Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

Analytical Laboratory Report Title

Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS via Gavage

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

at signing

Performing Laboratories

Urine Analyses

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

Feces Analyses

Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801

Liver Analyses

Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201

Serum Analyses

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

Project Identification

3M Medical Department Study: T-6295.14
Argus In-Life Study: 418-015
Analytical Report: FACT TOX-111
3M Environmental Laboratory Request No. U2994

Total Number of Pages

341

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS via Gavage

Study Identification Numbers: T-6295.14, FACT TOX-111, LRN-U2994

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. The analytical phase completed at the 3M Environmental Laboratory was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Exceptions to GLP compliance:

- In the study binder, there are numerous Chain of Custody sheets that lack a signature, time, and date.
- In the study binder, there are packing lists sent by Argus that have evidence of having been whited-out with "White-out."
- Mass spectrometry data for liver and urine were collected and processed with the Mass Lynx software system that was not fully validated.
- Separate study directors were initially assigned to lead the in vivo and analytical portions of the study, respectively. This was eventually corrected.
- On several occasions, data entries or corrections were not documented exactly as required by the GLPs.
- Not all reagents and solutions used in this study included all the fields required by GLPs.
- Dose confirmation analyses were not performed in compliance with GLP regulations.

(signatures on next page)

Marvin T. Case, D.M.V., Ph.D., *Study Director* — 4 May 2001 Date

Kris J. Hansen, Ph.D., *Analytical Investigator* — 05/04/01 Date

William Reagen, Ph.D., *Laboratory Manager* — 05/03/01 Date

GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Concentration of Perfluorooctanesulfonate (PFOS) in the Serum, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS via Gavage

Study Identification Numbers: T-6295.14, FACT TOX-111, LRN-U2994

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

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
10/7/99	Extraction	5/15/00	5/15/00
10/8/99	Analysis	5/15/00	5/15/00
8/29—9/1/00, 9/4—9/5/00	Data	9/6/00	9/6/00
10/12/00	Data	10/12/00	10/12/00
10/26—10/27/00	Draft Report	10/30/00	10/30/00
11/13/00	Draft Report	11/13/00	11/13/00

See Appendix G for the individual contractor quality assurance statements.

Tonya K Rudd — *QAU Representative*

5/4/01 — Date

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List of Tables

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Feces Analyses
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Location of Archives

All original raw data, the protocol, the analytical report, the test article and the analytical reference standard reserve samples, as well as the specimens pertaining to the analytical phase of this study, are archived at the 3M Environmental Laboratory for a minimum of ten years. Reserve samples of control matrices used at Battelle, Centre and ABS will be stored at the respective contract laboratories in such a manner that the control matrices will be preserved for use in ensuing studies.

Introduction and Purpose

The purpose of the analytical study is to determine the concentration of perfluoro-octanesulfonate (PFOS) in sera, liver, urine, and feces specimens taken from the Gavage Recovery Study of T-6295.14 (potassium perfluorooctanesulfonate (KPFOS), CAS Number: 2795-39-3) in Crl:CD®BR VAF/Plus® Rats. Rat dams were exposed to KPFOS via gavage prior to and during mating. Exposure to KPFOS was halted on the first day of presumed gestation. Pups were not directly exposed to KPFOS, but may have been exposed *in utero* and during lactation. The analytical phase of this study was initiated on 9 June 1999.

Test System

The test system species and strain selected was the Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat, received from Charles River Laboratories, Inc., and permanently identified using Monel® self-piercing ear tags. Generation F0 virgin female rats were approximately 60 days of age and weighed approximately 181 to 222 g when received. Male rats of the same source and strain were used only as breeders and were not administered the test article or considered part of the test system. Generation F1 rats were identified as part of Group I, II, or III litters, but were not individually tagged, and all parameters were evaluated in terms of the litter (Table 1).

The test system comprised Generation F0 rats and Generation F1 pups. In Generation F0, three groups of 8 female rats each (a total of 24) were used. Group I control F0 female rats were administered Tween®80 (carrier). All other F0 female rats were administered KPFOS in 0.5% Tween®80. At predetermined intervals during, and at the end of, the in-life phase of the study, sera, urine, feces, and liver specimens were collected from Generation F0 rats, and sera and liver specimens were collected from Generation F1 pups.

Table 1. Female Rat Population Demographics for Study #418-015

Population	Selected for Study	Total Litters	Selected for Study
Group I (Control) 0 mg/kg/day	8	7[a]	7[a]
Group II 0.1 mg/kg/day	8	8	8
Group III 1.6 mg/kg/day	8	7[b]	7[b]

[a] One Group I F0 rat died from injury on DG 15 and did not give birth.
[b] One Group III F0 rat did not give birth.

Specimen Collection and Analysis

In the analytical study reported here, 440 serum, liver, urine, and feces specimens were collected from adult female rats (Generation F0) and their offspring (Generation F1). The specimens were sent to the 3M Environmental Laboratory and contract laboratories to be analyzed for PFOS. Sera specimens were collected from all Generation F0 animals immediately

prior to cohabitation, on Gestation Days (DGs) 7, 15, and 21, and on Lactation Days (DLs) 14 and 22. Sera specimens were collected from pooled litter samples on DL 21. Urine and feces specimens were collected from all Generation F0 animals beginning one day before cohabitation was initiated through the morning cohabitation was initiated, from DGs 6 through 7, 14 through 15, and 20 through 21, and on DLs 21 through 22. On DL 22, all surviving Generation F0 and Generation F1 animals assigned to the study were sacrificed, and a liver specimen was collected from each animal. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Study Groups I through III (through 20 February 99):
Serum Specimens—161 specimens
Liver Specimens—45 specimens
Urine Specimens—117 specimens
Feces Specimens—117 specimens

Blood specimens were separated by refrigerated centrifuge. Resulting serum specimens were then transferred to labeled polypropylene tubes and immediately frozen on dry ice. Liver, urine, and feces specimens were collected and frozen on dry ice. All serum, liver, urine, and feces specimens were stored at -70 °C or below until they were shipped, on dry ice, to the 3M Environmental Laboratory. Specimens were then shipped to the contract analytical laboratories for analysis.

Urine samples were extracted beginning on 06 October 1999 using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response monitoring mode. PFOS levels were quantitated by external calibration. Analytical details are included in this report.

The methods and analytical equipment settings used by Battelle Memorial Institute, Advanced Bioanalytical Services, Inc. and Centre Analytical Laboratories, Inc. are presented in the respective contract laboratory reports (see Appendix G).

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received serum, liver, urine and feces specimens that were collected during the in-life phase of this study, #418-015 from Argus. All specimens were received frozen on dry ice and were immediately transferred to storage at -20 ± 10°C. Specimens that were analyzed at Battelle Memorial Institute (liver), at Advanced Bioanalytical Sciences—ABS (serum), or at Centre Analytical Laboratories (feces), were subsequently shipped frozen on dry ice. Table 2 lists specimen receipt information for this study.

Table 2. Custody Transfer of Specimens in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

Tissue/Sample	Generation	Number of Samples Received	Date Received	Date Shipped to Contract Laboratory
Liver	Dam	23	09 March 1999	19 August 1999
Serum	Dam	139	09 March 1999	15 June 1999
Urine	Dam	117	09 March 1999	—[a]
Feces	Dam	117	09 March 1999	27 June 2000
Liver	Fetal (pooled)	22	09 March 1999	19 August 1999
Serum	Fetal (pooled)	22	09 March 1999	15 June 1999

[a] Shipment date not applicable: urine specimens were analyzed at the 3M Environmental Laboratory.

Control matrices used in urine analyses were obtained from Lampire Biological Laboratories, Inc., and are presented in Appendix A.

Chemical Characterization of the Test Article

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

Chemical Formula: $C_8F_{17}SO_3^-K^+$ Molecular Weight: 538.0

Chemical characterization information on potassium perfluorooctanesulfonate, CAS Number 2795-39-3, used in this study is presented in Table 3.

Table 3. Characterization of Test Article in Study FACT TOX-111

	Test Article
Chemical Name	**KPFOS** Potassium Perfluorooctanesulfonate
Source	3M Toxicology Services
Expiration Date	31 August 2001
Storage Conditions	Ambient until 16 May 2000, then frozen ≤-10 °C
Chemical Lot #	217
Physical Description	White Crystalline Powder
Purity	86.9%

See the Certificate of Analysis in Appendix H for complete characterization information. Preliminary characterization had been performed by NMR only, showing a purity of 99.28%, which was initially used for all calculations. A more complete characterization was performed later, which determined the purity of 86.9%.

Procurement

Potassium perfluorooctanesulfonate was obtained in one lot (Lot number 217) from 3M Toxicology Services.

Stability Studies

The stability of potassium perfluorooctanesulfonate was not determined.

Dose Confirmation Analyses

Dose confirmation analyses were performed on test article samples (0.00, 0.02, and 0.32 mg/mL) collected on 22 November 1998 and 07 January 1999, during the in-life phase of the study. The results are presented in Appendix A. The dose confirmation data were collected according to a method that was not fully validated.

Dose confirmation was performed by diluting the KPFOS dose samples with methanol into the linear range of the instrument used for analysis. Samples were analyzed versus an unextracted 1/x-weighted calibration curve using HPLC-ES/MS/MS.

Method Summaries

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory and by contract laboratories. The methods used in this study are located in Appendices C and G.

The methods and analytical equipment settings used by Battelle Memorial Institute, Advanced Bioanalytical Services, Inc. and Centre Analytical Laboratories, Inc. are presented in the respective contract laboratory reports (see Appendix G).

3M Environmental Laboratory

PREPARATORY METHOD

- **ETS-8-96.0,** "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

  This method is used to extract PFOS from urine by the use of an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). An ion-pairing reagent (tetrabutyl ammonium hydrogen sulfate) is added to 2 mL of 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 0.5 mL of methanol, then filtered through a 0.2 µm nylon filter attached to a 3-mL plastic syringe, into glass autovials.

  Modification to ETS-8-96.0: Although the rat urine used to generate method validation data came from the same type of rat as the test animals, the validation rats and test animals were obtained from different suppliers. Upon analysis of urine extracts from the test animals, it was determined that an interferent, not present in the validation samples, was present in the urine extract of the test animals. The unidentified interferent proved problematic only in the analysis of the surrogate (THPFOS).

  As written, ETS-8-97.0 describes the quantitation of PFOS with respect to a surrogate. Due to the presence of the interferent in the surrogate analysis of the test animals, all quantitations for this study were conducted by external calibration (i.e., the surrogate was not used in any calculations).

ANALYTICAL METHODS

- **ETS-8-97.0,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/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 ($C_8F_{17}SO_3$-), selected as the primary ion for PFOS analysis, was fragmented further to produce ion 99 (FSO_3-). The characteristic product ion 99 was monitored for quantitative analysis.

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

  The analyses were performed by monitoring the perfluorooctanesulfonate (PFOS) parent anion, m/z=499, and a daughter ion, m/z=99, to verify the presence of PFOS in the samples using a tandem mass spectrometer.

- **Battelle Preparatory and Analytical Method:** Method for Analysis of Potassium Perfluorooctanesulfonate (PFOS) in Rat Liver by LC/MS/MS

- **ABS Preparatory and Analytical Method:** Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS

- **Centre Preparatory and Analytical Method:** 00M-023-003 (Revision 2), Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS

ANALYTICAL EQUIPMENT

The actual analytical equipment settings used in the analysis of urine samples varied slightly during actual data collection. The following is representative of the settings used during the analytical phase of this study by the 3M Environmental Laboratory.

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

Time (min)	%B
0.00	10.0
1.00	10.0
5.50	95.0
7.50	95.0
8.00	10.0

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.3
Cone Voltage: 60 V
Collision Gas Energy: 40–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 4. Negative Ions Monitored in 3M Laboratory Analyses for Study FACT TOX-111

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

* THPFOS is a surrogate.

Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Although the methods were validated using internal calibration, it was necessary to use external calibration for quantification in the study, due to an unknown contaminant that interfered with the surrogate response.

Deviations from the original protocol and methods are documented in Appendix B.

Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) for 3M Environmental Laboratory assays were indicated in the protocol for this study:

- **Linearity:** The coefficient of determination (R^2) ≥ 0.980.
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Acceptable Precision:** Precision is within 30% for the method.
- **Acceptable Spike Recoveries:** 50–150%
- **Demonstration of Specificity:** Specificity will be demonstrated by chromatographic retention time and mass spectral daughter ion characterization.

Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory protocol for FACT TOX-111 (see Appendix B) were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (R^2) of the standard curve was ≥0.980.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis (1/x-weighted) of two extracted matrix curves bracketing each group of samples, except as noted in the deviation summary. 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. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of the analyte was based on the response of one specific product ion using the multiple response-monitoring mode of the instrument (see Appendix C).

- **Limits of Quantitation (LOQ):** For urine, the LOQ is 0.00295 µg/g. It is equal to the lowest acceptable standard in the calibration curve, defined as a standard within ±30% of the theoretical value (see Appendix D).
- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest.
- **Precision:** Precision was not determined for this study.
- **Matrix Spike Recoveries:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs at the 3M Environmental Laboratory. Acceptable spike recoveries of 52 to 96% of expected values were achieved for all matrix spikes prepared in urine.
- **Surrogates:** The surrogate (THPFOS) was added to all urine samples and standards. External calibration was used to quantify urine values, because an unidentified compound interfered with the analysis of the surrogate in the samples.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data are quantitative to 50% or greater.

Refer to Appendix G for contract laboratory summaries of quality control analysis results.

