3.2.3.1 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
- 3.2.3.3 Pooled fetal tissue(s) – traceability information will be included in the final report

REASON:

Analysis of fetal tissue was added to the scope of the study following the issuance of the original protocol.

Protocol LRN-U2452
Amendment Number 1

Amendment Approval

Marvin T Case 1 March 2000
Marvin T. Case, D.V.M., Ph.D., Outgoing Sponsor Representative Date

John L. Butenhoff 1 March 2000
John L. Butenhoff, Ph.D., Incoming Sponsor Representative Date

Kristen Hansen 24-March-2000
Kristen J. Hansen, Ph.D., Outgoing Study Director Date

Marvin T Case 1 March 2000
Marvin T. Case, D.V.M., Ph.D., Incoming Study Director Date

Study Title

Oral (Stomach Tube) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-
ethanol in Rabbits

PROTOCOL AMENDMENT NO. 2

Amendment Date:

November 21, 2000

Performing Laboratory

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

Laboratory Project Identification

FACT TOX-097
ET&SS LRN-U2452
Argus Study: 418-010
3M Medical Department Study: T-6316.8

3M Environmental Laboratory

*Protocol FACT TOX-097
Amendment No. 2*

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

1. PROTOCOL READS:

There is not a principal analytical investigator assigned for this study.

AMEND TO READ:

The role of principal analytical investigator for the study was assigned to Kristen J. Hansen, Ph.D. as of the signing of this amendment.

REASON:

The role of principal analytical investigator was assigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements.

**3M Environmental Technology
and Services**

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



Study Title

Oral (Stomach Tube) Developmental Toxicity Study of 2(N-Ethylperfluorooctanesulfonamido)-ethanol in Rabbits

PROTOCOL AMENDMENT NO. 3

Amendment Date:

January 23, 2001

Performing Laboratory

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

Laboratory Project Identification

FACT TOX-097
ET&SS LRN-U2452
Argus Study: 418-010
3M Medical Department Study: T-6316.8

3M Environmental Laboratory

*Protocol FACT TOX-097
Amendment No. 3*

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

1. PROTOCOL READS:

Sections 3.0 and 4.0 identify PFOS as the analytical reference substance.

AMEND TO READ:

To include the additional analytical reference substances, PFOSA, PFOSAA, PFOSEA, and N-EtFOSE.

REASON:

To identify all the compounds that was analyzed in the analytical phase of the study.

2. CONSISTENCY:

The test article 2(N-Ethylperfluorooctanesulfonamido)-ethanol is given many different abbreviations in the protocol, study, raw data, and analytical report. Such as EtFOSE, N-EtFOSE, EtFOSE-OH, and N-EtFOSE-OH.

CLARIFICATION:

These different abbreviations are equivalent.

Protocol FACT TOX-097
Amendment No. 3

Amendment Approval

John L. Butenhoff 26 JAN 01
John L. Butenhoff, Ph.D., Sponsor Representative Date

Marvin T. Case 26 Jan 2001
Marvin T. Case, D.V.M., Ph.D., Study Director Date

Kristen J. Hansen 1/24/01
Kristen J. Hansen, Ph.D., Principal Analytical Investigator Date

Record of Deviation

I. Identification	
Study / Project No. FACT-TOX-097	Argus 418-010
Deviation Type <i>(Check one)</i>	<input type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <input type="checkbox"/> Protocol <input checked="" type="checkbox"/> Other:
Document Number(s): 21 cfr 58.120 (A) (11)	Date(s) of occurrence: 9/18/98
II. Description:	
Required Procedure/process: The Sponsor Representative approval of the analytical protocol is required prior to the Study Director's approval.	
Actual Procedure/process: The Study Director approved the analytical protocol before the Sponsor Representative.	
III. Actions Taken:	
<i>(such as amendment issued, SOP revision, etc.)</i>	
This deviation was written. In the future, analytical protocols will be signed by the Sponsor Representative before the Study Director.	
Recorded By <i>Lisa A. Clemens</i>	Date 12/14/00
IV. Impact on Study / Project	
This deviation will not adversely affect the outcome of this study.	
<i>by 12/14/00</i>	
Authorized By <i>(Study Director / Project Lead)</i> <i>John Z. Bistenhoff</i>	Date 15 Dec 2000

Sponsor Representative: John Bistenhoff
3M Environmental Laboratory
Form ETS-4-8.0

Study Director: Marv Case

Deviation No. 1

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

Record of Deviation

I. Identification	
Study / Project No. FACT-TOX-097	Argus 418-010
Deviation Type (Check one)	<input type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <input checked="" type="checkbox"/> Protocol <input type="checkbox"/> Other:
Document Number(s): FACT-TOX-097	Date(s) of occurrence: 10/17/98, 09/28/99
II. Description:	
Required Procedure/process: The protocol states the liver analytical method to follow for this study is FACT-M-2.0.	
Actual Procedure/process: On 10/17/98 no method was listed and on 09/28/99 method ETS-8-6.0 (the extraction method) was listed.	
III. Actions Taken: (such as amendment issued, SOP revision, etc.)	
This deviation was written.	
Recorded By <i>Alex A. Clemen</i>	Date 12/14/00
IV. Impact on Study / Project	
Although no method was listed on 10/17/98, method FACT-M-2.0 was followed as determined by parameters documented in the raw data. On 09/28/99 the extraction method was written in error - analytical method ETS-8-7.0 was used as determined by parameters listed in the raw data. This method is an improvement over FACT-M-2.0. No adverse affect on these data.	
Authorized By (Study Director / Project Lead) <i>John? Butenhoff DEC 15 2000</i>	Date <i>12/14/00</i>

Sponsor Representative: John Butenhoff Study Director: Marv Case

Record of Deviation

I. Identification	
Study / Project No. FACT-TOX-097	Argus 418-010
Deviation Type (Check one)	<input type="checkbox"/> SOP <input type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <input checked="" type="checkbox"/> Protocol <input type="checkbox"/> Other:
Document Number(s): FACT-TOX-097	Date(s) of occurrence: 10/20/98, 10/23/98, 07/29/99
II. Description:	
Required Procedure/process: The protocol states the sera analytical method to follow for this study is FACT-M-4.0.	
Actual Procedure/process: On 10/20/98 no method was listed, on 10/23/98 method FACT-M-4.1 was used, and on 07/29/99 method ETS-8-7.0 (a liver method) was listed.	
III. Actions Taken:	
(such as amendment issued, SOP revision, etc.)	
This deviation was written.	
Recorded By <i>Lisa A. Clemens</i>	Date 12/14/00
IV. Impact on Study / Project	
Although no method was listed on 10/20/98, method FACT-M-4.0 was followed as determined by parameters documented in the raw data. The method FACT-M-4.1, used on 10/23/98 is an improvement over FACT-M-4.0. Method ETS-8-7.0 was recorded in error. Method ETS-8-5.1 was used as determined by parameters listed in the raw data. ETS-8-5.1 is an improvement over FACT-M-4.0; there is no adverse affect on these data. <i>gh 12/14/00</i>	
Authorized By (Study Director / Project Lead) <i>John Z. Buterhoff DEC 15 2000</i> <i>Marv Case</i>	Date 15 Dec 2000

Sponsor Representative: John Buterhoff
3M Environmental Laboratory
Form ETS-4-8.0

Study Director: Marv Case

Deviation No. 3

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

Record of Deviation

I. Identification	
Study / Project No. TOX0097 (LIMS #U2452)	
Deviation type <input type="checkbox"/> SOP <input checked="" type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <i>(Check one)</i>	
<input type="checkbox"/> Protocol <input type="checkbox"/> Other:	
Document number FACT-M-2.1, ETS-8-5.1 and ETS-8-7.0	Date(s) of occurrence 11/25/98, 9/29/99 and 7/29/99
II. Description	
Required procedure/process: Section 14.2.1: Solvent blanks, method blanks, and matrix blanks must be below the lowest standard on the calibration curve.	
Actual procedure/process: Occasionally, the first solvent blank injected for a run was above the LOQ.	
III. Actions Taken <i>(such as amendment issued, SOP revision, etc.)</i>	
Deviation written.	
Recorded by <i>kh</i>	Date 11/20/00
IV. Impact on Study / Project <i>(completed by Study Director or Project Lead)</i>	
In each place a high solvent blank was analyzed, additional solvent blanks or method blanks were analyzed immediately following the high blank. This second injection was below the LOQ. Occassionally, the first injection of a run is high because it may immediately follow injection of a high standard from a previous run. For this reason, more than one blank is typically analyzed prior to the start of a calibration curve. These second and third, etc. blanks are below the LOQ and are more representative of the analytical conditions of the samples; the study data is not adversely affected. <i>kh 11/20/00</i>	
Authorized by <i>John Bulenhoff</i>	Date <i>11/20/00</i>

Sponsor: John Bulenhoff Study Director: Marc Case Deviation No. 4
(assigned by Study Director or Project Lead at the end of study or project)

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

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

FACT-M-3.0, "Extraction of Potassium Perfluorooctane or Other Anionic Fluorochemical Compounds from Serum or Other Fluids for Analysis Using HPLC-Electrospray/Mass Spectrometry," (8 pages)

ETS-8-04.1, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry," (14 pages)

ETS-8-06.0, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry," (14 pages)

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

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

ETS-8-05.1, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry," (9 pages)

ETS-8-07.0, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry," (10 pages)

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCETANESULFONATE OR OTHER ANIONIC FLUROCHEMICAL SURFACTANTS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

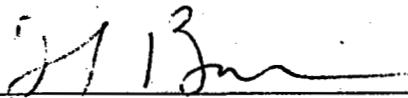
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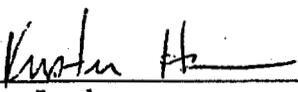
Adoption Date: 5/26/98

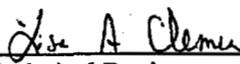
Revision Date: N/A

Author: Lisa Clemen

Approved By:

	5/26/98
Laboratory Manager	Date

	5/26/98
Group Leader	Date

	5/27/98
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

- 3.1 None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

5.0 INTERFERENCES

- 5.1 There are no known interferences at this time.

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS

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

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

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

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L nalgene bottle.
- 8.1.2 Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 1:10. Measure 10 mL of the 10N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL nalgene bottle.
- 8.1.3 Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-Q™ water. Adjust to pH 10 using approximately 64 mL 10N NaOH and dilute to volume with Milli-Q™ water. Add NaOH slowly while adding the last 1 mL of NaOH because the pH changes abruptly. Store in a 1 L nalgene bottle.
 - 8.1.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.
- 8.1.4 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker or equivalent), (Na₂CO₃/NaHCO₃) 0.25M: Weigh approximately 26.5 g of sodium carbonate (Na₂CO₃) and 21.0 g of sodium bicarbonate (NaHCO₃) into a 1 L volumetric flask and dilute to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.
- 8.1.5 PFOS (3M Specialty Chemical Division), molecular weight = 538.
- 8.1.6 Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.
- 8.1.7 Methanol, Omnisolv, glass distilled or HPLC grade.
- 8.1.8 Liver and control liver, received frozen from testing laboratory.
- 8.1.9 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.2 Standards

- 8.2.1 Prepare PFOS standards for the standard curve.