Summary of Sample Results

Following is information regarding sample analyses at each of the analytical laboratories involved in the present study:

Battelle Memorial Institute: Refer to the Battelle Final Report for information regarding liver analysis (Appendix G). For liver analysis data that has been corrected with the Certificate of Analysis PFOS purity factor, see Appendix E.

Advanced Bioanalytical Services, Inc.: Refer to the ABS Bioanalytical Report and the ABS Addendum to the Bioanalytical Report for information regarding serum analysis (Appendix G). For serum analysis data that has been corrected with the Certificate of Analysis PFOS purity factor, see Appendix E.

Centre Analytical Laboratories, Inc.: Refer to the Centre Analytical Report for information regarding feces analysis (Appendix G). Feces analysis data is also presented in Appendix E.

- **Samples from Control Animals:** Low levels of PFOS were detected in the urine of some control animals. These levels were significantly lower than those found in the low dose test animals.

- **Samples from Dosed Animals:** In general, PFOS levels found in the urine of the F0 Generation animals increased with dose group. Detailed sample data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix F for example calculations used to generate the urine sample data in FACT TOX-111.

Statement of Conclusion

Under the conditions of the present study, perfluorooctanesulfonate was observed in all sample types of all Generation 0 test system animals dosed with the test substance during the in-life phase of the study, and in all sample types of their offspring (Generation F1).

Appendix A: Control Matrices and Dose Confirmation Analyses

Table 5. Characterization of the Control Matrices Used for Sera, Liver, Urine, and Feces Analyses in Study FACT TOX-111

Location of Analyses	3M Environmental Laboratory	Battelle Memorial Institute	Advanced Bioanalytical Services, Inc	Centre Analytical Laboratories, Inc.
Control Matrix	**Human Urine**	**Rat and/or Rabbit Livers**	**Rat Serum**	**Rat Feces**
Source	Lampire Biological Laboratories, Inc.	Argus Research and/or Sigma	Harlan Bioproducts for Science, Inc..	Lampire Biological Laboratories, Inc.
Expiration Date	01 August 2008	N/A	N/A	N/R
Storage Conditions	Frozen	Frozen at -20 ± 10 °C	Frozen	Frozen at < -10 °C
Chemical Lot #	66-11691	N/A	R80128	N/R
Physical Description	Urine	Liver	Frozen Liquid	Rat feces

N/A=Not Available
N/R=Not Recorded

Table 6. Characterization of the Analytical Reference Substances Used for Liver, Urine, Sera, and Feces Analyses in Study FACT TOX-111

Location	3M Environmental Laboratory	Battelle Memorial Institute	Advanced Bioanalytical Services, Inc	Centre Analytical Laboratories, Inc.
Substance	**KPFOS** Potassium Perfluorooctanesulfonate	**KPFOS** Potassium Perfluorooctanesulfonate	**KPFOS** Potassium Perfluorooctanesulfonate	**KPFOS** Potassium Perfluorooctanesulfonate
Source	3M Toxicology Services	3M Toxicology Services	3M Toxicology Services	3M Toxicology Services
Expiration Date	31 August 2001	31 August 2001	31 August 2001	31 August 2001
Storage Conditions	Ambient	Ambient	Ambient	Ambient
Chemical Lot Number	171	171	171	217
Physical Description	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder	White Crystalline Powder
Purity	86.4%	86.4%	86.4%	86.9%

Table 7. Dose Confirmation Analyses for Perfluorooctanesulfonate for Test Samples from In-Life Study #418-015

	Sample Number	Target Conc. PFOS (µg/mL)	Expected Conc. PFOS (µg/mL)	Raw Measured Conc. PFOS (µg/mL)	Corrected Measured Conc. PFOS (µg/mL)	PFOS % Recovery Accuracy
Group I Control 0.0 mg/kg/day	B-418-015-A (11/22/98) Diluted 1/1	0.00	0.00	0.00	0.00	<LOD
	B-418-015-A (1/7/99) Diluted 1/1	0.00	0.00	0.00	0.00	<LOD
Group II 0.1 mg/kg/day 0.02 mg/mL	B-418-015-B (11/22/98) Diluted 1/40	20.0	16.1	0.543	17.4	108%
	B-418-015-B (1/7/99) Diluted 1/40	20.0	16.1	0.477	15.3	95%
Group III 1.6 mg/kg/day 0.32 mg/mL	B-418-015-C (11/22/98) Diluted 1/808	320	258	0.441	285	111%
	B-418-015-C (1/7/99) Diluted 1/808	320	258	0.358	232	90%

Limit of Quantitation Limit (LOQ) = 0.030 µg/mL

Formula used to calculate Expected Concentration from Target Concentration:

Target Concentration x PFOS Purity (Test Article) x PFOS Correction Factor = Expected Concentration PFOS

PFOS Purity (Test Article, Lot 217) = 86.9%
PFOS Correction Factor = 0.9275

320 µg/mL x 0.869 x 0.9275 = 258 µg/mL Expected Concentration

Formula used to calculate Corrected Measured Concentration of PFOS:

Raw Measured Concentration x Dilution Factor x PFOS Purity (Reference Substance) x PFOS Correction Factor = Corrected Measured Concentration of PFOS

PFOS Purity (Reference Substance, Lot 171) = 86.4%
PFOS Correction Factor = 0.9275

0.358 µg/mL x 808 x 0.864 x 0.9275 = 232 µg/mL Corrected Measured Concentration of PFOS

Appendix B: Protocol, Protocol Amendments and Deviation Summary

Table 8. Deviation Summary for FACT TOX-111

Deviation	Date(s) of Occurrence	Impact on Study
Additional control matrices were used: human urine and rat feces.	Entire Study	No negative impact on the study—A successful cross-validation was completed.
ETS-8-97.0 specifies the method of analysis requires the use of an internal calibration. The actual analyses used an external calibration method.	06 October 1999 to 04 November 1999	Unknown contaminant interfered with use of surrogate for internal calibration. Corrective action is an improvement. Data quality is satisfactory to ±50% accuracy.
The analysis of the dose samples was not conducted according to GLP regulations. ETS-8-5.1, a method validated for the analysis of sera extracts, was followed for the analysis of the Tween dose samples.	21 September 1999	The analysis of Tween samples was not conducted under GLPs (as per discussions with study director). This deficiency is included in the final report.

3M Environmental Technology and Services

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Protocol #FACT-TOX-111

3M

Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

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

Laboratory Project Identification
FACT-TOX-111
U2994

Study Identification

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

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

Sub-Contract Laboratories	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850 Battelle Memorial Institute 505 King Avenue Columbus Ohio 43201-2693
Proposed Study Timetable	
Study Initiation Date	June 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic recovery study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-015. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. . If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

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

4. QUALITY ASSURANCE

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

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

5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-015.

6. CONTROL MATRICES

6.1 *Identification* Rat liver and serum, and/or rabbit liver and serum traceability numbers will be recorded in the raw data and included in the final report

6.2 *Source* Argus Research and/or Sigma Chemical

6.3 *Physical Description* Rat liver and serum, and/or rabbit liver and serum

6.4 *Purity and Stability* Not applicable

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

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

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

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

7. REFERENCE MATERIAL

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

7.2 *Source* 3M Specialty Chemicals

7.3 *Physical Description* White powder

7.4 *Purity and Stability* Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 *Storage Conditions* Room temperature

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

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

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

8. Test System

Rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-015. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation until day 0 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	8	0	0	5
2	8	0.1	0.02	5
3	8	~~0.6~~ (i.e. 1.6 kh 9/22/99)	0.32	5

9. Specimen and Sample Receipt

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

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	Dam-Predose, Days 7, 14, 15, 21, and 22 Pup-Day 21	144 Dam 24 Pup (pooled)
Urine and Feces – Dam and Pup animals	Predose, Days 7, 15, 21, and 22	120 Urine and 120 Feces
Liver – Dam and Pup animals	Dam-At the termination of the study Pup-Day21	24 Dam 24 Pup (pooled)

Total number of expected specimens: 456
Total number of test animals: 16
Total number of control animals: 8

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

10. PREPARATORY METHODS

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

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

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

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

11. ANALYTICAL METHODS

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

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

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

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

12. DATA QUALITY OBJECTIVES

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

12.1 *Linearity* $r^2 \geq 0.98$

12.2 *Limits of detection / quantitation*

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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

12.3 ***Duplicate acceptable precision*** < 30% for the method

12.4 ***Spike acceptable recoveries*** 70% - 130%

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

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

13. Sub-Contracted Analysis

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

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

14. Statistical Analysis

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

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

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

15. Report

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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

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

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

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

16. Location of Raw Data, Records, and Final Report

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

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

- ***16.1.1*** Approved protocol and amendments
- ***16.1.2*** Study correspondence
- ***16.1.3*** Shipping records
- ***16.1.4*** Raw data
- ***16.1.5*** Approved final report (original signed copy)
- ***16.1.6*** Electronic copies of data

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

- ***16.2.1*** Training records
- ***16.2.2*** Calibration records
- ***16.2.3*** Instrument maintenance logs
- ***16.2.4*** Standard Operating Procedures, Equipment Procedures, and Methods

17. SPECIMEN RETENTION

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

18. PROTOCOL AMENDMENTS AND DEVIATIONS

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

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T. Case

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — 9 June 1999 Date

Kristen J. Hansen

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — 6/9/99 Date

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Protocol FACT-TOX111
Amendment No. 1

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

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

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

AMEND TO READ: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to:

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"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

The extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat ~~Sera~~ by LC/MS/MS, Version ~~1~~ 1.0" (1) Liver

REASON: The analytical methods at the sub-contract laboratories were not included in the original protocol.

(1) (RE)UAC 9/27/99

kjn 9/27/99

3M Environmental Laboratory

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

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

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

Amendment Approval

Marvin T Case — 17 August 1999

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

Kris Hansen — 8/18/99

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

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 30, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Protocol FACT-TOX111
Amendment No. 2

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

1. ***PROTOCOL READS:***
Section 2 states the study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and sera in rats.

AMEND TO READ:
The study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver, sera, and urine in rats.

REASON:
The urine analytical methods were validated and approved after approval of the original protocol.

2. ***PROTOCOL READS:***
Section 10.0 and 11.0 list the following methods to use for extraction and analysis:
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"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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"

AMEND TO READ::
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"
ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

REASON: The extraction and analytical methods ETS-8-96.0 and ETS-8-97.0, were approved after approval of the original protocol.

3. ***PROTOCOL READS:***
Section 6.1 lists control matrices as rat liver and serum or rabbit liver and serum. Source Argus and/or Sigma Chemical.

AMEND TO READ:
Control matrices identification rat liver and serum, rabbit liver and serum, human urine and/or rat urine. Source Argus, Sigma Chemical, Lampire Biologicals, Biological Specialty Corp. and/or Golden West Biologicals.

REASON:
Addition of control urine specifications.

4. ***PROTOCOL READS:***
Section 12.2.1 a) lists sera method detection limit as 1.75 ppb and b) lists liver method detection limit as 15 ppb.

AMEND TO READ:
The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

REASON:
The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

5. ***PROTOCOL READS:***
Section 16 states that 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, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that 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:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

6. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

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

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

Protocol FACT-TOX111
Amendment No. 2

Amendment Approval

Marvin Case, D.V.M., Ph.D., Sponsor Representative — 4 Oct 1999 Date

Kristen J. Hansen, Ph.D., Study Director — 5 Oct 1999 Date

3M Environmental Laboratory

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number 2759-39-3) in the Serum, Liver, and Urine of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS Via Gavage

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

Performing Laboratories

Performing Laboratories

Urine Analyses	Liver Analyses	Serum Analyses
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

3M Environmental Laboratory

Protocol LRN-U2994
Amendment Number 3

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

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

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

Amendment Approval

John L. Butenhoff — February 10, 2000

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

Kristen Hansen — 11-Feb-2000

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

Marvin T Case — 10 February 2000

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

Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:

20 April 2000

Performing Laboratories

URINE ANALYSES	FECES ANALYSES
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09, 935 Bush Avenue St. Paul, MN 55106	**Centre Analytical Laboratories, Inc.** 3048 Research Drive State College, PA 16801
LIVER ANALYSES	**SERUM ANALYSES**
Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	**Advanced Bioanalytical Services, Inc.** 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

3M Environmental Laboratory

Protocol LRN-U2994
Amendment Number 4

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

1. ***PROTOCOL READS:***
The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
AMEND TO READ:
This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
REASON:
The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:***
The amended section 6.0 lists rat or rabbit liver, serum, and urine.
AMEND TO READ:
Add: rat or rabbit feces with a physical description of rat or rabbit feces.
REASON:
Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. ***PROTOCOL READS:***
Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
AMEND TO READ:
Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
REASON:
Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. ***PROTOCOL READS:***
Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
AMEND TO READ:
The feces extraction and analytical method used by **Centre Analytical Laboratories** will be; 00M–023–003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
REASON:
The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Amendment Approval

John L. Butenhoff April 19, 2000

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

Marvin T. Case 19 April 2000

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

3M Environmental Laboratory

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 5

Amendment Date:
July 25, 2000

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

Laboratory Project Identification
ET&SS FACT-TOX-111
U2994

3M Environmental Laboratory

Protocol FACT TOX-111
Amendment No. 5

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

1. ***PROTOCOL READS:***
In section 8, Table 1, the dosage level for dosage group 3 is listed as 0.6 mg/kg/day.

AMEND TO READ:
Section 8, Table 1, the dosage level for dosage group 3 is 1.6 mg/kg/day.

REASON:
The dosage level for dosage group 3 was changed after the protocol was written.

2. ***PROTOCOL READS:***
The amended analytical protocol (Amendment No. 1) states the extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat Sera by LC/MS/MS, Version 1"

AMEND TO READ: The extraction and analytical method to follow at Battelle Memorial Institute is:

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

REASON: Liver analyses were sub-contracted to Battelle Memorial after the protocol was written; therefore the method used to analyze liver samples also changed. The Battelle method list in Amendment No. 1 contained a hand-written change, in that "sera" was crossed out and "liver" was written in. This hand-written change was dated after the study director had signed the amendment.

3. ***PROTOCOL READS:***
The Argus in-life protocol #418-015 lists the testing facility as Argus Research Laboratories, Inc.

AMEND TO READ:
Change the testing facility to 3M Toxicology Services—Medical Department. Address: 3M Center, Building 220-2E-02, St. Paul, MN 55144-1000. This change is retroactive to February 10, 2000.

REASON:
Per GLP regulations, the testing facility must be where the study director resides. There cannot be two testing facilities.

4. ***PROTOCOL READS:***
The cover page of FACT-TOX-111 lists the document type as a "Protocol." On page three, under Proposed Study Timetable, the "Study Initiation Date" and the "Study Completion Date" are listed.

AMEND TO READ:
Change the document type for FACT-TOX-111 to "Analytical Phase Protocol." Change "Study Initiation Date" to "Phase Initiation Date." Change "Study Completion Date" to "Phase Completion Date."

REASON:
FACT-TOX-111 is the protocol for the analytical phase, while the Argus protocol #418-015 is the protocol for the in-life phase.

5. ***PROTOCOL READS:***
The amended analytical protocol (Amendment No. 3) states that Marvin T. Case replaces Kristen J. Hansen as the study director. Kristen J. Hansen was reassigned to the role of *Principle* Analytical Investigator. The Argus Research Laboratories, Inc. in-life protocol #418-015 states that the study director for the in-life phase is Raymond York.

AMEND TO READ:
Marvin T. Case replaces both Kristen J. Hansen ***and*** Raymond York as study director. Raymond York has been reassigned to the role of Principal In-life Investigator. Kristen J. Hansen was reassigned to the role of *Principal* (corrected spelling) Analytical Investigator. These changes are retroactive to February 10, 2000.

REASON:
Per GLP regulations, only one study director is assigned to a study. Corrected spelling of *Principal*.

6. ***PROTOCOL READS:***
The amended analytical protocol (Amendment No 4) states that rat feces will be analyzed for PFOS, as well as rat urine, liver and serum. Centre Analytical Laboratories will be conducting the feces analyses.

AMEND TO READ:
Add that the Principal Analytical Investigator (PAI) at Centre analytical Laboratories is Enaksha Wickremesinhe.

REASON:
The PAI for Centre was not listed in Amendment No. 4.

Protocol FACT TOX-111
Amendment No. 5

Amendment Approval

John L Butenhoff by mCase 9/5/00

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

Marvin T Case 5 Sept 2000

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

3M Environmental Laboratory

Study Title
Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 6

Amendment Date:
October 17, 2000

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

Laboratory Project Identification
ET&SS FACT TOX-111
3M Laboratory Request No. U2994

3M Environmental Laboratory

Protocol FACT TOX-111
Amendment #6

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

1. ***PROTOCOL READS:***
   Data Quality Objectives, Section 12.4, Spike Acceptable Recoveries are required to be 70%–130%.

   AMEND TO READ:
   Spike Acceptable Recoveries are required to be 50–150%.

   REASON: The analytical method and resulting QC data support a 50–150% acceptable range for spike recoveries.

3M Environmental Laboratory

Amendment Approval

John L. Butenhoff 10/14/00

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

Marvin T Case 18 October 2000

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

Appendix C: Extraction and Analytical Methods

This Appendix includes the following methods:

3M Environmental Laboratory, **ETS-8-96.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry," (14 pages).

3M Environmental Laboratory, **ETS-8-97.0,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry," (10 pages).

3M Environmental Laboratory, **ETS-8-5.1,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry," (9 pages).

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-96.0

Adoption Date: 7-28-99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager	Date: 7/28/99
Group Leader	Date: 7/28/99
Technical Reviewer	Date: 7/27/99

1.0 SCOPE AND APPLICATION

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

1.2 **Applicable compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Human, rat, and monkey urine 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 fluorochemicals from urine, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, eight fluorochemicals are extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, M570, perfluorooctanoate (POAA), and surrogate standard (see ***3.0 Definitions***). An ion pairing reagent is added to two ml of 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 0.5 ml of methanol, then filtered through a 0.2 µm nylon filter attached to 3 cc plastic syringe into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-97.0 or other appropriate method.

3.0 Definitions

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

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

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

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

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

3.6 M570: $C_8F_{17}SO_2N(CH_3)CH_2COOH$

3.7 POAA: perfluorooctanoate $C_7F_{15}COO^-$

3.8 Surrogate standard THPFOS: 1H-1H-2H-2H perfluorooctane sulfonic acid, used as an internal standard in this method.

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 At this time, it is unknown how the extraction method is affected by potential interferences that may be present such as conjugated fluorochemicals (eg. Glucuronides). Conjugates may become deconjugated during extraction or analysis resulting in a high bias for reported results of target analytes.

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 2.5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 REAGENTS AND STANDARDS

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

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

8.9.6 M570 (3M Specialty Chemical Division), molecular weight = 571

8.9.7 POAA (3M Specialty Chemical Division), molecular weight = 452

8.9.8 THPFOS (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.9 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 = 10.0. 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 in the Standard Logbook.

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

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

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

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

8.12 Surrogate stock standard preparation

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

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

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 100-120 ppm. Record the actual volume transferred in the Standard Logbook.

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 2.0 ml methanol is used as a solvent blank.

10.1.2 Extract two 2.0 ml aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 2.0 ml aliquots of the urine 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 should 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 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 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 Transfer 2.0 ml of urine to a 15 ml centrifuge tube.

11.1.2 Record each sample volume on the extraction sheet.

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

11.1.4 Two 2.0 ml aliquots serve as matrix blanks.

11.1.5 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 twenty standards, two matrix blanks, and two method blanks.

11.1.6 Refer to validation report **FACT-TOX-131, W2067,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.7 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 10 ppb - 1500 ppb.

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

Table 1 Approximate spiking amounts for standards and spikes Using 2.0 ml of matrix			
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix	Approx. final conc. Of analyte in solvent
-	-	Blank	Blank
0.500 ppm	5	1.25 ppb	5.00 ppb
0.500 ppm	10	2.50 ppb	10.0 ppb
0.500 ppm	25	6.00 ppb	25.0 ppb
5.00 ppm	5	12.5 ppb	50.0 ppb
5.00 ppm	10	25.0 ppb	100 ppb
5.00 ppm	25	62.5 ppb	250 ppb
50.0 ppm	5	125 ppb	500 ppb
50.0 ppm	7.5	200 ppb	750 ppb
50.0 ppm	10	250 ppb	1000 ppb
50.0 ppm	15	375 ppb	1500 ppb

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 2.0 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 sample weight/volume worksheet. . See **Attachment D**. The original weight/volume worksheet is included in the study binder.

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 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 30 to 60 minutes.

12.11 Add 0.5 ml of methanol to each centrifuge tube using a graduated pipette. If excessive residue is present, add the methanol and allow the extract to sit for 30 minutes prior to vortexing.

12.17 Vortex mix for 30 seconds.

12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, fluorochemical components, extraction type, vial file archive number, and analyst(s) performing the extraction.

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

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

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/ml) of PFOS in matrix

14.0 Method Performance

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

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-8-97.0 for method performance criteria.

15.0 Pollution Prevention and Waste Management

15.1 Human and monkey sample waste is disposed in infectious biohazard waste containers, all other sample waste is disposed in noninfectious biohazard waste containers. Flammable solvent waste is disposed in high BTU containers. 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 Date

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

17.4 Attachment D, Sample weight/volume worksheet

18.0 References

18.1 The validation report associated with this method is **FACT-TOX-131, W2067.**

19.0 Affected Documents

19.1 ETS-8-97.0, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray Mass Spectrometry/Mass Spectrometry"

20.0 Revisions

Revision Number	Reason For Revision	Revision Date

3M Medical Department Study: T-6395.14 Extraction Worksheet ETS-8-96.0 Analytical Report: FACT TOX-111

LRN-U2994

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

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

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

MDL/LOQ values for human urine

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR)
PFOS	4.6	14.7	15 ppb - 1500 ppb (in final MeOH extract)
POAA	22.9	72.9	50 ppb – 1500 ppb (in final MeOH extract)
PFOSA	n/d	n/d	25 ppb - 1000 ppb (in final MeOH extract)
PFOSAA	n/d	n/d	25 ppb - 1000 ppb (in final MeOH extract)
EtFOSE-OH	n/d	n/d	25 ppb - 1000 ppb (in final MeOH extract)
M556	n/d	n/d	25 ppb - 1000 ppb (in final MeOH extract)
M570	n/d	n/d	25 ppb - 1000 ppb (in final MeOH extract)

n/d = not determined

NOTE: to calculate MDL, LOQ, and LCR values in ug/ml of urine divide the above values by 4.

MDL/LOQ values in rat and monkey urine were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the human urine curves to determine equivalence. Responses in the rat and monkey were similar to the human responses, therefore, their MDL and LOQ are assumed to be similar to the values determined for human urine.

If a suitable amount of clean, control matrix is available, samples will be evaluated versus a curve extracted from urine originating from the same species as the specimens.

Please see LOQ Summary and MDL study in **FACT-TOX-131, W2067** for further information.

Compound: PFOS

Human Urine	Prepared range of standards (ppb) (ng/ml)	LCR from curve (ppb) (ng/ml)	% Recovery Range	RSD Range
Full Range	n/d	n/d	n/d	n/d
Low Curve	n/d	n/d	n/d	n/d
High curve	n/d	n/d	n/d	n/d
1/X	2.5ppb – 1500 ppb	15 ppb – 1500 ppb	75-116	+/- 30%

Compound: POAA

Human Urine	Prepared range of standards (ppb) (ng/ml)	LCR from curve (ppb) (ng/ml)	% Recovery Range	RSD Range
Full Range	n/d	n/d	n/d	n/d
Low Curve	n/d	n/d	n/d	n/d
High curve	n/d	n/d	n/d	n/d
1/X, quadratic	2.5ppb – 1500 ppb	50 ppb – 1500 ppb	86-105	+/- 30%

Ion Pair Standard Curves – Urine

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

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

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	POAA Std conc ug/ml	M556 Std conc ug/ml	M570 Std conc ug/ml	All Am't spiked ml	All Final vol ml
0.500	0.501	0.500	0.501	0.499	0.500	0.501	0.005	2.0075
0.500	0.501	0.500	0.501	0.499	0.500	0.501	0.010	2.0125
0.500	0.501	0.500	0.501	0.499	0.500	0.501	0.025	2.0275
5.00	5.01	5.00	5.01	4.99	5.00	5.01	0.005	2.0075
5.00	5.01	5.00	5.01	4.99	5.00	5.01	0.010	2.0125
5.00	5.01	5.00	5.01	4.99	5.00	5.01	0.025	2.0275
50.0	50.1	50.0	50.1	49.9	50.0	50.1	0.005	2.0075
50.0	50.1	50.0	50.1	49.9	50.0	50.1	0.0075	2.0100
50.0	50.1	50.0	50.1	49.9	50.0	50.1	0.010	2.0125
50.0	50.1	50.0	50.1	49.9	50.0	50.1	0.015	2.0130

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	POAA Final conc ng/ml	M556 Final conc ng/ml	M570 Final conc ng/ml	Surrogate Std conc ng/ml	All Am't spiked ml
1.25	1.25	1.25	1.25	1.24	1.25	1.25	100	0.0025
2.48	2.49	2.48	2.49	2.48	2.48	2.49		
6.17	6.18	6.17	6.18	6.15	6.17	6.18	**Surrogate**	
12.5	12.5	12.5	12.5	12.4	12.5	12.5	Final conc	
24.8	24.9	24.8	24.9	24.8	24.8	24.9	ng/ml	
61.7	61.8	61.7	61.8	61.5	61.7	61.8	125	
125	125	125	125	124	125	125		
187	187	187	187	186	187	187		
248	249	248	249	248	248	249		
372	372	372	372	371	372	372		

Calculated concentrations of standards in methanol extract (0.5 ml final volume)

PFOS Final conc ng/ml	PFOSA Final conc ng/ml	PFOSAA Final conc ng/ml	EtFOSE Final conc ng/ml	POAA Final conc ng/ml	M556 Final conc ng/ml	M570 Final conc ng/ml	Surrogate Std conc ng/ml	All Am't spiked ml
5.00	5.01	5.00	5.01	4.99	5.00	5.01	100	0.0025
10.0	10.0	10.0	10.0	10.0	10.0	10.0		
25.0	25.1	25.0	25.1	25.0	25.0	25.1	**Surrogate**	
50.0	50.1	50.0	50.1	49.9	50.0	50.1	Final conc	
100	100	100	100	100	100	100	ng/ml	
250	251	250	251	250	250	251	500	
500	501	500	501	499	500	501		
750	752	750	752	749	750	752		
1000	1002	1000	1002	998	1000	1002		
1500	1503	1500	1503	1497	1500	1503		

Prep Date(s):
Analyst(s):
Sample Matrix:
Method/Revision:
Target Analyte(s):

Study Number:
Equipment Number:
Final Solvent & TN Number:
Matrix Blank/Identifier:
Box:

Sample ID	Initial Wt./Vol. g/mL/L			Comments

Summary of method:

Notes:

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS IN URINE EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY/MASS SPECTROMETRY

Method Number: ETS-8-97.0

Adoption Date: 7-28-99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne, Glenn Langenburg

Approved By:

Laboratory Manager	7/28/99 Date
Group Leader	7/28/99 Date
Technical Reviewer	7/27/99 Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of urine extracts for fluorochemicals using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemicals or other ionizable compounds.