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Matrix Spikes

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

10.2 Continuing Calibration Checks

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Liver Homogenate to Use for Standards

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

- 11.1.4 Add 1 mL of homogeneous solution to a 15 mL centrifuge tube. Re-suspend homogeneous solution by shaking between aliquots while preparing a total of sixteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.
- 11.1.5 Two 1 mL aliquots serve as matrix blanks. Use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

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

- 11.1.1 See section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 Extract spiked liver homogenates following 12.14-12.24 of this method. Use these standards to establish each initial curve on the mass spectrometer.

12.0 PROCEDURES

- 12.1 Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.
- 12.2 Cut approximately 1 g of liver using a dissecting scalpel.
- 12.3 Weigh the sample directly into a tared plastic sample vial.
- 12.4 Record the liver weight in the study notebook.
- 12.5 Label the sample vial with the study number, weight, liver ID, date and analyst initials.
- 12.6 Add 2.5 mLs of water to sample vial.
- 12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.
- 12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.
- 12.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.
- 12.10 Cap the sample and vortex for 15 seconds.

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

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

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

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

14.0 METHOD PERFORMANCE

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCETANESULFONATE OR OTHER ANIONIC FLUROCHEMICAL SURFACTANTS FROM SERUM FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-3.0

Adoption Date: 4/22/98

Revision Date: N/A

Author: Lisa Clemen

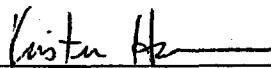
Approved By:



Laboratory Manager

4/22/98

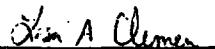
Date



Group Leader

4/22/98

Date



Technical Reviewer

4/22/98

Date

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

- 3.1 None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

5.0 INTERFERENCES

- 5.1 There are no known interferences at this time.

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS

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

- 7.11 Electronic pipettor, Eppendorf or equivalent
- 7.12 Timer
- 7.13 Disposable plastic 3 cc syringes
- 7.14 Filters, nylon syringe filters, 0.2 μm , 25 mm
- 7.15 Crimp cap autovials

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

8.0 REAGENTS AND STANDARDS

8.1 Reagents

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

8.2 Standards

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

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

10.2 Matrix Spikes

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

10.3 Continuing Calibration Checks

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

- 10.3.3 Prepare each continuing calibration check from the same serum used to prep the initial curve.
- 10.3.4 The expected concentration will fall within the mid-range of the initial calibration curve.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Serum Standards

- 11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.
- 11.1.2 If the majority of serum sample volumes are less than 1.0 mL, extract standards using serum volumes in the standards equal to the serum volumes in samples. Do not extract below 0.50 mL of serum. Record the serum volume on the extraction sheet.
- 11.1.3 Mix or shake between aliquots while preparing a total of sixteen aliquots of serum in 15 mL centrifuge tubes.
- 11.1.4 Two 1 mL or appropriate aliquots serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.
- 11.1.5 Refer to the validation report FACT-M-3.0-V-1 and FACT-M-4.0-V-1 which lists the working ranges for calibration curves.

Working Standard (Approx. Conc.)	μL	Approx. final conc. of PFOS in serum
-	-	Blank
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm

- 11.1.4 See section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 Extract spiked serum standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

12.0 PROCEDURES

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

14.0 METHOD PERFORMANCE

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

18.1 None

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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3M ENVIRONMENTAL LABORATORY

METHOD

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

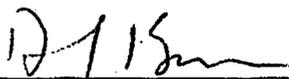
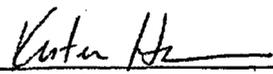
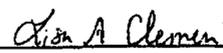
Method Number: ETS-8-4.1

Adoption Date: 03/01/99

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Approved By:

	4/27/99
Laboratory Manager	Date
	4/26/99
Group Leader	Date
	04/26/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

4.0 WARNINGS AND CAUTIONS

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

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

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

- 6.1.4 Nitrogen evaporator, Organomation
- 6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

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

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

8.0 REAGENTS AND STANDARDS

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na₂CO₃), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO₃), 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 ($\mu g/mL$).
- 8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

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

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, C₈F₁₃SO₃H 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.

Working standard (approx. conc.)	μL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 METHOD PERFORMANCE

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 ATTACHMENTS

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

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

<p>Prep date(s):</p> <p>Analyte(s):</p> <p>Sample matrix:</p> <p>Method/revision:</p> <p>Target analyte(s):</p> <p>FC mix std approx. 0.500 ppm:</p> <p>FC mix std approx. 5.00 ppm:</p> <p>FC mix std approx. 50.0 ppm:</p> <p>Surrogate std approx. 100 ppm:</p>	<p>Standard number:</p> <p>Equipment number:</p> <p>Final solvent and TN:</p> <p>Blank fluid/Identifier:</p>
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Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

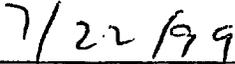
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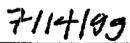
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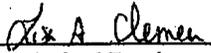
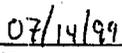
Revision Date: NR

Author: Lisa Clemen, Robert Wynne

Approved By:

	
_____ Laboratory Manager	_____ Date

	
_____ Group Leader	_____ Date

	
_____ Technical Reviewer	_____ Date

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

5.0 INTERFERENCES

- 5.1 There are no interferences known at this time.

6.0 EQUIPMENT

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

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

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

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

8.0 REAGENTS AND STANDARDS

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

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

8.11 Reagent preparation

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

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

8.12 Standards preparation

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

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

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

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, C₈F₁₃SO₃H into a 50 ml volumetric flask and record the actual weight.

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

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Matrix blanks and method blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration verifications

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

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

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

12.0 PROCEDURE

- 12.1 Obtain frozen liver samples.
- 12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.
- 12.3 Weigh the sample directly into a tared plastic sample vial.
- 12.4 Record the liver weight in the study notebook.
- 12.5 Return unused liver portions to freezer.
- 12.6 Add 2.5 mLs of water to sample vial.
- 12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.
- 12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.
- 12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.
- 12.10 Cap the sample and vortex for 15 seconds. Label the sample vial with the study number, weight, liver ID, date and analyst initials.

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

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

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

* refer to 13.1.1 for details.

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

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

*refer to 13.1.2 for details.

14.0 METHOD PERFORMANCE

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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MDL/LOQ values for rabbit liver

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

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

Prep date(s): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Blank liver/identifier:

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

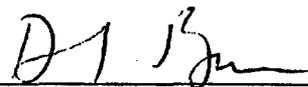
Method Number: FACT-M-2.0

Adoption Date: 5/26/98

Revision Date: N/A

Author: Lisa Clemen

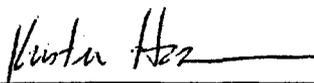
Approved By:



Laboratory Manager

5/26/98

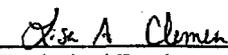
Date



Group Leader

5/26/98

Date



Technical Reviewer

5/27/98

Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

4.2 Cautions:

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

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

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

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1** Methanol, HPLC grade or equivalent.

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

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

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

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

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

10.2 Matrix Spikes

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

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

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

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

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

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

11.0 CALIBRATION AND STANDARDIZATION

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

12.2 Using the Autosampler

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

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

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

- 12.2.2.5** Press the "Start" button.

12.3 Instrument Sep-up

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

- 13.1.2 Calculate percent difference using the following equation:

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

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

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

14.0 METHOD PERFORMANCE

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

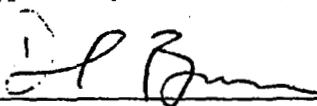
Method Number: FACT-M-4.0

Adoption Date: 4/22/98

Revision Date: N/A

Author: Lisa Clemen

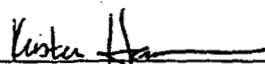
Approved By:



Laboratory Manager

4/22/98

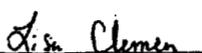
Date



Group Leader

4/22/98

Date



Technical Reviewer

4/14/98

Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

- 3.1** None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

4.2 Cautions:

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

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

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

8.0 REAGENTS AND STANDARDS

8.1 Reagents

- 8.1.1** Methanol, HPLC grade or equivalent.

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

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

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

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Matrix Blanks and Method Blanks

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

10.2 Matrix Spikes

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

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

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

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

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

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

11.0 CALIBRATION AND STANDARDIZATION

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

12.2 Using the Autosampler

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

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

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

- 12.2.2.5** Press the "Start" button.

12.3 Instrument Set-up

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

- 13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, anion in serum ($\mu\text{g/mL}$):

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

14.0 METHOD PERFORMANCE

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

14.2 The practical quantitation limit is equal to the lowest standard in the calibration curve.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

16.1 Store chromatograms in the study folder. Each chromatogram must have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and tape into appropriate study notebook.

16.6 Summarize data using suitable software and store in the study folder.

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Attachment A: FACT-M-4 Data reporting spreadsheet

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCCTANESULFONATE OR OTHER FLUROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

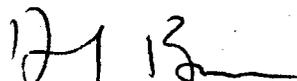
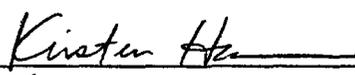
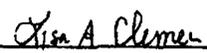
Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

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

1.0 SCOPE AND APPLICATION

- 1.1 **Scope:** This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.
- 1.2 **Applicable Compounds:** Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.
- 1.3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

4.2 Cautions:

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

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS**7.1 Supplies**

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

8.0 REAGENTS AND STANDARDS**8.1 Reagents**

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

8.2 Standards

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

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

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

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

10.2 Matrix Spikes

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

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

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

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

10.3 Continuing Calibration Verifications

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

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

11.0 CALIBRATION AND STANDARDIZATION

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

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

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

12.0 PROCEDURES**12.1 Acquisition Set up**

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

12.2 Using the Autosampler

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

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

- 12.2.2.5** Press the "Start" button.

12.3 Instrument Set-up

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

- 13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 METHOD PERFORMANCE

- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-4.1, Attachment B, for a listing of current validated MDL and LOQ values.
- 14.2 Solvent Blanks, Method Blanks, and Matrix Blanks
- 14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve
- 14.3 Calibration Curves
- 14.3.1 The r^2 value for the calibration curve must be 0.980 or better.
- 14.4 Matrix Spikes
- 14.4.1 Matrix spike percent recoveries are must be within $\pm 30\%$ of the spiked concentration.
- 14.5 Continuing Calibration Verifications
- 14.5.1 Continuing calibration verification percent recoveries must be $\pm 30\%$ of the spiked concentration.
- 14.6 If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.
- 14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

- 16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3 Plot the calibration curve by linear regression, weighted $1/x$, then print these graphs and store in the study folder.
- 16.4 Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Laboratory Study

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCCTANESULFONATE OR OTHER FLUROCHEMICALS IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-7.0

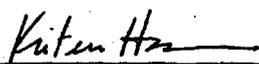
Adoption Date: 07/22/99

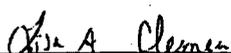
Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

	7/22/99
Laboratory Manager	Date

	7/14/99
Group Leader	Date

	07/14/99
Technical Reviewer	Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

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

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.