1.3 Matrices: Human, rat, and monkey urine, or other fluids as designated in the validation report.

2.0 Summary of Method

2.1 This method describes the analysis of fluorochemicals extracted from urine 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 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 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 WindowsNT 4.0 versions. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole 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 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 regulated to approximately 100 psi. (House air system)

7.1.2 High purity grade argon regulated to approximately 6 psi.

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

7.1.4 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 ASTM type I, 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

8.1.3.1 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 twenty matrix standards are prepared during the extraction procedure. See **ETS-8-96.0.**

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 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 solvent blank, method blank, and 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 regression (linear or otherwise, see **11.2**), weighted 1/x, not forced through zero, with IS reference (surrogate is used as an internal standard) using MassLynx or other suitable software.

11.2 Use the following parameters for determining a calibration curve for each compound:

Compound	Weighting	Regression Fit	Response type
PFOS	1/X	Linear	IS reference
POAA	1/X	Quadratic	IS reference

Suitability of curve fitting parameters for other fluorochemical compounds will be addressed by the analyst.

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

11.4 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 50 ppb of analyte, generate a calibration curve consisting of the standards from 25 ppb to 250 ppb rather than the full range of the curve (25 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 two digits 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 after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9.0 minutes

12.2.2.4 Solvent ramp =

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 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,** "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. Click on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

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

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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

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

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

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

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

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-96.0, 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 values 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 must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

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

14.6 If criteria listed in this 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 a linear or quadratic fit, referenced to the internal standard (surrogate), 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 7.0) 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-8-97.0 Data summary spreadsheet.

18.0 References

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

18.2 The validation report associated with this method is **FACT-TOX-131, W2067**

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-96.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

Laboratory Study

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

Group Dose	Sample#	Concentration 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

Method Modification

Method: ETS-8-97.0
Sections modified: 10.3.2 and 14.5.1

Method reads:

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

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

Modify method to read:

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

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

Effective date of modification: 7/28/99

Kitu Hon Sept. 21, 2000

Signature of PAI and date

Exact Copy of Original
UC 11/14/00
Initial Date

Method Modification

Methods: ETS-8-7.0, ETS-8-5.1, ETS-8-97.0
Section modified: add section 14.3.2

Method reads:

NA

Modify method to read:

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

Effective date of modification ETS-8-7.0: 7/22/99
Effective date of modification ETS-8-5.1: 4/26/99
Effective date of modification ETS-8-97.0: 7/28/99

Kristie Hz 11/09/00
Signature of PAI and date

Exact Copy of Original
RWW 11/13/00
Initial Date

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

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

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

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

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

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 Interferences

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

6.0 Equipment

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

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

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

7.0 Supplies and Materials

7.1 Supplies

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

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

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 Reagents and Standards

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

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

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

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

9.0 Sample Handling

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

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

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

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

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

10.2 Matrix Spikes

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

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

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

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

10.3 Continuing Calibration Verifications

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

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

11.0 CALIBRATION AND STANDARDIZATION

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

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

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

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

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

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

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

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

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

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

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

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

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 METHOD PERFORMANCE

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

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

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

14.5 Continuing Calibration Verifications

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

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

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

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

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

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

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

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Laboratory Study

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

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

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

Method Modification

Method: ETS-8-5.1
Sections modified: 10.3.2 and 14.5.1

Method reads:

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

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

Modify method to read:

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

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

Effective date of modification: 4/26/99

Kristen Hz 11/08/00
Signature of PAI and date

Exact Copy of Original
RWW 11/13/00
Initial Date

Appendix D: Data Summary Tables

Table 9. Average Results for the Analyses of Sera Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	DG0 PFOS Conc. (µg/mL)	**DG7** PFOS Conc. (µg/mL)	**DG15** PFOS Conc. (µg/mL)	**DG21** PFOS Conc. (µg/mL)	**DL14** PFOS Conc. (µg/mL)	**DL21** PFOS Conc. (µg/mL)	**DL22** PFOS Conc. (µg/mL)
Group I F0 0.0 mg/kg/day	0.100[a,b]	0.0796[a,b]	0.0742[a,b]	<LLQ[a,b]	0.0542[a,b]	NS	0.0492[a,b]
Group I F1	NS	NS	NS	NS	NS	0.0531[a,b]	NS
Group II F0 0.1 mg/kg/day	9.21[a,b]	7.24[a,b]	5.68[a,b]	2.58[a,b]	1.63[a,b]	NS	0.979[a,b]
Group II F1	NS	NS	NS	NS	NS	1.80[a,b]	NS
Group III F0 1.6 mg/kg/day	161[a,b]	129[a,b]	90.6[a,b]	39.5[a,b]	20.6[a,b]	NS	14.1[a,b]
Group III F1	NS	NS	NS	NS	NS	27.1[a,b]	NS

[a] PFOS concentrations are the average of samples within a dose group from all animals tested, not including samples that tested at or below the limit of quantitation (0.04 µg/mL). Individual animal data are located in Appendix E.

[b] Sera analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

<LLQ=Less than the Limit of Quantitation

NS=Not Sampled

Table 10. Average Results for the Analyses of Liver Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	DL22 PFOS Conc. (µg/g)
Group I F0 0.0 mg/kg/day	0.243[a,b]
Group I F1	0.174[a,b]
Group II F0 0.1 mg/kg/day	6.15[a,b]
Group II F1	5.00[a,b]
Group III F0 1.6 mg/kg/day	59.7[a,b]
Group III F1	56.2[a,b]

[a] PFOS concentrations are the average of samples within a dose group from all animals tested, not including samples that tested at or below the limit of quantitation (0.11 µg/g). Individual animal data are located in Appendix E.

[b] Liver analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

Table 11. Average Results for the Analyses of Urine Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	DG0 PFOS Conc. (µg/mL)	DG6/7 PFOS Conc. (µg/mL)	DG14/15 PFOS Conc. (µg/mL)	DG20/21 PFOS Conc. (µg/mL)	DL21/22 PFOS Conc. (µg/mL)
Group I F0 0.0 mg/kg/day	0.00819[a]	0.0100[a]	0.00685[a]	0.00614[a]	<LOQ[a]
Group II F0 0.1 mg/kg/day	0.0905[a]	0.0307[a]	0.0327[a]	0.0231[a]	0.00555[a]
Group III F0 1.6 mg/kg/day	2.11[a]	0.888[a]	0.613[a]	0.340[a]	0.0334[a]

[a] PFOS concentrations are the average of samples within a dose group from all animals tested, not including samples that tested at or below the limit of quantitation (0.00295 µg/mL). Individual animal data are located in Appendix E.

<LOQ=Less than Limit of Quantitation

Table 12. Average Results for the Analyses of Feces Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	DG0 PFOS Conc. (µg/g)	DG6/7 PFOS Conc. (µg/g)	DG14/15 PFOS Conc. (µg/g)	DG20/21 PFOS Conc. (µg/g)	DL21/22 PFOS Conc. (µg/g)
Group I F0 0.0 mg/kg/day	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
Group II F0 0.1 mg/kg/day	0.601[a,b]	0.399[a]	0.294[a]	0.119[a]	0.0522[a]
Group III F0 1.6 mg/kg/day	10.9[a]	8.39[a]	4.83[a]	2.06[a]	0.387[a]

[a] PFOS concentrations are the average of samples within a dose group from all animals tested, not including samples that tested at or below the limit of quantitation (0.0100 µg/g). Individual animal data are located in Appendix E. and Appendix G.

[b]Animal 13745F result not included in average.

ND=Not Detected

Table 13. LOQ Values Used in FACT TOX-111 Analysis by Method and Usage Dates

Sample	LOQ	Laboratory	Method(s)	Usage Date
Urine	0.00295 µg/mL	3M Environmental Laboratory	ETS-8-96.0 ETS-8-97.0	6 October 1999
Dosage Confirmation	0.030 µg/mL	3M Environmental Laboratory	ETS-8-5.1	21 September 1999
Liver	0.11 µg/g	Battelle Memorial Institute	N003604-A	29 October 1999
Serum	0.04 µg/mL	Advanced Bioanalytical Services, Inc.	99VDJA01.MI.DOC	9 July 1999
Feces	0.0100 µg/g	Centre Analytical Laboratories, Inc.	00M-023-003, revision 2	6 July 1999

Appendix E: Data Spreadsheets

Table 14. Results of Analyses of Sera Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage.

	Sample #	DG0 PFOS Conc. (µg/mL)	DG7 PFOS Conc. (µg/mL)	DG15 PFOS Conc. (µg/mL)	DG21 PFOS Conc. (µg/mL)	DL14 PFOS Conc. (µg/mL)	DL21 PFOS Conc. (µg/mL)	DL22 PFOS Conc. (µg/mL)
Group I 0.0 mg/kg/day	13726	0.186[a]	0.152[a]	0.145[a]	0.0734[a]	0.0710[a]	NS	0.0516[a]
	13727	0.105[a]	0.0721[a]	0.0805[a]	0.0507[a]	0.0551[a]	NS	0.0447[a]
	13728	0.0804[a]	0.0614[a]	0.0523[a]	<LLQ[a]	0.0458[a]	NS	<LLQ[a]
	13731	0.0727[a]	0.0754[a]	0.0527[a]	NS	NS	NS	NS
	13734	0.102[a]	0.0556[a]	0.0575[a]	<LLQ[a]	0.0513[a]	NS	<LLQ[a]
	13735	0.0818[a]	0.0605[a]	0.0496[a]	<LLQ[a]	<LLQ[a]	NS	<LLQ[a]
	13736	0.0976[a]	0.0933[a]	0.0599[a]	<LLQ[a]	<LLQ[a]	NS	0.0511[a]
	13737	0.0758[a]	0.0663[a]	0.0959[a]	0.0459[a]	0.0478[a]	NS	0.0492[a]
	13726P	NS	NS	NS	NS	NS	0.0615[a]	NS
	13727P	NS	NS	NS	NS	NS	<LLQ[a]	NS
	13728P	NS	NS	NS	NS	NS	<LLQ[a]	NS
	13734P	NS	NS	NS	NS	NS	<LLQ[a]	NS
	13735P	NS	NS	NS	NS	NS	<LLQ[a]	NS
	13736P	NS	NS	NS	NS	NS	<LLQ[a]	NS
	13737P	NS	NS	NS	NS	NS	0.0448[a]	NS
Group II 0.1 mg/kg/day	13739	8.45[a]	8.21[a]	5.08[a]	1.56[a]	1.57[a]	NS	1.15[a]
	13740	10.5[a]	6.78[a]	6.77[a]	3.46[a]	1.72[a]	NS	1.27[a]
	13741	10.0[a]	6.56[a]	5.76[a]	2.32[a]	1.30[a]	NS	1.03[a]
	13744	8.54[a]	7.93[a]	5.22[a]	2.63[a]	1.69[a]	NS	0.762[a]
	13745	8.54[a]	6.39[a]	5.01[a]	2.02[a]	2.20[a]	NS	0.942[a]
	13746	9.59[a]	7.34[a]	5.75[a]	2.45[a]	1.75[a]	NS	0.816[a]
	13748	8.81[a]	NS	4.63[a]	3.40[a]	1.17[a]	NS	0.814[a]
	13749	9.24[a]	7.44[a]	7.26[a]	2.83[a]	1.62[a]	NS	1.05[a]
	13739P	NS	NS	NS	NS	NS	2.02[a]	NS
	13740P	NS	NS	NS	NS	NS	1.83[a]	NS
	13741P	NS	NS	NS	NS	NS	1.62[a]	NS
	13744P	NS	NS	NS	NS	NS	1.98[a]	NS
	13745P	NS	NS	NS	NS	NS	1.62[a]	NS
	13746P	NS	NS	NS	NS	NS	2.05[a]	NS
	13748P	NS	NS	NS	NS	NS	1.50[a]	NS
	13749P	NS	NS	NS	NS	NS	1.79[a]	NS

[a] Sera analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

<LLQ=Less than the Limit of Quantitation (0.04 (µg/mL))

NS=No sample

Table 14. Results of Analyses of Sera Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	Sample #	DG0 PFOS Conc. (µg/mL)	DG7 PFOS Conc. (µg/mL)	DG15 PFOS Conc. (µg/mL)	DG21 PFOS Conc. (µg/mL)	DL14 PFOS Conc. (µg/mL)	DL21 PFOS Conc. (µg/mL)	DL22 PFOS Conc. (µg/mL)
Group III 1.6 mg/kg/day	13751	134[a]	129[a]	82.6[a]	33.3[a]	18.3[a]	NS	14.8[a]
	13752	142[a]	146[a]	94.2[a]	30.1[a]	20.8[a]	NS	16.8[a]
	13753	184[a]	136[a]	96.8[a]	28.2[a]	18.7[a]	NS	15.7[a]
	13754	171[a]	131[a]	89.0[a]	32.1[a]	18.3[a]	NS	8.19[a]
	13755	165[a]	119[a]	108[a]	96.8[a]	NS	NS	NS
	13756	162[a]	137[a]	93.3[a]	34.9[a]	26.8[a]	NS	17.2[a]
	13758	162[a]	124[a]	75.1[a]	32.0[a]	23.9[a]	NS	12.4[a]
	13759	171[a]	115[a]	85.9[a]	28.5[a]	17.6[a]	NS	13.3[a]
	13751P	NS	NS	NS	NS	NS	30.2[a]	NS
	13752P	NS	NS	NS	NS	NS	29.3[a]	NS
	13753P	NS	NS	NS	NS	NS	24.5[a]	NS
	13754P	NS	NS	NS	NS	NS	20.2[a]	NS
	13756P	NS	NS	NS	NS	NS	41.0[a]	NS
	13758P	NS	NS	NS	NS	NS	22.1[a]	NS
	13759P	NS	NS	NS	NS	NS	22.7[a]	NS