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

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

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

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

4.2 Cautions:

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

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

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

8.0 REAGENTS AND STANDARDS

8.1 Reagents

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

8.2 Standards

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Method Blanks and Matrix Blanks

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

10.2 Matrix Spikes

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

10.3 Continuing Calibration Checks

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

11.0 CALIBRATION AND STANDARDIZATION

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

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

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 μ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

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

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

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

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

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

12.3.4 Turn on the nitrogen.

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

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

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

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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

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

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

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

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

- 13.1.5 Calculate percent difference using the following equation:

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

- 13.1.6 Calculate actual concentrations in matrix ($\mu\text{g/g}$):

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

14.0 METHOD PERFORMANCE

- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to ETS-8-6.0, Attachment B for a listing of current validated MDL and LOQ values.
- 14.2 Solvent Blanks, Method Blanks and Matrix Blanks
- 14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.
- 14.3 Calibration Curves
- 14.3.1 The r^2 value for the calibration must be 0.980 or better.
- 14.4 Matrix Spikes
- 14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.5 Continuing Calibration Verification
- 14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

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

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

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

Appendix D: Data Summary Tables

Table 6. Rabbit Sera F0 PFOS, PFOSA and PFOSAA Data for FACT-TOX-097

Group Dose	Sample #	PFOS µg/mL	PFOSA µg/mL	PFOSAA* µg/mL
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	<LOQ (0.0458)	<LOQ (0.00490)	<LOQ (0.0124)
	8683F	<LOQ (0.0458)	<LOQ (0.00490)	<LOQ (0.0124)
	8684F	<LOQ (0.0458)	<LOQ (0.00490)	<LOQ (0.0124)
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	0.484	0.0155	0.975
	8686F	0.919	0.00696	1.00
	8687F	0.839	0.0154	1.15
	8688F	0.622	0.0132	0.660
	8689F	0.806	0.00988	1.01
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	4.79	0.0954	9.19
	8691F	3.66	0.0561	6.67
	8692F	8.66	0.242	11.6
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	24.4	0.378	27.6
	8694F	14.1	0.433	20.7
	8695F	9.88	0.460	22.7
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	22.6	0.489	44.3
	8697F	21.5	0.592	59.8
	8698F	18.8	1.01	54.1
	8699F	19.5	0.450	45.6
	8700F	14.8	1.41	45.4

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

Table 7. Rabbit Sera F0 N-EtFOSE and PFOSEA Data for FACT-TOX-097

Group Dose	Sample #	N-EtFOSE* µg/mL	PFOSEA µg/mL
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	<LOQ (0.0216)	<LOQ (0.00487)
	8683F	<LOQ (0.0216)	<LOQ (0.00487)
	8684F	<LOQ (0.0216)	<LOQ (0.00487)
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	<LOQ (0.0216)	<LOQ (0.00487)
	8686F	<LOQ (0.0216)	<LOQ (0.00487)
	8687F	<LOQ (0.0216)	<LOQ (0.00487)
	8688F	<LOQ (0.0216)	<LOQ (0.00487)
	8689F	<LOQ (0.0216)	<LOQ (0.00487)
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	<LOQ (0.0216)	<LOQ (0.00487)
	8691F	0.0368	<LOQ (0.00487)
	8692F	<LOQ (0.0216)	<LOQ (0.00487)
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	<LOQ (0.0216)	<LOQ (0.00487)
	8694F	<LOQ (0.0216)	<LOQ (0.00487)
	8695F	<LOQ (0.0216)	<LOQ (0.00487)
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	0.0294	<LOQ (0.00487)
	8697F	0.0279	<LOQ (0.00487)
	8698F	0.0386	<LOQ (0.00487)
	8699F	<LOQ (0.0216)	<LOQ (0.00487)
	8700F	0.132	<LOQ (0.00487)

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

Table 8. Rabbit Liver F0 PFOS, PFOSA and PFOSAA Data for FACT-TOX-097

Group Dose	Sample #	PFOS µg/g	PFOSA µg/g	PFOSAA* µg/g
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	0.0519	<LOQ (0.0120)	0.0458
	8683F	0.0346	<LOQ (0.0120)	0.119
	8684F	<LOQ (0.0279)	0.0120	0.0680
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	1.83	0.147	5.32
	8686F	1.58	0.0947	6.32
	8687F	1.64	0.156	5.74
	8688F	1.41	0.0663	4.47
	8689F	1.44	0.100	3.91
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	10.8	1.01	36.2
	8691F	7.89	1.04	25.0
	8692F	13.4	1.98	37.5
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	24.3	6.85	65.4
	8694F	22.2	4.27	67.5
	8695F	26.0	4.61	90.9
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	37.5	7.83	123
	8697F	48.5	8.65	259
	8698F	33.2	11.9	148
	8699F	32.1	5.61	88.5
	8700F	25.2	18.3	133

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

Table 9. Rabbit Liver F0 N-EtFOSE and PFOSEA Data for FACT-TOX-097

Group Dose	Sample #	N-EtFOSE* µg/g	PFOSEA µg/g
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	<LOQ (0.0525)	<LOQ (0.0298)
	8683F	<LOQ (0.0525)	<LOQ (0.0298)
	8684F	<LOQ (0.0525)	<LOQ (0.0298)
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	<LOQ (0.0525)	<LOQ (0.0298)
	8686F	<LOQ (0.0525)	<LOQ (0.0298)
	8687F	<LOQ (0.0525)	<LOQ (0.0298)
	8688F	<LOQ (0.0525)	<LOQ (0.0298)
	8689F	<LOQ (0.0525)	<LOQ (0.0298)
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	<LOQ (0.0525)	<LOQ (0.0298)
	8691F	0.621	<LOQ (0.0298)
	8692F	<LOQ (0.0525)	<LOQ (0.0298)
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	0.161	<LOQ (0.0298)
	8694F	0.123	<LOQ (0.0298)
	8695F	0.140	<LOQ (0.0298)
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	0.417	<LOQ (0.0298)
	8697F	0.477	<LOQ (0.0298)
	8698F	93.5	<LOQ (0.0298)
	8699F	0.235	<LOQ (0.0298)
	8700F	30.8	<LOQ (0.0298)

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

Table 10. FACT-TOX-097 Data Summary of Average Sera Concentration ($\mu\text{g/mL}$) and Standard Deviation ($\pm\text{SD}$)

Dosage Group	PFOS Average $\pm\text{SD}$	PFOSA Average $\pm\text{SD}$	PFOSAA* Average $\pm\text{SD}$	N-EtFOSE* Average $\pm\text{SD}$	PFOSEA Average $\pm\text{SD}$
Group 1 0.0 mg/kg/day 0.0 mg/mL	<LOQ NA (n=3)	<LOQ NA (n=3)	<LOQ NA (n=3)	<LOQ NA (n=3)	<LOQ NA (n=3)
Group 2 0.1 mg/kg/day 0.02 mg/mL	0.734 ± 0.177 (n=5)	0.0128 ± 0.00277 (n=5)	0.959 ± 0.180 (n=5)	<LOQ NA (n=5)	<LOQ NA (n=5)
Group 3 1.0 mg/kg/day 0.2 mg/mL	5.70 ± 2.62 (n=3)	0.131 ± 0.0977 (n=3)	9.15 ± 2.46 (n=3)	0.0267 0.00875 (n=3)	<LOQ NA (n=3)
Group 4 2.5 mg/kg/day 0.5 mg/mL	16.1 ± 7.47 (n=3)	0.423 ± 0.0497 (n=3)	23.7 ± 3.51 (n=3)	<LOQ NA (n=3)	<LOQ NA (n=3)
Group 5 3.75 mg/kg/day 0.75 mg/mL	19.4 ± 2.98 (n=5)	0.790 ± 0.411 (n=5)	49.8 ± 6.82 (n=5)	0.0499 0.0464 (n=3)	<LOQ NA (n=5)

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

NA = Not Applicable

Table 11. FACT-TOX-097 Data Summary of Average Liver Concentration ($\mu\text{g/g}$) and Standard Deviation ($\pm\text{SD}$)

Dosage Group	PFOS Average $\pm\text{SD}$	PFOSA Average $\pm\text{SD}$	PFOSAA* Average $\pm\text{SD}$	N-EtFOSE* Average $\pm\text{SD}$	PFOSEA Average $\pm\text{SD}$
Group 1 0.0 mg/kg/day 0.0 mg/mL	0.0381 ± 0.0124 (n=3)	<LOQ NA (n=3)	0.0776 ± 0.0375 (n=3)	<LOQ NA (n=3)	<LOQ NA (n=3)
Group 2 0.1 mg/kg/day 0.02 mg/mL	1.58 ± 0.171 (n=5)	0.113 ± 0.0376 (n=5)	5.15 ± 0.968 (n=5)	<LOQ NA (n=5)	<LOQ NA (n=5)
Group 3 1.0 mg/kg/day 0.2 mg/mL	10.7 ± 2.78 (n=3)	1.34 ± 0.550 (n=3)	32.9 ± 6.86 (n=3)	0.242 ± 0.328 (n=3)	<LOQ NA (n=3)
Group 4 2.5 mg/kg/day 0.5 mg/mL	24.1 ± 1.92 (n=3)	5.24 ± 1.40 (n=3)	74.6 ± 14.1 (n=3)	0.141 ± 0.0187 (n=3)	<LOQ NA (n=3)
Group 5 3.75 mg/kg/day 0.75 mg/mL	35.3 ± 8.60 (n=5)	10.5 ± 4.93 (n=5)	150 ± 64.8 (n=5)	25.1 ± 40.4 (n=5)	<LOQ NA (n=5)

*Non-qualitative screening data only.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to 30% or greater.

NA = Not Applicable

Table 12. Approximate LOQ Values Used in FACT-TOX-097

Matrix	Compound	LOQ
Liver	PFOS	0.0279 $\mu\text{g/g}$
	PFOSA	0.0120 $\mu\text{g/g}$
	PFOSAA*	0.0324 $\mu\text{g/g}$
	N-EtFOSE*	0.0525 $\mu\text{g/g}$
	PFOSEA	0.0298 $\mu\text{g/g}$
Sera	PFOS	0.0458 $\mu\text{g/mL}$
	PFOSA	0.00490 $\mu\text{g/mL}$
	PFOSAA*	0.0124 $\mu\text{g/mL}$
	N-EtFOSE*	0.0216 $\mu\text{g/mL}$
	PFOSEA	0.00487 $\mu\text{g/mL}$

*Non-qualitative screening data only.