[a] Sera analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

LLQ= Limit of Quantitation (0.04 µg/mL)

NS=No sample

Table 15. Results of Analyses of Liver Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	Sample #	DL22 PFOS Conc. (µg/g)
Group I 0.0 mg/kg/day	13726	0.295[a]
	13727	0.371[a]
	13728	0.258[a]
	13731	0.257[a]
	13734	0.188[a]
	13735	0.217[a]
	13736	0.165[a]
	13737	0.189[a]
	13726P	0.367[a]
	13727P	0.137[a]
	13728P	0.122[a]
	13734P	0.135[a]
	13735P	0.137[a]
	13736P	BLOQ[a]
	13737P	0.148[a]
Group II 0.1 mg/kg/day	13739	5.22[a]
	13740	5.84[a]
	13741	7.19[a]
	13744	5.41[a]
	13745	5.91[a]
	13746	5.97[a]
	13748	5.86[a]
	13749	7.77[a]
	13739P	5.49[a]
	13740P	5.11[a]
	13741P	4.19[a]
	13744P	5.47[a]
	13745P	5.30[a]
	13746P	5.24[a]
	13748P	4.34[a]
	13749P	4.90[a]

[a] Liver analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

BLOQ=Below Concentration of Lowest Standard in Calibration Curve

LOQ=0.11 µg/g

Table 15. Results of Analyses of Liver Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Liver, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	Sample #	DL22 PFOS Conc. (µg/g)
Group III 1.6 mg/kg/day	13751	69.6[a]
	13752	88.1[a]
	13753	43.5[a]
	13754	42.9[a]
	13756	56.2[a]
	13758	49.0[a]
	13759	68.3[a]
	13751P	50.7[a]
	13752P	54.6[a]
	13753P	49.8[a]
	13754P	62.9[a]
	13756P	72.0[a]
	13758P	50.6[a]
	13759P	52.6[a]

[a] Liver analyses were conducted at a contract laboratory, but were corrected by 3M to reflect the official purity values from the Certificate of Analysis. A revised final report from the contract laboratory will be added as an amendment to this report.

BLOQ=Below Concentration of Lowest Standard in Calibration Curve
LOQ=0.11 µg/g

Table 16. Results of Analyses of Urine Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	Sample #	DG0 PFOS Conc. (µg/mL)	DG6/7 PFOS Conc. (µg/mL)	DG14/15 PFOS Conc. (µg/mL)	DG20/21 PFOS Conc. (µg/mL)	DL21/22 PFOS Conc. (µg/mL)
Group I 0.0 mg/kg/day	13726F	0.00381	<LOQ	<LOQ	<LOQ	<LOQ
	13727F	<LOQ	<LOQ	<LOQ	0.00614	<LOQ
	13728F	0.00464	0.00312	<LOQ	<LOQ	<LOQ
	13731F	0.00650	<LOQ	<LOQ	NS	NS
	13734F	0.0108	<LOQ	<LOQ	<LOQ	<LOQ
	13735F	0.0117	<LOQ	<LOQ	<LOQ	<LOQ
	13736F	0.0117	0.0168	<LOQ	<LOQ	<LOQ
	13737F	<LOQ	<LOQ	0.00685	<LOQ	<LOQ
Group II 0.1 mg/kg/day	13739F	0.0852	0.0454	0.0135	0.00531	<LOQ
	13740F	0.202	0.0296	0.0444	0.0230	0.00981
	13741F	0.0587	0.0256	0.0335	0.0368	0.00391
	13744F	0.0807	0.0257	0.0184	0.0259	<LOQ
	13745F	0.0520	0.0394	0.0244	0.0298	<LOQ
	13746F	0.0517	0.0223	0.0642	0.0187	0.00328
	13748F	0.101	0.0248	0.0253	0.0266	<LOQ
	13749F	0.0929	0.0331	0.0384	0.0188	0.00520
Group III 1.6 mg/kg/day	13751F	1.91	1.47	0.653	0.419	0.0539
	13752F	1.49	0.479	0.549	0.482	0.0472
	13753F	3.33	1.69	0.520	0.0898	0.0433
	13754F	1.48	SL	NE	0.183	0.0118
	13755F	0.859	0.403	0.953	0.363	NS
	13756F	2.55	0.597	0.798	0.416	0.0279
	13758F	2.74	0.438	0.273	0.558	0.0328
	13759F	2.50	1.14	0.548	0.208	0.0169

<LOQ=Less than the Limit of Quantitation (0.00295(µg/mL)
ND=Not Detected
NS=No Sample
SL=Sample lost during extraction
NE=No sample extract remaining to perform dilution

Table 17. Results of Analyses of Feces Samples in the Study of the Determination of the Presence and Concentration of PFOS in the Sera, Livers, Urine, and Feces of Crl:CD®BR VAF/Plus® Rats Exposed to T-6295.14 via Gavage

	Sample #	Prior to Cohabitation PFOS Conc. (µg/g)	DG6/7 PFOS Conc. (µg/g)	DG14/15 PFOS Conc. (µg/g)	DG20/21 PFOS Conc. (µg/g)	DL21/22 PFOS Conc. (µg/g)
Group I 0.0 mg/kg/day	13726F	ND	ND	ND	ND	0.0204
	13727F	ND	ND	ND	ND	ND
	13728F	ND	ND	ND	ND	ND
	13731F	ND	ND	ND	NS	NS
	13734F	ND	ND	ND	ND	ND
	13735F	ND	ND	ND	ND	ND
	13736F	ND	ND	ND	ND	ND
	13737F	ND	ND	ND	ND	ND
Group II 0.1 mg/kg/day	13739F	0.810	0.514	0.298	0.133	0.0674
	13740F	0.664	0.537	0.311	0.0668	0.0693
	13741F	0.394	0.318	0.308	0.165	0.0389
	13744F	0.402	0.309	0.416	0.0883	0.0729
	13745F	2.72[a]/0.904[b]	0.382	0.294	0.0650	0.0339
	13746F	0.688	0.389	0.345	0.113	0.0609
	13748F	0.703	0.322	0.204	0.157	0.0508
	13749F	0.546	0.420	0.176	0.163	0.0231
Group III 1.6 mg/kg/day	13751F	10.8	8.51	5.00	1.61	0.513
	13752F	10.8	9.61	6.31	2.08	0.731
	13753F	12.3	9.03	4.02	1.42	0.327
	13754F	13.5	8.86	3.80	1.69	0.295
	13755F	8.57	12.2	5.65	4.29	NS
	13756F	10.8	6.70	5.13	2.02	0.336
	13758F	9.41	7.65	5.30	1.97	0.211
	13759F	10.9	4.47	3.41	1.38	0.296

[a]Questionable residue found: re-extracted for confirmation.

[b]Re-extracted value. Animal 13745F values were not included in the average values in Table 12.

LOQ=0.0100 µg/g

ND=Not Detected

NS=No Sample

Appendix F: Example Calculations

Formula Used for Urine Analyses in Study FACT TOX-111

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

Calculation Used for Group II DG 14-15, Animal Number 13739F

$$16.79\ \text{(ng/mL)} \times 1 \times 0.9275 \times \frac{1.0\mu g}{1000ng} \times 0.864 = 0.0135\ \text{(µg PFOS/mL urine)}$$

AR—Analytical result from MassLynx summary
DF—Dilution factor
SC—PFOS salt correction constant (0.9275)
PC—PFOS purity correction factor (0.864)

Appendix G: Contract Laboratory Reports

This appendix includes the following contract laboratory reports:

Advanced Bioanalytical Services, Inc., Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-111 Using Turbo Ion Spray LC/MS, ABS Report Number 99ADJA03.MI.DOC (19 pages).

Advanced Bioanalytical Services, Inc., "Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS," ABS Report Number 99VDJA01.MI.DOC (50 pages).

Battelle Memorial Institute, Study Number N003296-G, Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats Final Report, (58 pages).

Centre Analytical Laboratories, Inc., Centre Study Number 023-017, Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats, (93 pages).

ADVANCED
BIOANALYTICAL
SERVICES, INC.

BIOANALYTICAL REPORT

Title:	Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-111 Using Turbo Ion Spray LC/MS
Date:	11 October 1999
Authors:	David J. Anderson, M.S. Amie J. Prince, B.S. Holly D. Ross, M.S.
Prepared For:	3M Environmental Technology and Safety Services St. Paul, MN 55133-3331
ABS Report No.:	99ADJA03.MI.DOC
Study Protocol:	FACT-TOX-111
Number of Pages:	19

Summary and Conclusions

The concentration of perfluorooctanesulfonate (PFOS) was determined in rat serum samples collected during the study #FACT-TOX-111 using a sensitive, specific, accurate, and reproducible analytical method developed by Advanced BioAnalytical Services, Inc., Ithaca, NY.

Rat serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate PFOS and the internal standard, 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). Following evaporation and reconstitution, sample extracts were analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

All samples were successfully analyzed within four runs. The lower limit of quantitation was 0.05 µg/mL for PFOS. The precision of this assay (RSD) as determined from the analysis of quality control samples for PFOS was ≤3.61%. The precision of this assay (RSD), as determined from the calibration standards for PFOS was ≤5.08%. The relative error (RE) of the assay, as determined from the analysis of the quality control samples ranged from 2.38 to 12.7%. The RE of the assay, as determined from the analysis of calibration standards ranged from -5.31 to 10.5%.

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SIGNATURE PAGE

Title: Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-111 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA03.MI.DOC

Reported by: _____ 11 OCT 1999
David J. Anderson, M.S. — Date
Research Scientist

Reviewed by: Melissa Mapes 11 OCT 99
Melissa Mapes, A.S. — Date
Associate Auditor

Authorized for Release by: _____ 11 OCT 99
John R. Perkins, Ph.D. — Date
Assistant Scientific Director

Accepted by: _____ _____
Kris Hanson, Ph.D. — Date
3M Study Director

QAU STATEMENT

Periodic inspections of the bioanalytical portion of study #FACT-TOX-111 were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:

25 June 1999; 7, 21 July 1999; 31 August 1999.

Results of the inspection were reported to ABS Management and Project Team Leader on:

25 June 1999; 7, 21 July 1999; 31 August 1999.

Results of the inspections were reported to the Study Director on 2 September 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Melissa Mapes 11OCT 99

Melissa Mapes, A.S. Date
Associate Auditor

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1. INTRODUCTION

1.1. Study Description and Objective

The objective of this study was to determine the concentrations of perfluorooctanesulfonate (PFOS) in rat serum samples collected during the study #FACT-TOX-111, "Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats." Rat serum samples were analyzed using a liquid-liquid extraction and turbo ion spray liquid chromatography/mass spectrometry (LC/MS) assay.

2. METHODS

2.1. Analytical Procedure(s)

PFOS concentrations were determined in rat serum according to the validated LC/MS method (1). Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure using ethyl acetate to isolate PFOS and the internal standard (IS), 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS) from rat serum. Following evaporation to dryness and subsequent reconstitution, sample extracts were separated by reversed-phase chromatography on a 2 x 50 mm (5 µm) Betasil™ C_{18} column (Keystone Scientific, Inc., Bellefonte, PA) with an initial mobile phase of 55% Eluent A (10:90 methanol:2 mM ammonium acetate) and 45% Eluent B (90:10 methanol:2 mM ammonium acetate). PFOS concentrations were determined by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in selected ion monitoring (SIM) mode. The negative ions monitored were:

m/z = 499.0 for PFOS $[M-K]^-$
m/z = 427.0 for Tetra-H-PFOS $[M-H]^-$

Study sample concentrations were determined from a weighted ($1/y^2$), quadratic regression of peak area ratios (peak area of PFOS/peak area of Tetra-H-PFOS) versus nominal concentration of ten calibration standards. The nominal concentrations of the calibration standards were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 µg/mL PFOS.

The lower limit of quantitation (LLQ) for this assay was determined to be 0.05 µg/mL PFOS. Quality control (QC) serum samples at three different concentrations (0.2, 6, and 18 µg/mL PFOS) were analyzed with each assay batch in replicates of four. Dilution QC samples (QC4, 100 µg/mL) were prepared at each dilution that was used during a particular assay and were analyzed with each assay batch in replicates of four as needed.

The acceptance criteria for calibration standards stipulated that the back-calculated concentrations of at least three-fourths of the individual calibration standard replicates must not deviate more than ±15% from their nominal concentration (except at the LLQ). At least one duplicate at the LLQ must exhibit a deviation within ±20% of the nominal

concentration . The coefficient of determination (r^2) must be ≥0.98. The acceptance criteria followed are in accordance with ABS Standard Operating Procedures.

The acceptance criteria for the quality control samples stipulated that at least two-thirds of the individual QC sample replicates must not deviate more than ±15% from their nominal concentrations. At least one replicate at each QC concentration must exhibit a deviation within ±15%.

A summary of the sample and assay information for study #FACT-TOX-111 is given in Table 1.

2.2. Assay Site

All samples were analyzed at Advanced BioAnalytical Services, Inc., Ithaca, NY.

2.3. Data Processing

The data were collected using turbo ion spray LC/MS selected ion monitoring (SIM) in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the ABS file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson (v 5.3.1.01 PSS, Inc., Wayne, PA 19087). Calculations were performed on unrounded numbers. All calibration standard and QC results were rounded to no less than three significant figures before reporting.