Appendix E: Data Spreadsheets

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFPOSE in Rabbits
 Product Number(Test Substance): EFPOSE-OH (T-4316.3)
 Matrix: Rabbit Serum
 Method/Revision: FACT-M-3.0 & FACT-M-4.0 - Linear regression, weighted 1/x
 Analytical Equipment System Number: Arctis 062494, Madeline 041096, Soap 020199
 Instrument Software/Version: Mast 3.1
 File name: See listing to the right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Extraction/Anlyst: 10/16/98 LAS
 Dates of Analysis/Anlyst: 10/20/98, 10/23/98, 07/29/99 HJ/MBS/KJH/DBB
 Date of Data Reduction/Anlyst: 10/17/00, 11/22/00 KJH

Filmaram 4.94 ng/mL, 9.87 ng/mL, 4.90 ng/mL
 PFOA
 Bks M10209806-7 & 52-53 M10209806-7 & 52-53
 Grp 1 10209818-30 10209818-30
 Grp 2 102398034-42 10209823-37
 Grp 3 102398043-45 10209830-32
 Grp 4 102398024-28 10209836-38
 Grp 5 102398029-35 102398033,46-51
 MS, MSD-0482F 10209840-50 10209840-50

Sample Data
 RABBIT SERA F0

Group Desc	Sample #	Extraction Vol. Ratio	PFOA Std Correction Factor	PFOA Purity Correction Factor	PFOA Dilution Factor	PFOA Conc. ng/mL	Concentration of PFOA ng/mL or % Rec.	Mean PFOA ng/mL	RSD Std. Dev. MS/MSD RFD	PFOA Purity Correction Factor	PFOA Dilution Factor	PFOA Conc. ng/mL	Concentration of PFOA ng/mL or % Rec.	Mean PFOA ng/mL	RSD Std. Dev. MS/MSD RFD
Method BR:	H30 BR-1	1	0.9275	Unknown	1	0.00	<LOQ (0.0438 ng/mL)			Unknown	1	0.00	<LOQ (0.0438 ng/mL)		
	H30 BR-2	1	0.9275	Unknown	1	0.00	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA	Unknown	1	0.00	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA
Matrix BR:	Rabbit Serum BR-1	1	0.9275	Unknown	1	0.00	<LOQ (0.0438 ng/mL)			Unknown	1	0.00	<LOQ (0.0438 ng/mL)		
	Rabbit Serum BR-2	1	0.9275	Unknown	1	0.00	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA	Unknown	1	0.00	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA
QC:500 ppb	8683F-M8	1	NA	NA	1	433	88%			NA	1	499	101%		
	8683F-M8D	1	NA	NA	1	435	88%		1%	NA	1	538	110%		
Group 1	8683F	1	0.9275	Unknown	1	16.6	<LOQ (0.0438 ng/mL)			Unknown	1	0.00	<LOQ (0.0438 ng/mL)		
0.8 mg/kg/day	8683F	1	0.9275	Unknown	1	2.02	<LOQ (0.0438 ng/mL)			Unknown	1	0.00	<LOQ (0.0438 ng/mL)		
0.0 ng/mL	8684F	1	0.9275	Unknown	1	2.16	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA	Unknown	1	0.00	<LOQ (0.0438 ng/mL)	<LOQ (0.0438 ng/mL)	NA
Group 2	8685F	1	0.9275	Unknown	10	52.5	0.484			Unknown	1	15.6	0.0153		
0.1 mg/kg/day	8685F	1	0.9275	Unknown	10	99.5	0.919			Unknown	1	6.99	0.00696		
0.02 ng/mL	8687F	1	0.9275	Unknown	10	90.9	0.829			Unknown	1	15.5	0.0154		
	8688F	1	0.9275	Unknown	10	47.4	0.622			Unknown	1	13.3	0.0132		
	8689F	1	0.9275	Unknown	10	87.4	0.806	0.734	34.1 0.177	Unknown	1	9.93	0.00988	0.0128	21.6 0.00277
Group 3	8690F	1	0.9275	Unknown	10	519	4.70			Unknown	1	95.9	0.0954		
1.0 mg/kg/day	8691F	1	0.9275	Unknown	10	396	3.66		46.0	Unknown	1	56.4	0.0564		74.6
0.2 ng/mL	8692F	1	0.9275	Unknown	10	939	8.66	5.70	34.2	Unknown	1	243	0.242	0.131	0.977
Group 4	8693F	1	0.9275	Unknown	100	264	24.4			Unknown	1	380	0.378		
2.5 mg/kg/day	8694F	1	0.9275	Unknown	100	152	14.1		46.4	Unknown	1	433	0.433		8.0
0.5 ng/mL	8695F	1	0.9275	Unknown	100	107	9.89	16.1	7.47	Unknown	1	460	0.460	0.423	0.619
Group 5	8696F	1	0.9275	Unknown	100	244	22.6			Unknown	2	345	0.489		
3.75 mg/kg/day	8697F	1	0.9275	Unknown	100	213	21.5			Unknown	2	296	0.392		
0.75 ng/mL	8698F	1	0.9275	Unknown	100	263	18.8			Unknown	2	507	1.01		
	8699F	1	0.9275	Unknown	100	210	19.5		15.3	Unknown	2	225	0.450		52.0
	8700F	1	0.9275	Unknown	100	160	14.8	19.4	2.98	Unknown	100	14.1	1.41	0.750	0.411

Original PFOA, PFOA, EFPOSE LOQs (4.94 ng/mL, 9.87 ng/mL, 23.1 ng/mL, 24.3 ng/mL) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Correction factors not applicable for MS/MSD QC data

Date Entered By: 10/27/00, 11/27/00, 12/01/00 LAC
 Date Verified By: 10/30/00, 12/04/00 KJH

PFOA = Perfluorooctanoic acid
 PFOA = Perfluorooctanoic acid
 PFOA = Perfluorooctanoic acid
 EFPOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamide ethyl alcohol
 PFOA = Perfluorooctane sulfonamide
 B256 = Perfluorooctanoic acid ethylamine CAS#17302N(0)(CHECOO)

Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-097
Argus# 418-010

Product Number/Test Substance: EPOSE-OH (T-6316.5)
 Matrix: Rabbit Serum
 Method/Revision: FACT-M-3.0 & FACT-M-4.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Amelco 902409, Modeline 041096, Soap 020199
 Instrument Software/Version: MMSLsys 3.1
 Filename: See listing to the right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Extraction/Analyze: 10/16/98 IAS
 Dates of Analysis/Analyze: 10/20/98, 10/23/98, 07/29/99 H01/06E/KH/RWD
 Date of Data Reduction/Analyze: 10/17/00, 11/23/00 KH

Fluorence 23.1 ng/mL 24.3 ng/mL
 PFOCSAA EPOSE
 Bites 341020906-7 & 52-53 M1020906-7 & 52-53
 Grp 1 10209018-30 10209018-30
 Grp 2 102398036-42 10209023-37
 Grp 3 102398021-23 10209030-32
 Grp 4 102398024-28 10209036-38
 Grp 5 102398014-18 10209042-46
 MS, MSD-8682F 1029849-30 1029849-30
 High recovery confirmed 7/29/99 analysis

RABBIT SERA FB

Group Dose	Sample #	lot 617		lot 956		Concentration of PFOCSAA ng/mL or % Rec.	Mean PFOCSAA ng/mL	RSD Std. Dev. MS/MSD RPD	EPOSE Purity Correction Factor	EPOSE Dilution Factor	EPOSE Conc. ng/mL	Concentration of EPOSE ng/mL or % Rec.	Mean EPOSE ng/mL	RSD Std. Dev. MS/MSD RPD
		PFOCSAA Correction Factor	PFOCSAA Dilution Factor	PFOCSAA Conc. ng/mL	EPOSE Conc. ng/mL									
Method DR	H2O BB-1	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
	H2O BB-3	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
Matrix DR	Rabbit Serum BB-1	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
	Rabbit Serum BB-2	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)	0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
QC-500 ppb	8682F-M8	NA	1	411	43%			NA	1	938	194%			
	8682F-MSD	NA	1	348	111%		97%	28%	NA	957	196%		2%	
Group 1	8682F	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)		0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
0.0 mg/kg/day	8683F	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)		0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
0.0 mg/mL	8684F	0.5382	1	0.00	<LOQ (0.0124 ng/mL)	<LOQ (0.0124 ng/mL)		0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
Group 2	8685F	0.5382	10	182	0.973			0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)		
0.1 mg/kg/day	8686F	0.5382	10	187	1.00			0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)		
0.02 mg/mL	8687F	0.5382	10	214	1.13			0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)		
	8688F	0.5382	10	123	0.660			18.7	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
	8689F	0.5382	10	189	1.01	0.559		0.180	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
Group 3	8690F	0.5382	100	172	0.919			0.8890	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)		
1.0 mg/kg/day	8691F	0.5382	100	125	6.67			0.8890	1	41.6	0.0568		32.8	
0.2 mg/mL	8692F	0.5382	100	217	11.6	9.13		2.46	1	0.00	<LOQ (0.0216 ng/mL)	0.0267	0.00875	
Group 4	8693F	0.5382	100	315	27.6			0.8890	1	11.2	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
2.5 mg/kg/day	8694F	0.5382	100	396	20.7			14.8	1	5.75	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
0.5 mg/mL	8695F	0.5382	100	423	22.7	23.7		3.51	1	0.00	<LOQ (0.0216 ng/mL)	<LOQ (0.0216 ng/mL)	NA	
Group 5	8696F	0.5382	1000	51.3	44.3			0.8890	1	33.1	0.0294			
3.75 mg/kg/day	8697F	0.5382	1000	111	39.8			0.8890	1	31.4	0.0279			
0.75 mg/mL	8698F	0.5382	1000	101	54.1			0.8890	1	43.4	0.0364			
	8699F	0.5382	1000	84.8	43.6			13.7	1	14.0	<LOQ (0.0216 ng/mL)		92.8	
	8700F	0.5382	1000	84.3	43.4	49.8		6.82	1	149	0.132	0.0499	0.0464	

Original PFOCSAA, EPOSE LOQs (4.94 ng/mL, 9.87 ng/mL, 23.1 ng/mL, 34.3 ng/mL) updated to reflect correction factor information on 01/30/01. LAC 01/30/01

Correction factors not applicable for MS/MSD QC data

Date Entered By: 10/27/00, 11/27/00, 12/01/00 LAC
 Date Verified By: 10/30/00, 12/04/00 KH

Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

PFOCS = Perfluorooctanesulfonate
 PFOCSA = Perfluorooctanesulfonamide
 PFOCSAA = Perfluorooctanesulfonamide
 EPOSE = Hexam Range 14-Ethyl Perfluorooctanesulfonamide ethyl alcohol
 PFOSEA = Perfluorooctanesulfonamide ethyl alcohol
 M556 = Perfluorooctanesulfonamide hexanoate C8F17SO2N(H)CH2COO

FACT-TOX-097
Argus# 418-010

Product Number/Test Substance: EPOSE-OH (T-6316.9)
 Matrix: Rabbit Serum
 Method/Revision: FACT-M-3.0 & FACT-M-4.0 - Linear regression, weighted 1/x
 Analytical Equipment System Number: Arctis 063498, Madeline 041098, Soap 020199
 Instrument Software/Version: MassLynx 3.1
 Filtrate: See listing to the right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Estimation/Anlyst: 10/16/08 IAS
 Dates of Analysis/Anlyst: 10/20/08, 10/21/08, 07/29/09 HCV/MS/EK/RJ/DB
 Date of Data Reduction/Anlyst: 10/17/00, 11/23/00 KRJ

Filtrates: 23.1 ug/mL, 4.87 ug/mL
 MS56 PFOSEA DR
 Riba M10209806-7 & 52-53 M10209806-7 & 52-53
 Grp 1 10209818-20 10209818-20 1/1, 1/1, 1/1, 1/1, 1/1
 Grp 2 10209823-27 10209823-27 1/10, 1/1, 1/10, 1/1, 1/1, 1/1
 Grp 3 10209830-32 10209830-32 1/10, 1/1, 1/100, 1/1, 1/1, 1/1
 Grp 4 10209836-38 10209836-38 1/100, 1/1, 1/100, 1/1, 1/1, 1/1
 Grp 5 10209842-46 10209842-46 1/100, 1/2&100, 1/1000, 1/1, 1/1, 1/1
 MS, MSD-86827 10209849-50 072099015-16 1/1, 1/1, 1/1, 1/1, 1/1

RABBIT SERA F0

Based on PFOSEA Curve

Lot TN-A-1885

Group Data	Sample #	M556 Purity Correction Factor	M556 Dilution Factor	M556 Conc. ug/mL	Concentration of M556 ug/mL or % Res.	Mean M556 ug/mL	ESD Std. Dev. MS/MSD RPD	PFOSEA Purity Correction Factor	PFOSEA Dilution Factor	PFOSEA Conc. ug/mL	Concentration of PFOSEA ug/mL or % Res.	Mean PFOSEA ug/mL	ESD Std. Dev. MS/MSD RPD
Method Blk	1D0 Blk-1	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	H2O Blk-2	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Matrix Blk	Rabbit Serum Blk-1	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	Rabbit Serum Blk-2	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
QC-500 ppb	8683F-M8	NA	1	NA	NA	NA	NA	NA	1	511	105%	100%	7%
	8682F-M5D	NA	1	NA	NA	NA	NA	NA	1	550	113%		
Group 1	8682F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.0 mg/kg/day	8683F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.0 mg/mL	8684F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 2	8685F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.1 mg/kg/day	8686F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.02 mg/mL	8687F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	8688F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	8689F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 3	8690F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
1.0 mg/kg/day	8691F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.2 mg/mL	8692F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 4	8693F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
2.5 mg/kg/day	8694F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.5 mg/mL	8695F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 5	8696F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
3.75 mg/kg/day	8697F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
0.75 mg/mL	8698F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	8699F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	8700F	NA	1	NA	NA	NA	NA	Unknown	1	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA

Original PFO6,PFO5AA, EPOSE LOQs (4.94 ug/mL, 9.87 ug/mL, 33.1 ug/mL), updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Correction factors not applicable for MS/MSD QC data

PFO6 = Perfluorooctanesulfonate
 PFO5A = Perfluorooctanesulfonamide
 PFO5AA = Perfluorooctanesulfonamideacetate
 EPOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamide ethyl alcohol
 PFOSEA = Perfluorooctanesulfonylethyl ethylamide
 M556 = Perfluorooctanesulfonamideacetate CRF17802N(8DCH2COO)

Date Entered By: 10/27/00, 11/27/00, 12/01/00 LAC
 Date Verified By: 10/30/00, 12/04/00 KRJ

Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-097
Argun# 418-010

Study: Argun 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSE in Rabbits
 Product Number/Test Substrate: EFOSE-C11 (T-8316.5)
 Matrix: Rabbit Serum
 Method/Revision: FACT-M-3.0 & FACT-M-4.0 - Linear regression, weighted 1/x
 Analytical Equipment System Number: Amelin 062408, Modeline 041028, Somp 020199
 Instrument Software/Version: MetLynx 3.1
 Filename: See listing to the right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Date of Estimation/Analyst: 10/15/00 LAC
 Date of Analysis/Analyst: 10/20/00, 10/25/00, 07/29/99 HO/AGEE/KJH/ORB
 Date of Data Reduction/Analyst: 10/17/00, 11/22/00 KJH

Sample Data
RABBIT SERA F0

Group	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Res.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD	PFOSA Conc. ug/mL	Concentration of PFOSA ug/mL or % Res.	Mean PFOSA ug/mL	RSD Std. Dev. MS/MSD RPD	PFOSAA Conc. ug/mL	Concentration of PFOSAA ug/mL or % Res.	Mean PFOSAA ug/mL	RSD Std. Dev. MS/MSD RPD	EFOSE Conc. ug/mL	Concentration of EFOSE ug/mL or % Res.	Mean EFOSE ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	HEO Blk-1	0.00	<LOQ (0.0458 ug/mL)	<LOQ (0.0458 ug/mL)	NA	0.00	<LOQ (0.00490 ug/mL)	<LOQ (0.00490 ug/mL)	NA	0.00	<LOQ (0.0124 ug/mL)	<LOQ (0.0124 ug/mL)	NA	0.00	<LOQ (0.0216 ug/mL)	<LOQ (0.0216 ug/mL)	NA
Matrix Blk	Rabbit Serum Blk-1	0.00	<LOQ (0.0458 ug/mL)	<LOQ (0.0458 ug/mL)	NA	0.00	<LOQ (0.00490 ug/mL)	<LOQ (0.00490 ug/mL)	NA	0.00	<LOQ (0.0124 ug/mL)	<LOQ (0.0124 ug/mL)	NA	0.00	<LOQ (0.0216 ug/mL)	<LOQ (0.0216 ug/mL)	NA
QC-500 ppb	8682F-MS3	435	88%	483	1%	518	101%	493	9%	411	93%	458	28%	928	196%	928	2%
Group 1	8682F	14.6	<LOQ (0.0458 ug/mL)			0.00	<LOQ (0.00490 ug/mL)			0.00	<LOQ (0.0124 ug/mL)			0.00	<LOQ (0.0216 ug/mL)		
0.0 mg/kg/day	8681F	2.02	<LOQ (0.0458 ug/mL)		NA	0.00	<LOQ (0.00490 ug/mL)		NA	0.00	<LOQ (0.0124 ug/mL)		NA	0.00	<LOQ (0.0216 ug/mL)		NA
0.0 mg/mL	8684F	2.16	<LOQ (0.0458 ug/mL)	<LOQ (0.0458 ug/mL)	NA	0.00	<LOQ (0.00490 ug/mL)	<LOQ (0.00490 ug/mL)	NA	0.00	<LOQ (0.0124 ug/mL)	<LOQ (0.0124 ug/mL)	NA	0.00	<LOQ (0.0216 ug/mL)	<LOQ (0.0216 ug/mL)	NA
Group 2	8685F	52.5	0.484			15.6	0.0155			182	0.975			0.00	<LOQ (0.0216 ug/mL)		
0.1 mg/kg/day	8686F	99.5	0.919			6.99	0.00696			187	1.00			0.00	<LOQ (0.0216 ug/mL)		
0.02 mg/mL	8687F	90.9	0.839			13.3	0.0134			214	1.15			0.00	<LOQ (0.0216 ug/mL)		
	8688F	67.4	0.622	0.734	34.1	13.3	0.0132		21.6	123	0.660		18.7	0.00	<LOQ (0.0216 ug/mL)		NA
	8689F	17.4	0.206		0.177	9.29	0.0998	0.0128	0.00277	189	1.01	0.959	0.180	0.00	<LOQ (0.0216 ug/mL)	<LOQ (0.0216 ug/mL)	NA
Group 3	8690F	519	4.79			56.3	0.0954			172	9.19			0.00	<LOQ (0.0216 ug/mL)		
1.0 mg/kg/day	8691F	396	3.64		46.0	54.4	0.0561		74.6	125	4.67		36.9	41.6	0.0368		33.8
0.2 mg/mL	8692F	939	8.64	5.70	2.02	343	0.242	0.131	0.0977	217	11.6	9.15	3.46	0.00	<LOQ (0.0216 ug/mL)	0.0267	0.00875
Group 4	8693F	264	24.4			380	0.378			515	27.6			11.2	<LOQ (0.0216 ug/mL)		
2.5 mg/kg/day	8694F	132	14.1		44.4	433	0.433		9.90	386	30.7		14.8	5.75	<LOQ (0.0216 ug/mL)		NA
0.5 mg/mL	8695F	107	9.81	16.1	7.47	469	0.469	0.423	0.0419	425	22.7	23.7	3.51	0.00	<LOQ (0.0216 ug/mL)	<LOQ (0.0216 ug/mL)	NA
Group 5	8696F	244	22.6			345	0.449			523	44.3			33.1	0.0294		
3.75 mg/kg/day	8697F	132	21.5			296	0.392			111	39.8			31.4	0.0279		
0.75 mg/mL	8698F	263	18.8			307	1.01			101	54.1			43.4	0.0386		
	8699F	210	19.5		15.3	225	0.450		52.0	84.8	45.6		13.7	14.0	<LOQ (0.0216 ug/mL)		92.8
	8700F	160	14.8	19.4	2.98	14.1	1.41	0.790	0.411	84.3	45.4	49.8	6.82	149	0.132	0.0499	0.0464

Original PFOS, PFOSA, EFOSE LOQs (4.94 ug/mL, 9.87 ug/mL, 23.1 ug/mL, 34.3 ug/mL) updated to reflect correction factor information on 01/30/01. LAC 01/30/01

Correction factors not applicable for MS/MSD QC data

Date Entered/By: 10/17/00, 11/27/00, 12/01/00 LAC
 Date Verified/By: 10/30/00, 12/04/00 KJH

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 PFOSAA = Perfluorooctanesulfonamideacetate
 EFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamide ethyl alcohol
 PFOSIA = Perfluorooctanesulfonamide ethylamide
 MS56 = Perfluorooctanesulfonamideacetate CFF17802N(01)CL3C00

Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSBE in Rabbits
 Product Number(Test Substance): EFOSBE-OH (T-6316.5)
 Matrix: Rabbit Serum
 Method/Revision: FACT-M-3.0 & FACT-M-4.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Amelco 962408, Model# 041099, Smp 020199
 Instrument Software/Version: MestLyn 5.1
 Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Extraction/Anlyst: 10/16/98 IAS
 Dates of Analyis/Anlyst: 10/20/98, 10/23/98, 07/29/99 HJM/BE/KRH/DRS
 Date of Data Reduction/Anlyst: 10/17/00, 11/22/00 KJH

Group Dose	Sample #	MSS6 Conc. ug/mL	Concentration of MSS6 ug/mL or % Rec.	Mean MSS6 ug/mL	RSD Std. Dev. MS/MSD RPD	PFOSAA Conc. ug/mL	Concentration of PFOSAA ug/mL or % Rec.	Mean PFOSAA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk:	IDO Blk-1	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	IDO Blk-2	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
Matrix Blk	Rabbit Serum Blk-1	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	Rabbit Serum Blk-2	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
QC-500 ppb	8682F-348	NA	NA	NA	NA	311	103%		
	8682F-482D	NA	NA	NA	NA	520	113%		
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8683F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
Group 2 0.1 mg/kg/day 0.02 mg/mL	8683F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
	8687F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
Group 3 1.0 mg/kg/day 0.2 mg/mL	8688F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8689F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 4 2.5 mg/kg/day 0.5 mg/mL	8690F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8691F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 5 3.75 mg/kg/day 0.75 mg/mL	8692F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8693F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 6 0.75 mg/mL	8694F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8695F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA
Group 7 0.75 mg/mL	8700F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)		
	8701F	NA	NA	NA	NA	0.00	<LOQ (0.00487 ug/mL)	<LOQ (0.00487 ug/mL)	NA

Original PFOS,PFOSAA, EFOSBE LOQs (4.94 ug/mL, 9.87 ug/mL, 23.1 ug/mL, 24.3 ug/mL) updated to reflect correction factor information on 01/30/01. LAC: 01/30/01
 Correction factors not applicable for MS/MSD QC data

Date Entered/By: 10/27/00, 11/27/00, 12/01/00 LAC
 Date Verified/By: 10/30/00, 12/04/00 KJH

PFOS = Perfluorooctanesulfonic acid
 PFOSAA = Perfluorooctanesulfonamide
 PFOSAAA = Perfluorooctanesulfonamideacetate
 EFOSBE = Narrow Range N-Ethyl Perfluorooctanesulfonamide ethyl alcohol
 PFOSBEA = Perfluorooctanesulfonamide ethyl alcohol
 MSS6 = Perfluorooctanesulfonamidehexane C8F17SO2N(H)C12H20O2
 MSS5 = Perfluorooctanesulfonamidehexane C8F17SO2N(H)C12H20O2

Extraction Volume Ratio = Initial volume/final volume. For all samples and standards the initial volume is equal to the final volume for an extraction volume ratio of 1.

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSE in Rabbits
 Product Number (Test Substance): EFOSE-OH (T-6316.5)
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Andia 062498 and Modeline 041098
 Instrument Software/Version: MasLynx 3.1
 Date of Extraction/Analyst: 10/16/98 SAE/DCP
 Date of Analysis/Analyst: 10/17/98, 11/25/98, 09/28/99 HOJ/JAS
 Date of Data Reduction/Analyst: 10/23/98, 12/01/98, 09/29/99 HOJ/JAS-DEI
 Filenames: See list to right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Filenames: 30.1 ng/g, 60.3 ng/g
 PFO8
 A10179804-5 & 53-54
 Gcp 1 10179816-18
 Gcp 2 112598029-033
 Gcp 3 112598040-042
 Gcp 4 112598061-063
 Gcp 5 112598103-107
 MS, MSD 10179850-51

RABBIT LIVER FO

Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFO8 Std Correction Factor	PFO8 Purity Correction Factor	PFO8 Conc. ng/g	PFO8 Dilution Factor	PFO8 Calc. Conc. ng/g	Concentration of PFO8 ng/g or % Rec.	Mean PFO8 ng/g	ESD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	1.0000	NA	0.9275	Unknown	4.71	1	4.37	<LOQ (0.0279 ng/g)		
	H2O Blk-2	1.0000	NA	0.9275	Unknown	10.8	1	9.98	<LOQ (0.0279 ng/g)	<LOQ (0.0279 ng/g)	NA
Matrix Blk	Rabbit Liver Blk-1	1.0000	NA	0.9275	Unknown	0.00	1	0.00	<LOQ (0.0279 ng/g)		
	Rabbit Liver Blk-2	1.0000	NA	0.9275	Unknown	0.00	1	0.00	<LOQ (0.0279 ng/g)	<LOQ (0.0279 ng/g)	NA
QC - 500 ppb	8682F-MS	1.0113	NA	NA	NA	612	1	554	99%	94%	2%
	8682F-MSD	1.0113	NA	NA	NA	626	1	568			
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	1.0113	NA	0.9275	Unknown	56.6	1	51.9	0.9519		32.4
	8683F	0.9964	NA	0.9275	Unknown	37.2	1	34.6	0.0346		0.0124
	8684F	1.0131	NA	0.9275	Unknown	22.9	1	21.0	<LOQ (0.0279 ng/g)	0.0381	
Group 2 0.1 mg/kg/day 0.01 mg/mL	8685F	1.0187	NA	0.9275	Unknown	201	10	1931	1.83		
	8686F	1.0072	NA	0.9275	Unknown	172	10	1585	1.58		
	8687F	1.0184	NA	0.9275	Unknown	180	10	1636	1.64		
	8688F	1.0197	NA	0.9275	Unknown	155	10	1407	1.41		10.8
	8689F	1.0052	NA	0.9275	Unknown	156	10	1436	1.44	1.58	0.171
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	1.0200	NA	0.9275	Unknown	119	100	10784	10.8		26.0
	8691F	1.0157	NA	0.9275	Unknown	86.4	100	7889	7.89		2.78
	8692F	0.9998	NA	0.9275	Unknown	145	100	13445	13.4	10.7	
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	0.9997	NA	0.9275	Unknown	262	100	24280	24.3		7.95
	8694F	0.9972	NA	0.9275	Unknown	238	100	22155	22.2		1.92
	8695F	1.0142	NA	0.9275	Unknown	284	100	25987	26.0	24.1	
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	1.0161	NA	0.9275	Unknown	411	100	37478	37.5		
	8697F	0.9917	NA	0.9275	Unknown	519	100	48502	48.5		
	8698F	1.0109	NA	0.9275	Unknown	362	100	33213	33.2		24.3
	8699F	1.0179	NA	0.9275	Unknown	353	100	32139	32.1		
	8700F	1.0059	NA	0.9275	Unknown	273	100	25193	25.2	35.3	8.60

PFO8 = Perfluorooctanoic acid
 Correction Factors not applicable to MS/MSD QC data
 The curve (2 for PFO8AA, and EFOSE-OH did not meet criteria, these data and MS/MS data (which was calculated using the PFO8AA curve) will be considered qualitative data. LAC 12/13/00
 Original PFO8, PFO8AA, EFOSE LOQs (0.1 ng/g,) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Date Entered/Analyst: 10/27/00 LAC
 Date Verified/Analyst: 10/30/00 KKH

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSE in Rabbits
 Product Number(Test Substance): EFOSE-OH (T-616.5)
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - Linear regression, weighted 1/x
 Analytical Equipment System Number: Anachis 062498 and Machine 041098
 Instrument Software/Version: Masslynx 3.1
 Date of Extraction/Analyst: 10/16/98 SAH/DCP
 Date of Analysis/Analyst: 10/17/98, 11/25/98, 09/28/99 HJJAAS
 Date of Data Reduction/Analyst: 10/23/98, 12/01/98, 09/29/99 HJJAAMH

60.2 ng/g
 Elements Qualitative Data Only
 PFOsAA
 Bkt: A112598003-4 & 201-202
 Grp 1 092899016, 112598016-017
 Grp 2 112598021,30-033
 Grp 3 112598040-042
 Grp 4 112598056-058
 Grp 5 112598092-096
 MS, MSD 092899017-18

RABBIT LIVER FU

lot 617

Group Dose	Sample #	PFOsAA Purty Correction Factor	PFOsAA Conc. ng/g	PFOsAA Dilution Factor	PFOsAA Calc. Conc. ng/g	Concentration of PFOsAA ng/g or % Res.	Mean PFOsAA ng/g	RSD Std. Dev. MS/MSD RPD
Method Bkt	H2O Bkt-1	0.5382	0.00	1	0.00	<LOQ (0.0324 ng/g)		
	H2O Bkt-2	0.5382	0.00	1	0.00	<LOQ (0.0324 ng/g)	<LOQ (0.0324 ng/g)	NA
Matrix Bkt	Rabbit Liver Bkt-1	0.5382	0.00	1	0.00	<LOQ (0.0324 ng/g)		
	Rabbit Liver Bkt-2	0.5382	0.00	1	0.00	<LOQ (0.0324 ng/g)	<LOQ (0.0324 ng/g)	NA
QC - 500 ppb	8682F-MS	NA	748	1	695	117%		
	8682F-MSD	NA	849	1	795	134%	125%	13%
Group 1 0.0 mg/kg/day 0.0 mg/mL	8683E	0.5382	86.1	1	45.8	0.0458		
	8683F	0.5382	220	1	119	0.119		48.3
	8684F	0.5382	128	1	68.0	0.0680	0.0776	0.0375
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685E	0.5382	101	100	5324	5.32		
	8686F	0.5382	1182	10	6318	6.32		
	8687F	0.5382	1087	10	5742	5.74		
	8688F	0.5382	848	10	4474	4.47		18.8
Group 3 1.0 mg/kg/day 0.2 mg/mL	8689F	0.5382	729	10	3906	3.91	5.15	0.568
	8690F	0.5382	686	100	36175	36.2		
	8691F	0.5382	472	100	25003	25.0		20.8
Group 4 2.5 mg/kg/day 0.5 mg/mL	8692F	0.5382	696	100	37474	37.5	32.9	6.86
	8693F	0.5382	121	1000	65389	65.4		
	8694F	0.5382	125	1000	67485	67.5		19.0
Group 5 3.75 mg/kg/day 0.75 mg/mL	8695F	0.5382	171	1000	90855	90.9	74.6	14.1
	8696F	0.5382	233	1000	123403	123		
	8697E	0.5382	478	1000	259480	259		
	8698E	0.5382	278	1000	147948	148		
	8699F	0.5382	167	1000	88494	88.5		43.1
	8700F	0.5382	248	1000	132664	133	150	64.8

PFOs = Perfluorooctanesulfonate

Correction Factors not applicable to MS/MSD QC data

The curve fit for PFOsAA, and EFOSE-OH did not meet criteria, these data and MS56 data (which was calculated using the PFOsAA curve) will be considered qualitative data. LAC 12/13/00