3. RESULTS

3.1. Assay Performance

The performance of the assay for PFOS as determined from the analysis of daily quality control samples is documented in Table 2. The inter-assay precision (RSD) of quality control samples ranged from 2.78 to 3.61% for PFOS. There was no marked inaccuracy in the results from these quality control samples; the relative error (RE) ranged from 2.38 to 12.7%.

The performance of daily calibration curves is documented in Table 3. The inter-assay precision (RSD) of the standards ranged from 1.52 to 5.08% for PFOS. There was no marked inaccuracy in the results from these standards; the relative error (RE) ranged between -5.31 to 10.5%.

The slope, y-intercept, and coefficient of determination (r^2) for each analytical run are presented in Table 4. The r^2 values ranged from 0.9959 to 0.9977 for PFOS in rat serum.

All ABS personnel assigned to analyze samples for this project were required to successfully extract quality control (QC) samples and calibration standards. Results of the extraction were reviewed by a trained analyst against the pre-defined acceptance criteria for this assay. Results are maintained in the study records of ABS.

3.2. Analytical Results for Study #FACT-TOX-111

PFOS concentrations in rat serum from Study #FACT-TOX-111 are presented in Tables 5 through 7. All data were rounded to no less than three significant figures before reporting in Tables 5 through 7. Study samples requiring repeat analysis for Study #FACT-TOX-111 are presented in Table 8.

4. SUMMARY AND CONCLUSIONS

The concentration of PFOS was determined in rat serum samples collected during the study #FACT-TOX-111.

PFOS was isolated from serum by a liquid-liquid extraction procedure and determined by LC/MS.

The quality of the determinations was satisfactory throughout. The LLQ was 0.05 µg/mL PFOS. The precision of the assay, as determined from the analysis of quality control samples was ≤3.61% for PFOS.

5. DATA RETRIEVAL

The calculated concentration data from the analysis of rat serum samples for Study #FACT-TOX-111 are maintained on file in the archives of the Advanced BioAnalytical Services, Inc., Ithaca, NY in ABS Notebook 2325.

6. REFERENCES

1. Advanced BioAnalytical Services Validation Report 99VDJA01.MI.DOC. Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS. David J. Anderson, M.S. Amie J. Prince, B.S., and Holly D. Ross, M.S. 26 August 1999.

7. TABLES

Table 1: Summary of Sample and Assay Information for Protocol FACT-TOX-111

Species/Matrix:	Rat/Serum
Sample Collection and Storage Information:	
Anticoagulant/Stabilizer:	None
Reported Sample Collection Dates:	12 January 1999 to 20 February 1999
Dates Received at ABS:	16 June 1999
Storage Temperature at ABS:	–20 °C
Assay Information:	
Assay Period:	9 July 1999 to 14 July 1999
Analyte:	**Potassium perfluorooctanesulfonate (PFOS)**
Analytical Standard:	PFOS
Lot No:	171
Source:	3M
Internal Standard:	1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS)
Lot No:	59909
Source:	3M
Calibration Range:	
Regression Method:	quadratic
Weighting Factor:	$1/y^2$
Lower Limit of Quantitation:	0.05 µg/mL
Upper Limit of Quantitation:	20 µg/mL

Table 2: Inter-Assay Precision and Accuracy for PFOS Quality Control Samples in Rat Serum for Study #FACT-TOX-111

	PFOS Concentration (µg/mL)				
Run No.	QC1 0.2	QC2 6	QC3 18	QC4 100 (1 in 10 Dilution)	QC4 100 (1 in 20 Dilution)
1	0.232	6.17	18.2	NA	NA
	0.212	6.28	18.4	NA	NA
	0.210	6.11	18.2	NA	NA
	0.216	5.97	18.2	NA	NA
2	0.216	6.00	18.1	NA	NA
	0.227	6.09	19.0	NA	NA
	0.224	6.07	19.8	NA	NA
	0.219	6.18	18.5	NA	NA
3	0.211	5.97	18.8	116	NA
	0.210	5.84	18.2	114	NA
	0.217	6.07	18.8	113	NA
	0.214	5.99	17.9	106	NA
4	0.222	6.48	19.3	NA	112
	0.233	6.23	19.7	NA	109
	0.214	6.54	19.6	NA	117
	0.225	6.30	18.5	NA	113
Mean	0.219	6.14	18.7	112	113
RSD (%)	3.43	3.08	3.28	3.61	2.78
RE	9.39	2.38	3.86	12.3	12.7

RSD = (SD/Mean) x 100%
RE = [(Mean-Nominal)/Nominal] x 100%
NA: Not applicable.

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Table 3: Inter-Assay Precision and Accuracy for PFOS Calibration Standards in Rat Serum for Study #FACT-TOX-111

	PFOS Concentration (µg/mL)									
Run No.	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8	STD9	STD10
	0.05	0.1	0.25	0.5	1	2	4	8	16	20
1	0.0496	0.0993	0.238	0.493	0.957	2.13	4.39	7.72	16.0	20.4
	0.0513	0.101	0.243	0.491	0.963	2.12	4.38	7.76	15.5	20.0
2	0.0470	0.101	0.244	0.542	0.899	2.03	4.30	7.67	16.0	20.6
	0.0538	0.101	0.244	0.539	0.928	2.03	4.43	7.77	15.8	19.8
3	0.0490	0.105	0.254	0.482	0.980	2.15	4.49	7.80	16.9	20.4
	0.0511	0.0980	0.242	0.487	0.937	2.01	4.39	7.39	15.8	19.2
4	0.0522	0.0950	0.246	0.484	0.953	2.11	4.47	7.59	15.9	20.6
	0.0491	0.104	0.246	0.481	0.958	2.13	4.50	7.89	15.3	20.0
Mean	0.0504	0.100	0.245	0.500	0.947	2.09	4.42	7.70	15.9	20.1
RSD (%)	4.24	3.04	1.80	5.08	2.66	2.67	1.52	1.98	2.98	2.34
RE	0.775	0.425	-2.16	-0.0325	-5.31	4.49	10.5	-3.76	-0.651	0.626

RSD = (SD/Mean) x 100%

RE = [(Mean-Nominal)/Nominal] x 100%

Table 4: Calibration Curve Statistics for the Determination of PFOS in Rat Serum for Study #FACT-TOX-111

Run No.	Quadratic Coefficient (A)	Linear Coefficient (B)	Intercept (C)	Coefficient of Determination (r^2)
1	-0.0004154	0.1025	-0.0006090	0.9977
2	-0.0005419	0.1001	-0.0004172	0.9959
3	-0.0006070	0.1046	-0.0001313	0.9968
4	-0.0002665	0.1036	-0.0004533	0.9966
Mean	-0.0004577	0.1027	-0.0004027	0.9968

$y = Ax^2 + Bx + C$, weighted $1/y^2$

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Table 5: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 0 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*							
	13726	13726P	13727	13727P	13728	13728P	13731	13734
0	0.215	NS	0.121	NS	0.0930	NS	0.0842	0.118
7	0.176	NS	0.0835	NS	0.0711	NS	0.0873	0.0644
14	0.0822	NS	0.0638	NS	0.0530	NS	NS	0.0594
15	0.168	NS	0.0932	NS	0.0605	NS	0.0610	0.0665
21	0.0849	0.0712	0.0587	<LLQ[0:0452]<(0.05)	<LLQ[0.0385]<(0.05)	<LLQ[0.0343]<(0.05)	NS	<LLQ[0.0390]<(0.05)
22	0.0597	NS	0.0517	NS	<LLQ[0.0431]<(0.05)	NS	NS	<LLQ[0.0446]<(0.05)

*Rat Number with a "P" indicates pup sample.
<LLQ = Less than the Lower Limit of Quantitation (0.05 µg/mL).
NS: No sample.

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Table 5: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 0 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*						
	13734P	13735	13735P	13736	13736P	13737	13737P
0	NS	0.0947	NS	0.113	NS	0.0877	NS
7	NS	0.0700	NS	0.108	NS	0.0767	NS
14	NS	<LLQ[0.0492]<(0.05)	NS	<LLQ[0.0474]<(0.05)	NS	0.0553	NS
15	NS	0.0574	NS	0.0693	NS	0.111	NS
21	<LLQ[0.0409]<(0.05)	<LLQ[0.0418]<(0.05)	<LLQ[0.0401]<(0.05)	<LLQ[0.0491]<(0.05)	<LLQ[0.0409]<(0.05)	0.0531	0.0518
22	NS	<LLQ[0.0370]<(0.05)	NS	0.0592	NS	0.0570	NS

*Rat Number with a "P" indicates pup sample.
<LLQ = Less than the Lower Limit of Quantitation (0.05 µg/mL).
NS: No sample.

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Table 6: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 0.1 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*											
	13739	13739P	13740	13740P	13741	13741P	13744	13744P	13745	13745P	13746	13746P
0	9.78	NS	12.1	NS	11.6	NS	9.88	NS	9.88	NS	11.1	NS
7	9.50	NS	7.85	NS	7.59	NS	9.18	NS	7.40	NS	8.49	NS
14	1.82	NS	1.99	NS	1.51	NS	1.96	NS	2.55	NS	2.03	NS
15	5.88	NS	7.83	NS	6.67	NS	6.04	NS	5.80	NS	6.65	NS
21	1.81	2.34	4.00	2.12	2.69	1.88	3.04	2.29	2.34	1.88	2.84	2.37
22	1.33	NS	1.47	NS	1.19	NS	0.882	NS	1.09	NS	0.945	NS

*Rat Number with a "P" indicates pup sample.
NS: No sample.

Table 6: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 0.1 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL)			
	Rat Number*			
	13748	13748P	13749	13749P
0	10.2	NS	10.7	NS
7	NS	NS	8.61	NS
14	1.35	NS	1.87	NS
15	5.36	NS	8.40	NS
21	3.94	1.74	3.27	2.07
22	0.942	NS	1.22	NS

*Rat Number with a "P" indicates pup sample.
NS: No sample.

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Table 7: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 1.6 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*											
	13751	13751P	13752	13752P	13753	13753P	13754	13754P	13755	13756	13756P	13758
0	155	NS	164	NS	213	NS	198	NS	191	188	NS	188
7	149	NS	169	NS	157	NS	152	NS	138	158	NS	143
14	21.2	NS	24.1	NS	21.6	NS	21.2	NS	NS	31.0	NS	27.7
15	95.6	NS	109	NS	112	NS	103	NS	125	108	NS	86.9
21	38.5	35.0	34.8	33.9	32.6	28.3	37.1	23.4	112	40.4	47.4	37.0
22	17.1	NS	19.5	NS	18.2	NS	9.48	NS	NS	19.9	NS	14.3

*Rat Number with a "P" indicates pup sample.
NS: No sample.

Table 7: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-111 Following the 1.6 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL)		
	Rat Number*		
	13758P	13759	13759P
0	NS	198	NS
7	NS	133	NS
14	NS	20.4	NS
15	NS	99.4	NS
21	25.6	33.0	26.3
22	NS	15.4	NS

*Rat Number with a "P" indicates pup sample.
NS: No sample.

Table 8: Repeat Analysis of Rat Serum Samples from Study #FACT-TOX-111

Rat Number	Treatment	Time	Original Conc. µg/ml	Original Curve Number	Reason for Reassay	Reassay Conc. µg/ml	Reassay Curve Number	Reported Conc. µg/ml	Reason for Reported Conc.
13753	3	Day 0	>ULQ-222.9138>(200)	3	1	213	4	213	1

>ULQ: Greater than the Upper Limit of Quantitation.
REASONS FOR REASSAY:
1). Run 3 concentration exceeded calibration range.
REASONS FOR REPORTED CONC:
1). Run 4 concentration within calibration range.

METHOD VALIDATION REPORT

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

DATE: 26 August 1999

REPORT: 99VDJA01.MI.DOC

AUTHORS: David J. Anderson, M.S.
Amie J. Prince, B.S.
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PREPARED FOR: 3M Environmental Technology and Safety Services
St. Paul, MN 55133-3331

NUMBER OF PAGES: 50

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AUTHORS: **David J. Anderson, M.S.**
Amie J. Prince, B.S.
Holly D. Ross, M.S.

FOR: **3M Environmental Technology and Safety Services**

DATE: **26 August 1999**

TITLE: **METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS**

ABSTRACT

A sensitive, specific, accurate, and reproducible analytical method was developed by Advanced BioAnalytical Services, Inc., Ithaca, New York to quantitate perfluorooctanesulfonate (PFOS) in rat serum samples. Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate the analyte from rat serum. Sample extracts were reduced to dryness, reconstituted, and analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode. The assay demonstrated a lower limit of quantitation (LLQ) of 0.05 µg/mL using 50-µL sample aliquots. The calibration curves were fit from 0.05 µg/mL to 20 µg/mL for PFOS by a weighted ($1/y^2$) quadratic equation. The coefficients of determination of the calibration curves ranged from 0.9962 to 0.9965.

Precision and accuracy quality control (QC) samples were prepared at concentrations of 0.2, 6, and 18 µg/mL PFOS. Quality control (QC) samples were prepared at a concentration of 100 µg/mL PFOS for partial volume analysis. The intra- and inter-assay precision (RSD) results calculated from all QC samples ranged from 1.92% to 4.87% for PFOS. The intra- and inter-assay accuracies (RE) calculated from QC samples ranged from -3.58% to 5.58% for PFOS. The mean extraction recoveries were from 87.6% to 100% for PFOS and 89.9% for the internal standard (IS).