Original PFOs,PFOsAA, EFOSE LOQs (30.1 ng/g,) updated to reflect correction factor information on 01/30/01. LAC 01/30/01

Date Entered/Analyst: 10/27/00 LAC
 Date Verified/Analyst: 10/30/00 KJH

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EPOSE in Rabbits
 Product Number(Test Substance): EPOSE-OH (T-6316.5) 60.3 ug/g 12.0 ug/g 29.8 ug/g
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Amelab 062498 and Modeline 041098
 Instrument Software/Version: Maxam, v3.1
 Date of Extractions/Analyst: 10/16/98 SAH/KCP
 Date of Analysis/Analyst: 10/17/98, 11/25/98, 09/28/99 HJ/JAS
 Date of Data Reduction/Analyst: 10/23/98, 12/01/98, 09/29/99 HJ/MADH
 Sample Data
 MS56 PFO5A PFO5A
 A112598003-4 & 201-2 A112598003-4 & 201-202 A10179804-5 & 53-54
 112598015-17 112598015-17 10179816-18
 112598029-33 1125980157-161 10179822-26
 112598047-049 112598047-049 10179830-32
 112598056-58 112598081-085 10179836-38
 112598092-96 112598114-118 10179842-46
 NS 112598136-137 10179850-51

Based on PFO5AA Curve

Group	Sample #	M556 Purity Correction Factor	M556 Conc. ug/g	M556 Dilution Factor	M556 Calc. Conc. ug/g	Concentration of M556 ug/g or % Rec.	Mean M556 ug/g	RSD Std. Dev. MS/MSD RPD	PFO5A Purity Correction Factor	PFO5A Conc. ug/g	PFO5A Dilution Factor	PFO5A Calc. Conc. ug/g	Concentration of PFO5A ug/g or % Rec.	Mean PFO5A ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	1120 Blk-1 1120 Blk-2	NA NA	-90.6 -90.6	1 1	-0.0906 -0.0906	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown	3.02 4.03	1 1	3.02 4.03	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g)	<LOQ (0.0120 ug/g)	NA
Matrix Blk	Rabbit Liver Blk-1 Rabbit Liver Blk-2	NA NA	-90.6 -90.6	1 1	-0.0906 -0.0906	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown	1.62 2.49	1 1	1.62 2.49	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g)	<LOQ (0.0120 ug/g)	NA
QC - 500 ppb	8682F-MS 8682F-MSD	NA NA	NS NS	NA NA	NA NA	NS NS	NS	NA	NA NA	688 594	1 1	669 577	113% 98%	105%	15%
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F 8683F 8684F	NA NA NA	-90.6 -90.6 -90.6	1 1 1	-0.0906 -0.0906 -0.0906	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown Unknown	11.0 9.35 12.2	1 1 1	10.9 9.38 12.0	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g) 0.0120	<LOQ (0.0120 ug/g)	NA NA
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F 8686F 8687F 8688F	NA NA NA NA	78.1 48.5 10.8 0.885	10 10 10 10	0.701 0.485 0.108 0.00885	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown Unknown Unknown	149 95.3 159 67.6	1 1 1 1	147 94.7 156 66.3	0.147 0.0947 0.156 0.0663	0.113	33.4 0.0376
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F 8691F 8692F	NA NA NA	724 696 1223	10 10 10	7.24 6.96 12.2	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown Unknown	104 105 198	10 10 10	1015 1038 1980	1.01 1.04 1.98	1.34	40.9 0.550
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F 8694F 8695F	NA NA NA	248 60.2 223	100 100 100	24.8 6.02 22.3	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown Unknown	685 426 467	10 10 10	6848 4272 4608	6.85 4.27 4.61	5.24	26.7 1.40
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F 8697F 8698F 8700F	NA NA NA NA	473 913 808 359	50 50 50 50	23.7 45.7 40.4 17.9	<LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g) <LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA	Unknown Unknown Unknown Unknown	159 172 240 114	50 50 50 50	7828 8650 11883 5615	7.83 8.65 11.9 5.61	10.5	47.2 4.93

PFO5 = Perfluorooctanesulfonate
 Correction Factors not applicable to MS/MSD QC data
 The curve fit for PFO5AA, and EPOSE-OH did not meet criteria, these data and M556 data (which was calculated using the PFO5AA curve) will be considered qualitative data. LAC 12/13/00
 Original PFO5, PFO5AA, EPOSE LOQs (30.1 ng/g,) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Date Entered/Analyst: 10/27/00 LAC
 Date Verified/Analyst: 10/30/00 KJH

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFPOSE in Rabbits
 Product Number/Test Substance: EFPOSE-OH (T-6316.5)
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Analaia 062498 and Madeline 041098
 Instrument Software/Version: MassLynx 3.1
 Date of Extraction/Analyze: 10/16/98 SAH/KCP
 Date of Analysis/Analyze: 10/17/98, 11/25/98, 09/28/99 HQJ/LAS
 Date of Data Reduction/Analyze: 10/23/98, 12/01/98, 09/29/99 HQJ/M-DH
 Sample Data

Filename: See Attachments
 R-Separated Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

59.0 ng/g
 Filenames: Qualitative Data Only
 EFPOSE
 Blks A11259803-4 & 201-202
 Grp 1 112598015-17
 Grp 2 112598157-161
 Grp 3 112598168-170
 Grp 4 112598175-179
 Grp 5 112598105,114,186-88,192
 MS, MSD 092899017-18
 Dilutions
 1/1, 1/1, 1/1, 1/1, 1/1, 1/1
 1/1, 1/1, x, 1/1, 1/1
 1/10, 1/10, x, 1/1, 1/1
 1/100, 1/100, x, 1/10, 1/1
 1/100, 1/1000, x, 1/10, 1/1
 1/100, 1/1000, x, 1/50, 1/1 (50 & 100)
 1/1, 1/1, x, 1/1, 1/1

Group Dose	Sample #	FFOSEA Purity Correction Factor	FFOSEA Conc. ng/g	FFOSEA Dilution Factor	FFOSEA Calc. Conc. ng/g	Concentration of FFOSEA ng/g or % Rec.	Mean FFOSEA ng/g	RSD Std. Dev. MS/MSD RPD	EFPOSE Purity Correction Factor	EFPOSE Conc. ng/g	EFPOSE Dilution Factor	EFPOSE Calc. Conc. ng/g	Concentration of EFPOSE ng/g or % Rec.	Mean EFPOSE ng/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
	H2O Blk-2	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	53.2	1	53.2	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
Matrix Blk	Rabbit Liver Blk-1	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
	Rabbit Liver Blk-2	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
QC - 500 ppb	8682F-MS	NA	647	1	640	109%	114%	9%	NA	402	1	398	68%	72%	10%
	8682F-MSD	NA	707	1	699	119%			NA	445	1	440	75%		
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8683F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8684F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8686F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8687F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8688F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
	8689F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	92.1	1	90.2	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	136
	8691F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	630	1	621	0.621	0.242	0.328
	8692F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	0.00	1	0.00	<LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	0.161
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	161	1	161	0.161	0.141	13.2
	8694F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	123	1	123	0.123		0.0187
	8695F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	142	1	140	0.140		
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	424	1	417	0.417		161
	8697F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	473	1	477	0.477		40.4
	8698F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	945	100	9479	93.5		
	8699F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	239	1	235	0.235		
	8702F	Unknown	0.00	1	0.00	<LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.8890	621	50	30843	30.8	25.1	

FFOS = Pfluorooctanoic acid
 Correction Factors not applicable to MS/MSD QC data
 The curve for FFOSAA, and EFPOSE-OH did not meet criteria, these data and MS56 data (which was calculated using the FFOSAA curve) will be considered qualitative data. LAC 12/13/00
 Original FFOS, FFOSAA, EFPOSE LOQs (30.1 ng/g.) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Date Entered/Analyze: 10/27/00 LAC
 Date Verified/Analyze: 10/30/00 KJH

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSB in Rabbits
 Product Number(Test Substance): EFOSB-OH (T-6316.5)
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Amulco 662498 and Medchem 041098
 Instrument Software/Version: Mastlynx 3.1
 Date of Extraction/Analyst: 10/16/98 SAH/CP
 Date of Analysis/Analyst: 10/17/98, 11/25/98, 09/28/99 HJM/AS
 Date of Data Reduction/Analyst: 10/23/98, 12/01/98, 09/29/99 HJM/AMH
 Sample Data

Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

RABBIT LIVER F0

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	ESD Std. Dev. MS/MSD RPD	PFOSAA Calc. Conc. ng/g	Concentration of PFOSAA ug/g or % Rec.	Mean PFOSAA ug/g	ESD Std. Dev. MS/MSD RPD	M556 Calc. Conc. ng/g	Concentration of M556 ug/g or % Rec.	Mean M556 ug/g	ESD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	4.37	<LOQ (0.0279 ug/g)			0.00	<LOQ (0.0324 ug/g)			-0.0906	<LOQ (0.0602 ug/g)		
	H2O Blk-2	9.98	<LOQ (0.0279 ug/g)	<LOQ (0.0279 ug/g)	NA	0.00	<LOQ (0.0324 ug/g)	<LOQ (0.0324 ug/g)	NA	-0.0906	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
Matrix Blk	Rabbit Liver Blk-1	0.00	<LOQ (0.0279 ug/g)			0.00	<LOQ (0.0324 ug/g)			-0.0906	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
	Rabbit Liver Blk-2	0.00	<LOQ (0.0279 ug/g)	<LOQ (0.0279 ug/g)	NA	0.00	<LOQ (0.0324 ug/g)	<LOQ (0.0324 ug/g)	NA	-0.0906	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
QC - 500 ppb	8682F-M5	554	93%			695	117%			NA	NS		
	8682F-M5SD	568	95%	94%	2%	795	134%	125%	13%	NA	NS	NS	NA
Group 1 0.0 mg/kg/day 0.0 mg/mL	8682E	51.9	0.0519			45.8	0.0458			-0.0906	<LOQ (0.0602 ug/g)		
	8683F	34.6	0.0346		32.4	119	0.119			-0.0906	<LOQ (0.0602 ug/g)		
	8684E	21.0	<LOQ (0.0279 ug/g)	0.0381	0.0124	68.0	0.0680	0.0776	48.3	-0.0906	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F	1831	1.83			5324	5.32			-0.0906	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
	8686F	1585	1.58			6318	6.32			0.701	<LOQ (0.0602 ug/g)		
	8687F	1636	1.64			5742	5.74			0.108	<LOQ (0.0602 ug/g)		
	8688E	1407	1.41		10.8	4474	4.47			0.00885	<LOQ (0.0602 ug/g)		NA
Group 3 1.0 mg/kg/day 0.2 mg/mL	8689F	1436	1.44	1.58	0.171	3906	3.91	5.15	0.968	0.293	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
	8690F	10784	10.8			36175	36.2			7.24	<LOQ (0.0602 ug/g)		
	8691F	7889	7.89		26.0	25003	25.0			6.96	<LOQ (0.0602 ug/g)		NA
	8692E	13445	13.4	10.7	2.78	37474	37.5	32.9	6.86	12.2	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F	24280	24.3			65389	65.4			24.8	<LOQ (0.0602 ug/g)		
	8694E	22155	22.2		7.95	67485	67.5			6.02	<LOQ (0.0602 ug/g)		NA
	8695E	25987	26.0	24.1	1.92	90855	90.9	74.6	19.0	14.1	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F	37478	37.5			123403	123			23.7	<LOQ (0.0602 ug/g)		
	8697F	48502	48.5			259440	259			45.7	<LOQ (0.0602 ug/g)		
	8698F	33213	33.2		24.3	147948	148			40.4	<LOQ (0.0602 ug/g)		
	8699F	32139	32.1		24.3	88494	88.5			17.9	<LOQ (0.0602 ug/g)		NA
	8700F	25193	25.2	35.3	8.60	132664	133	150	43.1	64.8	<LOQ (0.0602 ug/g)	<LOQ (0.0602 ug/g)	NA