PFOS was measured as stable in rat serum for up to 24 hours at ambient temperature. PFOS was measured as stable in rat serum at –20 °C, currently for up to 33 days, and

after three freeze/thaw cycles. Reliable results were obtained for sample extracts reinjected 27 hours after initial reconstitution.

QAU STATEMENT

Periodic inspections of the method validation for the quantitation of PFOS in rat serum were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to ABS Management on:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to the Study Director on 5 August 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Kathleen Cormack
Kathleen Cormack, B.S.
Quality Auditor

26Aug99
Date

SIGNATURE PAGE

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

Report Number: 99VDJA01.MI.DOC

Reported by: David J. Anderson, M.S. — Research Scientist — 26 August 1999 (Date)

Reviewed by: Kathleen Cormack, B.S. — Associate Auditor — 26Aug99 (Date)

Authorized for Release by: John R. Perkins, Ph.D. — Assistant Scientific Director — 26 AUGUST 99 (Date)

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1. INTRODUCTION

The purpose of this report is to describe the method validation for the quantitative determination of perfluorooctanesulfonate (PFOS) in rat serum samples after modification of a previous method (1). The objectives of the method validation were to validate a simple extraction procedure, determine the lower limit of quantitation for routine analysis, determine the extraction recoveries of the analyte and internal standard (IS), determine the analyte stability in rat serum at ambient temperature, long-term freezer storage, and over three freeze/thaw cycles, and to provide specific, accurate, and reproducible quantitative results by turbo ion spray liquid chromatography/mass spectrometry (LC/MS).

2. EXPERIMENTAL

2.1. CHEMICALS AND MATERIALS

Perfluorooctanesulfonate (PFOS, Lot# 171) was obtained from 3M, Inc. The internal standard (IS) for PFOS was 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). The internal standard (Lot# 59909) was obtained from 3M, Inc. A detailed list of chemicals and materials is found in Appendix A.

Stock and working solutions which were used to prepare the calibration curves for the analytes were prepared as described in Appendix A. Stock solutions used in the preparation of quality control (QC) samples were prepared separately from those used in preparation of the calibration curves.

Preparation of all solutions used during extraction and analysis are described in Appendix A.

2.2. LC/MS INSTRUMENTATION

The liquid chromatography/mass spectrometry system consisted of two LC-10AD pumps (Shimadzu, Columbia, MD 21046), a SCL-10A pump controller (Shimadzu,

Columbia, MD 21046), a WISP 717plus autosampler (Waters Associates, Millipore Corporation, Milford, MA 01757), a Betasil C_{18} (2 x 50 mm, 5 µm) column (Keystone Scientific, Inc., Bellefonte, PA 16823), and a PE SCIEX API 365 mass spectrometer (PE SCIEX, Concord, Ontario). A detailed list of the instrumentation and instrument conditions is found in Appendix A.

2.3. SAMPLE PREPARATION AND EXTRACTION PROCEDURE

For each analytical run (tray), duplicate 50-µL aliquots of the calibration curve samples were prepared as described in Appendix A. The nominal (theoretical) concentrations of PFOS in the calibration curves were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 µg/mL.

Rat serum quality control samples were prepared in advance of the validation study at nominal (theoretical) concentrations of 0.2, 6, and 18 µg/mL for QC1, QC2, and QC3, respectively, as detailed in Appendix A. A dilution QC (QC4, 100 µg/mL) was prepared at a concentration exceeding the upper limit of the calibration curve range (20 µg/mL), and was assayed using a 10-fold dilution for partial volume analysis.

Calibration standards and QC samples were extracted by the procedure detailed in Appendix A.

2.4. RAT SERUM VALIDATION DATA

The data were collected using selected ion monitoring (SIM) turbo ion spray LC/MS in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the Advanced BioAnalytical Services (ABS) file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson v 5.3.1.01 (PSS, Inc., Wayne, PA 19087). All data were rounded to no less than three significant figures by ABS prior to reporting in Tables 1 through 11. The data for the rat serum validation are stored in ABS Notebook 2304.

All calculations were based on the peak area ratio of the analyte to the internal standard. Concentrations of each analyte in quality control samples were determined by inverse prediction from the calibration curve.

2.5. ASSAY EVALUATION

2.5.1. Intra- and Inter-Assay Accuracy

The intra- and inter-assay accuracy of the method was assessed by determining the relative error observed in the analysis of quality control samples. The mean concentration for each quality control level was divided by the theoretical concentration. One was subtracted from the result, converted to percent and expressed as RE. QC1 through QC3 were assayed in replicates of five. In addition, the RE was reported for standards at all levels over three runs.

2.5.2. Intra- and Inter-Assay Precision

The intra- and inter-assay precision of the method was assessed by determining the Relative Standard Deviation (RSD) observed for quality control sample data. The mean concentration and RSD were calculated for the first run and over three runs for intra-assay and inter-assay precision, respectively. QC1 through QC3 were assayed in replicates of five. In addition, the RSD was reported for standards at all levels over three runs.

2.5.3. Partial Volume Analysis

The effect of dilution on the analysis of PFOS in rat serum was determined by partial volume analysis. QC4 (100 µg/mL) was prepared containing PFOS at approximately five times the upper limit of quantitation (ULQ). Five replicates of QC4 were diluted ten-fold and analyzed. The RSD and RE were reported.

2.5.4. Lower Limit of Quantitation (LLQ)

Rat serum control samples from six separate individuals were spiked with PFOS at a concentration of 0.05 µg/mL. The LLQ was determined by obtaining the back-calculated concentrations from these control samples. The overall mean, RSD, and RE were also calculated.

2.5.5. Selectivity

The selectivity of the assay was determined by LC/MS. To monitor for interference from the biological matrix, rat serum samples containing neither the analyte nor the internal standard (control blank) were assayed with all experiments.

2.5.6. Carryover Evaluation

The carryover of analyte from one injection to the next was assessed by analyzing a control blank injected immediately after a high calibration standard (STD10, 20 µg/mL).

2.6. STABILITY OF PFOS IN QUALITY CONTROL SAMPLES

QC1 through QC3 were used to determine the stability of the analyte during sample storage, extraction, and analysis.

2.6.1. Ambient-Temperature Stability of PFOS in Rat Serum

The stability of PFOS was evaluated by storing QC samples at each concentration level at ambient temperature (ca. 25 °C) for nominal timepoints of 0, 2.5, 6, and 24 hours after thawing. All QCs were assayed in replicates of five.

2.6.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The freezer stability of PFOS was evaluated by storing QC samples at each concentration level at –20 °C for 7, 13, and 33 days. Additional freezer stability timepoints will be reported in an addendum to this report. All QCs were assayed in replicates of four.

2.6.3. Freeze/Thaw Stability in Rat Serum

The stability of PFOS was evaluated after three freeze/thaw cycles. The mean concentrations of the QC samples after three freeze/thaw cycles (i.e., at least 2 hours frozen storage at the nominal temperature of –20 °C followed by thawing at ambient temperature for 30 minutes) were compared to the mean concentrations of freshly thawed QC samples. All QCs were assayed in replicates of five.

2.7. Reproducibility of Reinjecting Extracted Samples

The reproducibility of reinjecting reconstituted serum extracts was investigated by reinjecting a set of previously-assayed standards and QC samples which had been stored after injection at approximately 25 °C for 27 hours. The RE of the QC samples was used to assess processed sample stability in the reconstitution solution. All QCs were assayed in replicates of five.

2.8. Extraction Recovery

The extraction recovery of PFOS from rat serum was determined by comparing the peak area ratio (PAR) of samples (0.25, 4, and 16 µg/mL) spiked after extraction (post-extract) with the PAR of samples spiked before extraction (pre-extract). The internal standard was spiked post-extraction for all samples. The recovery of the internal standard (4 µg/mL) was assessed following a similar approach using PFOS as the reference. The extraction recovery (% Recovery) was determined by dividing the pre-extract PAR by the post-extract PAR and expressing the result as a percentage. Five replicates were used at each concentration level.

3. RESULTS AND DISCUSSION

A quantitative analytical procedure using turbo ion spray LC/MS in the negative ion mode was developed to meet the high sensitivity, specificity, and reproducibility requirements for the determination of PFOS in rat serum. The full-scan single MS mass spectrum of PFOS showed an abundant $[M-K]^-$ ion at m/z = 499 (Figure 1). The full-scan single MS mass spectrum of Tetra-H-PFOS showed an abundant $[M-H]^-$ ion at m/z = 427 (Figure 2).

The following selected ion monitoring (SIM) was used to quantify the analytes in rat serum:

PFOS	m/z = 499.0
Tetra-H-PFOS (IS)	m/z = 427.0

The peak labeling of full-scan data does not accurately represent the performance of the instrument in SIM mode. The SIM ions were derived from separate experiments using narrow-range scanning, which more accurately depicts the operation of the instrument in the SIM mode. The mass chromatograms of a representative control blank rat serum extract are shown in Figure 3. Mass chromatograms from a representative control rat serum extract containing only the internal standard (zero sample), are shown in Figure 4.

Figures 5 and 6 are mass chromatograms of representative extracts from calibration standards 1 and 10, respectively.

3.1. ASSAY EVALUATION RESULTS

3.1.1. Intra- and Inter-Assay Accuracy

The intra-assay accuracy (RE) data from QC samples ranged from -2.35 to 5.58% for PFOS (Table 1). The inter-assay accuracy ranged from -3.58 to 4.95% for PFOS (Table 2). These data indicate acceptable intra- and inter-assay accuracy for the determination of PFOS in rat serum.

3.1.2. Intra- and Inter-Assay Precision

The intra-assay precision data (RSD) ranged from 1.99 to 3.28% for PFOS at all QC concentration levels (Table 1). The inter-assay precision results from QC samples ranged from 1.92 to 4.87% for PFOS (Table 2). The RSD ranged from 0.613 to 4.38% for calibration standards at all levels (Table 3). These data indicate acceptable intra-and inter-assay precision for the determination of PFOS in rat serum.

3.1.3. Linearity

The calibration curves were fit by a weighted ($1/y^2$) quadratic regression. Coefficients of determination (r^2) were ≥0.9962 for PFOS in rat serum. The calibration curve statistics are shown in Table 4.

3.1.4. Partial Volume Analysis

The results of partial volume analysis of QC4 is shown in Table 5. The precision (RSD) of QC4 samples diluted 1 in 10 was 2.71%. The accuracy was 5.89%. These data indicate acceptable accuracy and precision for partial volume analysis for the determination of PFOS in rat serum.

3.1.5. Lower Limit of Quantitation (LLQ)

Table 6 shows the lower limit of quantitation (LLQ) data. The serum LLQ experiment demonstrated that 0.05 µg/mL is an acceptable LLQ for PFOS. The precision (RSD) was 4.95% for PFOS. The RE was 4.57%.

3.1.6. Selectivity

The assay was specific for PFOS. No chromatographic interferences were observed in any of the control serum samples analyzed (Figure 3). The control blanks and zero samples did show some evidence of small chromatographic peaks at the retention times of the analyte. These peaks were not quantifiable as they were below the lower limit of quantitation (LLQ).

3.1.7. Carryover Evaluation

Carryover of PFOS and the IS was evaluated by injection of an extracted rat serum control blank following an injection of an extracted high standard (STD 10). The response for the small chromatographic peak at the retention time for PFOS was comparable to the response of a rat serum control blank injected before the high standard. Thus, carryover is negligible for PFOS. There was no evidence of carryover for the internal standard.

3.2. Stability of PFOS in Quality Control Samples

3.2.1. Ambient Temperature Stability of PFOS in Rat Serum

The results of the ambient stability of PFOS in QC samples are shown in Table 7. The mean predicted concentrations deviated from -4.45 to 10.5% from the 0-hour values for all QC levels and time points. Based on these data, PFOS was stable in rat serum for up to 24 hours at ambient temperature.

3.2.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The results of the long-term stability of PFOS in QC samples stored at –20 °C are presented in Table 8. The PFOS concentrations deviated from 0-hour values from -2.76 to 12.3% for all QC levels stored up to 33 days.

Results of freezer storage after two, four, and eight months will be reported as an addendum to this report.

3.2.3. Freeze/Thaw Stability of PFOS in Rat Serum

The results of the freeze/thaw stability of PFOS in QC samples after three freeze/thaw cycles is shown in Table 9. PFOS was stable after three freeze/thaw cycles with deviations ranging from –1.38% to 10.1% from the 0-cycle samples for all QC levels (Table 9).

3.3. Reproducibility of Reinjecting Extracted Samples

The results of reinjecting reconstituted samples containing PFOS after storage for 27 hours at approximately 25 °C in reconstitution solution are shown in Table 10. Reinjecting processed samples after 27 hours in reconstitution solution was appropriate with RE values ranging from -6.33 to 4.59% at 27 hours for all QC levels.

3.4. Extraction Recovery

The mean recoveries of PFOS and Tetra-H-PFOS are shown in Table 11. The mean recoveries ranged from 87.6 to 100% for PFOS. The mean recovery for Tetra-H-PFOS was 89.9%.

4. CONCLUSIONS

The turbo ion spray LC/MS assay procedure for the determination of PFOS in rat serum has proven to be sensitive, specific, accurate, and reproducible. Its high sensitivity allows reliable and reproducible quantitation of PFOS down to a level of 0.05 μg/mL in rat serum based on 50-μL samples.

5. DATA RETRIEVAL

The data for the rat serum validation are stored in ABS Notebook 2304 and in the ABS Archives.

6. REFERENCES

1. Advanced BioAnalytical Services, Inc. Report 98AGKP02.MMM. Analytical Report for the Determination of Perfluorooctanoate and Perfluorooctanesulfonate in Human Serum by LC/MS. Grace K. Poon, Ph.D., David Hardwick, B.S., and Ellen Pace, M.A.T., 6 February 1998.