PFOS = Perfluorooctanesulfonic acid
 Correction Factors not applicable to MS/MSD QC data
 The curve r2 for PFOSAA, and EFOSB-OH did not meet criteria, these data and M556 data (which was calculated using the PFOSAA curve) will be considered qualitative data. LAC 12/13/00
 Original PFOS,PFOSAA, EFOSB LOQs (0.1 ng/g,) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Data Entered/Analyst: 10/27/00 LAC
 Data Verified/Analyst: 10/30/00 KKH

FACT-TOX-097
Argus# 418-010

Study: Argus 418-010, Oral (Stomach Tube) Development Toxicity Study of N-EFOSE in Rabbits
 Product Number(Test Substance): EFOSE-OH (T-6316.5)
 Matrix: Rabbit Liver
 Method/Revision: FACT-M-1.0 & FACT-M-2.0 - linear regression, weighted 1/x
 Analytical Equipment System Number: Autecha 062498 and Machine 041098
 Instrument Software/Version: MetLab.jmx 1.1
 Date of Extraction/Anlyst: 10/16/98 SAH/ACP
 Date of Analysis/Anlyst: 10/17/98, 11/25/98, 09/28/99 HOJ/AS
 Date of Data Reduction/Anlyst: 10/23/98, 12/01/98, 09/29/99 HOJ/AMH
 Sample Data

Films: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

RABBIT LIVER #0

Group	Sample #	PFOSAA Calc. Conc. ug/g	Concentration of PFOSAA ug/g or % Rec.	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	PFOSAA Calc. Conc. ug/g	Concentration of EFOSE ug/g or % Rec.	Mean EFOSE ug/g	RSD Std. Dev. MS/MSD RPD	EFOSE Calc. Conc. ug/g	Concentration of EFOSE ug/g or % Rec.	Mean EFOSE ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1 H2O Blk-2	3.01 4.01	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g)	<LOQ (0.0120 ug/g)	NA	0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.00 51.2	<LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
Matrix Blk	Rabbit Liver Blk-1 Rabbit Liver Blk-2	1.62 2.49	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g)	<LOQ (0.0120 ug/g)	NA	0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.00 0.00	<LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	<LOQ
QC - 500 ppb	8682F-MS 8682F-MSD	669 577	113% 98%	105%	15%	640 699	109% 119%	398 440	3%	398 440	68% 75%	72%	10%
Group 1 0.0 mg/kg/day 0.0 mg/mL	8683F 8684F	10.9 9.38 12.0	<LOQ (0.0120 ug/g) <LOQ (0.0120 ug/g) 0.0120	<LOQ (0.0120 ug/g)	NA	0.00 0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.00 0.00 0.00	<LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA NA
Group 2 0.1 mg/kg/day 0.02 mg/mL	8685F 8686F 8687F 8688F 8689F	147 94.7 156 66.3 100	0.147 0.0947 0.156 0.0663 0.100	0.113	33.4 0.0376	0.00 0.00 0.00 0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	0.00 0.00 0.00 0.00 0.00	<LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g)	<LOQ (0.0525 ug/g)	NA NA
Group 3 1.0 mg/kg/day 0.2 mg/mL	8690F 8691F 8692F	1015 1038 1980	1.015 1.04 1.98	1.34	40.9 0.550	0.00 0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	90.2 621 0.00	<LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g) <LOQ (0.0525 ug/g)	0.621 0.242	136 0.328
Group 4 2.5 mg/kg/day 0.5 mg/mL	8693F 8694F 8695F	6848 4272 4608	6.85 4.27 4.61	5.24	26.7 1.40	0.00 0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	161 123 140	0.161 0.123 0.140	0.141	13.2 0.0187
Group 5 3.75 mg/kg/day 0.75 mg/mL	8696F 8697F 8698F 8699F 8700F	7828 8650 11883 5615 18315	7.83 8.65 11.9 5.61 18.3	10.5	47.2 4.93	0.00 0.00 0.00 0.00 0.00	<LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g) <LOQ (0.0298 ug/g)	<LOQ (0.0298 ug/g)	NA	417 477 93479 235 30843	0.417 0.477 93.5 0.235 30.8	25.1	161 40.4

PFOS = Perfluorooctanesulfonic acid
 Correction Factors not applicable to MS/MSD QC data
 The curve fit for PFOSAA, and EFOSE-OH did not meet criteria, these data and MS/MSD data (which was calculated using the PFOSAA curve) will be considered qualitative data. LAC 12/13/00
 Original PFOS,PFOSAA, EFOSE LOQs (30.1 ng/g,) updated to reflect correction factor information on 01/30/01. LAC 01/30/01
 Date Entered/Anlyst: 10/27/00 LAC
 Date Verified/Anlyst: 10/30/00 KJE

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT-TOX-097

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

Calculation Used for Group 3, Animal ID 8690F (PFOS)

$$519 \text{ ng/mL} \times 10 \times 0.9275 \times \frac{1 \text{ mL}}{1.005 \text{ mL}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = 4.79 \mu\text{g/mL}$$

AR— Analytical result from MassLynx summary

DF— Dilution factor

SC—PFOS salt correction constant (0.9275)

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

EV—Volume of sera extracted

Formula Used for Liver Analyses in Study FACT-TOX-097

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

⁽¹⁾ $\partial \text{ curve}$ is assumed to be: $\frac{1 \text{ g liver}}{5 \text{ mL H}_2\text{O}}$

Calculation Used for Group 3, Animal ID 8690F (PFOS)

$$119 \text{ ng/g} \times \frac{1 \text{ g} / 5 \text{ mL}}{1.0200 \text{ g} / 5 \text{ mL}} \times 0.9275 \times 100 \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = 10.8 \mu\text{g/g}$$

AR— Analytical result from MassLynx summary

$\partial \text{ curve}$ —Density of the liver standard curve, assumed to be 1g liver/ 5 ml water

$\partial \text{ sample}$ —Density of the liver sample (g sample/ 5 mL H₂O)

SC—PFOS salt correction constant (0.9275)

DF— Dilution factor

Appendix G: Interim Certificate(s) of Analysis



Centre Analytical Laboratories, Inc.

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

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1

3M Product: EtFOSE-OH

Test Control Reference #: SD-013

Purity: 88.9%

Test Name	Specifications	Result
Purity ¹		88.9%
Appearance	Yellow-white, waxy solid	Conforms
Identification NMR		Positive
Metals (ICP/MS)		
1. Calcium		1. <0.001 wt./wt.%
2. Magnesium		2. <0.001 wt./wt.%
3. Sodium		3. <0.001 wt./wt.%
4. Potassium		4. 0.002 wt./wt.%
5. Nickel		5. <0.001 wt./wt.%
6. Iron		6. <0.001 wt./wt.%
7. Manganese		7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.90 wt./wt.%
Total % Impurity (LC/MS)		None Quantified
Total % Impurity (GC/MS)		10.21 wt./wt.%
Related Compounds – POAA		0.03 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		87.6 wt./wt.%
Inorganic Anions (IC)		
1. Chloride		1. <0.015 wt./wt.%
2. Fluoride		2. <0.005 wt./wt.%
3. Bromide		3. <0.040 wt./wt.%
4. Nitrate		4. <0.009 wt./wt.%
5. Nitrite		5. <0.006 wt./wt.%
6. Phosphate		6. <0.007 wt./wt.%
7. Sulfate		7. <0.040 wt./wt.%
Organic Acids ² (IC)		
1. TFA		1. <0.1 wt./wt.%
2. PFPA		2. <0.1 wt./wt.%
3. HFBA		3. <0.1 wt./wt.%
4. NFPA		4. <0.25 wt./wt.%
Elemental Analysis ³ :		
1. Carbon	1. Theoretical Value = 25.2%	1. 24.42 wt./wt.%
2. Hydrogen	2. Theoretical Value = 1.75%	2. 1.78 wt./wt.%
3. Nitrogen	3. Theoretical Value = 2.45%	3. 2.72 wt./wt.%
4. Sulfur	4. Theoretical Value = 5.60%	4. 9.34 wt./wt.%
5. Fluorine	5. Theoretical Value = 56.6%	5. 58.4 wt./wt.%



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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1

3M Product: EtFOSE-OH

Test Control Reference #: SD-013

Date of Last Analysis: 11/26/00

Expiration Date: 11/26/01

Storage Conditions: <-10 °C

Re-assessment Date: 11/26/01

¹Purity = 100% - (total metal impurities, 0.002% + total NMR impurities, 0.90% + GC/MS impurities, 10.21 + POAA, 0.03%)

Total impurity from all tests = 11.14%

Purity = 100% - 11.14% = 88.9%

² TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

³Theoretical value calculations based on the empirical formula, C₁₂H₁₀F₁₇NO₃S
(MW=571)



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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1

3M Product: EtFOSE-OH

Test Control Reference #: SD-013

GC/MS Purity Profile

Peak #	Retention Time (min)	Identity	% Impurity
1	6.163	Unknown	0.12
2	8.011	Unknown	0.23
3	8.206	Unknown	0.51
4	9.065	Unknown	0.21
5	9.844	Unknown	0.34
6	13.93	Unknown	0.62
7	14.238	Unknown	0.11
8	15.130	C2	0.11
9	15.52	C3	1.11
10	15.941	C4	1.55
11	16.379	C5	1.07
12	16.801	C6	3.30
13	17.222	C7	0.93
Total	-	-	10.21

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

Prepared By: David S. Bell

David S. Bell
Scientist
Centre Analytical Laboratories

11/27/00
Date

Reviewed By: John M. Flaherty

John Flaherty
Laboratory Manager
Centre Analytical Laboratories

11/27/00
Date