7. TABLES

Table 1: Intra-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Mean	0.204	6.34	17.6
RSD (%)	2.93	1.99	3.28
RE (%)	1.77	5.58	-2.35

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 2: Inter-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples from Three Validation Runs

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Run 10	0.187	6.44	16.9
	0.186	6.44	17.3
	0.182	6.32	17.5
	0.189	6.19	18.0
	0.182	6.31	17.4
Run 11	0.194	6.17	18.2
	0.197	6.13	17.1
	0.184	6.41	17.6
	0.192	6.15	17.7
	0.184	6.24	17.0
Mean	0.193	6.30	17.5
RSD (%)	4.87	1.92	2.66
RE (%)	-3.58	4.95	-2.73

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 3: Accuracy and Precision of the PFOS Assay in Rat Serum for Calibration Standards from Three Validation Runs

	PFOS Calibration Standard Concentration (µg/mL)									
Theoretical Conc.	STD1 0.05	STD2 0.1	STD3 0.25	STD4 0.5	STD5 1	STD6 2	STD7 4	STD8 8	STD9 16	STD10 20
Run 9	0.0483	0.0972	0.251	0.480	0.942	2.10	4.48	7.61	15.8	20.0
	0.0530	0.101	0.252	0.483	0.938	2.13	4.55	7.70	16.2	19.9
Run 10	0.0491	0.0951	0.239	0.469	0.954	2.16	4.58	8.05	15.9	19.6
	0.0542	0.101	0.251	0.479	0.942	2.11	4.43	7.87	15.7	20.0
Run 11	0.0510	0.0960	0.251	0.469	0.945	2.21	4.40	7.64	15.8	20.3
	0.0510	0.0995	0.252	0.477	0.948	2.13	4.53	7.78	15.6	20.0
Mean	0.0511	0.0983	0.249	0.476	0.945	2.14	4.50	7.78	15.8	20.0
RSD (%)	4.38	2.65	2.07	1.15	0.613	1.90	1.62	2.13	1.22	1.08
RE (%)	2.20	-1.67	-0.207	-4.78	-5.53	7.05	12.4	-2.80	-1.00	-0.0764

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 4: Calibration Curve Parameters for PFOS in Rat Serum

Run #	Quadratic Coefficient (A)[a]	Coefficient Linear (B)	Intercept (C)	Coefficient of Determination (r^2)
9	-0.0010769	0.13730	0.00042009	0.9965
10	-0.0010516	0.14535	0.0014910	0.9962
11	-0.0012359	0.15173	0.00088515	0.9964
Mean	-0.0011215	0.14479	0.00093206	0.9964

a: $y = Ax^2 + Bx + C$, weighted $1/y^2$

Table 5: Partial Volume Analysis of PFOS in Rat Serum

	PFOS Conc. (µg/mL)
Theoretical Conc.	QC4 100 (1 in 10 dilution)
Run 9	111 105 105 104 104
Mean	106
RSD (%)	2.71
RE (%)	5.89

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 6: Lower Limit of Quantitation of PFOS in Rat Serum

Run 11 Theoretical Concentration	PFOS Conc. Found (µg/mL)
LLQ (0.05 µg/mL)	0.0548
	0.0489
	0.0499
	0.0554
	0.0527
	0.0520
Mean	0.0523
RSD (%)	4.95
RE (%)	4.57

RSD = (SD/Mean) x 100

RE = [(Mean/Theoretical)-1] x 100

Table 7: Ambient-Temperature Stability of PFOS in Rat Serum After 24 Hours

Run 11	PFOS (µg/mL)			
QC (Theoretical Conc.)	0 Hour	2.5 Hour	6 Hour	24 Hour
QC 1 (0.2 µg/mL)	0.194	0.187	0.177	0.190
	0.197	0.181	0.179	0.187
	0.184	0.184	0.180	0.191
	0.192	0.180	0.183	0.182
	0.184	0.178	0.189	0.190
Mean	0.190	0.182	0.182	0.188
RSD (%)	3.03	1.91	2.52	2.01
%DEV from 0 Hour	NA	-4.20	-4.45	-1.16
QC 2 (6 µg/mL)	6.17	6.17	6.36	6.55
	6.13	6.30	6.38	6.53
	6.41	6.26	6.35	6.85
	6.15	6.28	6.22	6.66
	6.24	6.24	6.11	6.81
Mean	6.22	6.25	6.28	6.68
RSD (%)	1.82	0.797	1.86	2.16
%DEV from 0 Hour	NA	0.525	1.05	7.42
QC 3 (18 µg/mL)	18.2	18.0	18.1	19.1
	17.1	18.0	18.0	19.4
	17.6	17.7	17.6	19.2
	17.7	17.9	18.2	19.4
	17.0	18.0	18.0	19.7
Mean	17.5	17.9	18.0	19.4
RSD (%)	2.84	0.719	1.26	1.19
%DEV from 0 Hour	NA	2.33	2.39	10.5

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(X Hour Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100

Table 8: Freezer Stability of PFOS in Rat Serum at -20 °C

QC Nominal Concentration	PFOS (ug/mL)			
	0 Hour (Run 2)*	7 Days (Run 6)*	13 Days (Run 3)**	33 Days (Run 16)
QC 1 (0.2 ug/mL)	0.213	0.225	0.211	0.245
	0.209	0.222	0.210	0.234
	0.208	0.218	0.217	0.228
	0.208	0.219	0.214	0.232
	0.207			
Mean	0.209	0.221	0.213	0.235
RSD (%)	1.24	1.32	1.55	3.17
%DEV from 0 Hour	NA	5.72	1.84	12.3
QC 2 (6 ug/mL)	6.02	6.22	5.97	6.45
	5.71	6.15	5.84	6.13
	6.00	6.39	6.07	6.58
	6.06	6.27	5.99	6.37
	5.69			
Mean	5.89	6.26	5.97	6.38
RSD (%)	3.08	1.62	1.58	2.97
%DEV from 0 Hour	NA	6.19	1.20	8.28
QC 3 (18 ug/mL)	20.2	19.1	18.8	18.6
	18.6	19.5	18.2	18.8
	18.9	19.8	18.8	18.7
	18.2	19.3	17.9	18.7
	18.9			
Mean	18.9	19.4	18.4	18.7
RSD (%)	3.96	1.37	2.42	0.395
%DEV from 0 Hour	NA	2.52	-2.76	-1.30

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100
*From Protocol FACT-TOX-110
**From Protocol FACT-TOX-111

Table 9: Stability of PFOS in Rat Serum After Three Freeze/Thaw Cycles

Run 10	PFOS (µg/mL)	
QC (Theoretical Conc.)	0 Cycle	3 Cycles
QC 1 (0.2 µg/mL)	0.187	0.177
	0.186	0.177
	0.182	0.185
	0.189	0.185
	0.182	0.189
Mean	0.185	0.182
RSD (%)	1.70	3.12
%DEV from 0 Cycle	NA	-1.38
QC 2 (6 µg/mL)	6.44	6.59
	6.44	6.61
	6.32	6.44
	6.19	6.39
	6.31	6.40
Mean	6.34	6.49
RSD (%)	1.66	1.63
%DEV from 0 Cycle	NA	2.35
QC 3 (18 µg/mL)	16.9	19.2
	17.3	19.1
	17.5	19.2
	18.0	19.6
	17.4	18.7
Mean	17.4	19.2
RSD (%)	2.31	1.80
%DEV from 0 Cycle	NA	10.1

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Cycle = [(3 Cycle - 0 Cycle)/0 Cycle] x 100

Table 10: Reproducibility of Reinjecting Extracted Samples Containing PFOS after 27 Hours in Reconstitution Solution

	PFOS (µg/mL)	
	t=0 Hours (Run 10)	t=27 Hours (Run 12)
QC 1 (0.2 µg/mL)	0.187	0.187
	0.186	0.182
	0.182	0.188
	0.189	0.188
	0.182	0.190
Mean	0.185	0.187
RSD (%)	1.70	1.61
RE (%)	-7.50	-6.33
QC 2 (6 µg/mL)	6.44	6.39
	6.44	6.02
	6.32	6.19
	6.19	6.55
	6.31	6.22
Mean	6.34	6.28
RSD (%)	1.66	3.23
RE (%)	5.62	4.59
QC 3 (18 µg/mL)	16.9	17.3
	17.3	18.2
	17.5	18.1
	18.0	17.3
	17.4	17.6
Mean	17.4	17.7
RSD (%)	2.31	2.40
RE (%)	-3.29	-1.73

NA: Not Applicable
RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 11: Extraction Recovery of PFOS from Rat Serum

Theoretical Conc. in serum (µg/mL)	Pre-Extraction Response (Area Ratio)	Post-Extraction Response (Area Ratio)	% Recovery*
PFOS	0.03160	0.02880	110
Spike 1	0.02850	0.03010	94.7
(0.25 µg/mL)	0.02930	0.02950	99.3
Run 14	0.02790	0.02930	95.2
	0.03050	0.02960	103
Mean	0.02956	0.02946	100
PFOS	0.4597	0.5270	87.2
Spike 2	0.4751	0.5304	89.6
(4 µg/mL)	0.4776	0.5335	89.5
Run 14	0.4724	0.5298	89.2
	0.4772	0.5392	88.5
Mean	0.4724	0.5320	88.8
PFOS	1.649	1.874	88.0
Spike 3	1.679	1.857	90.4
(16 µg/mL)	1.719	1.869	92.0
Run 14	1.535	1.887	81.4
	1.612	1.864	86.5
Mean	1.639	1.870	87.6
Tetra-H-PFOS	0.4068	0.4460	91.2
Spike IS	0.3884	0.4423	87.8
(4 µg/mL)	0.4043	0.4445	91.0
Run 13	0.3921	0.4512	86.9
	0.4078	0.4412	92.4
Mean	0.3999	0.4450	89.9

*(Individual Response for Pre-Ext./Individual Response for Post-Ext.) x 100

8. FIGURES

Figure 1: Full-Scan Single MS Mass Spectrum of PFOS

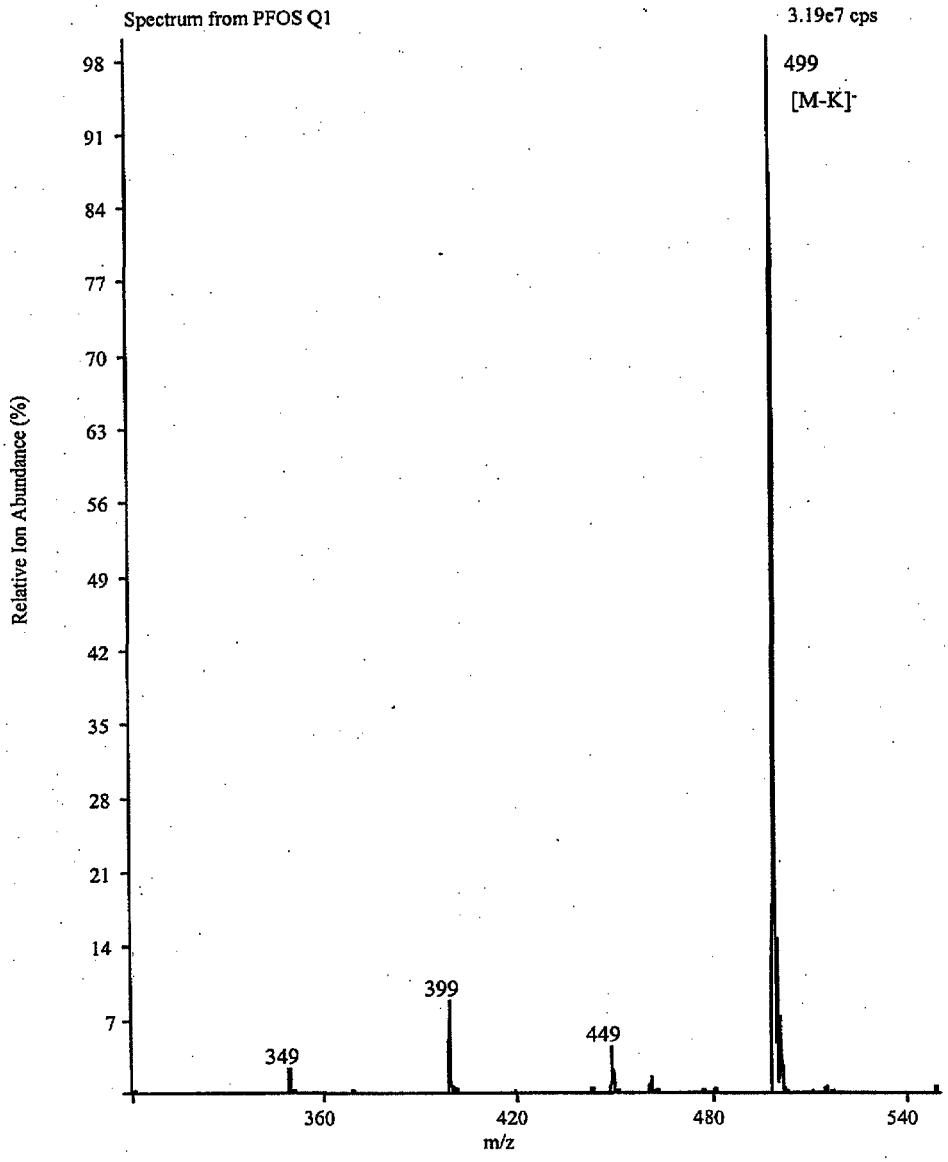


Figure 2: Full-Scan Single MS Mass Spectrum of Tetra-H-PFOS

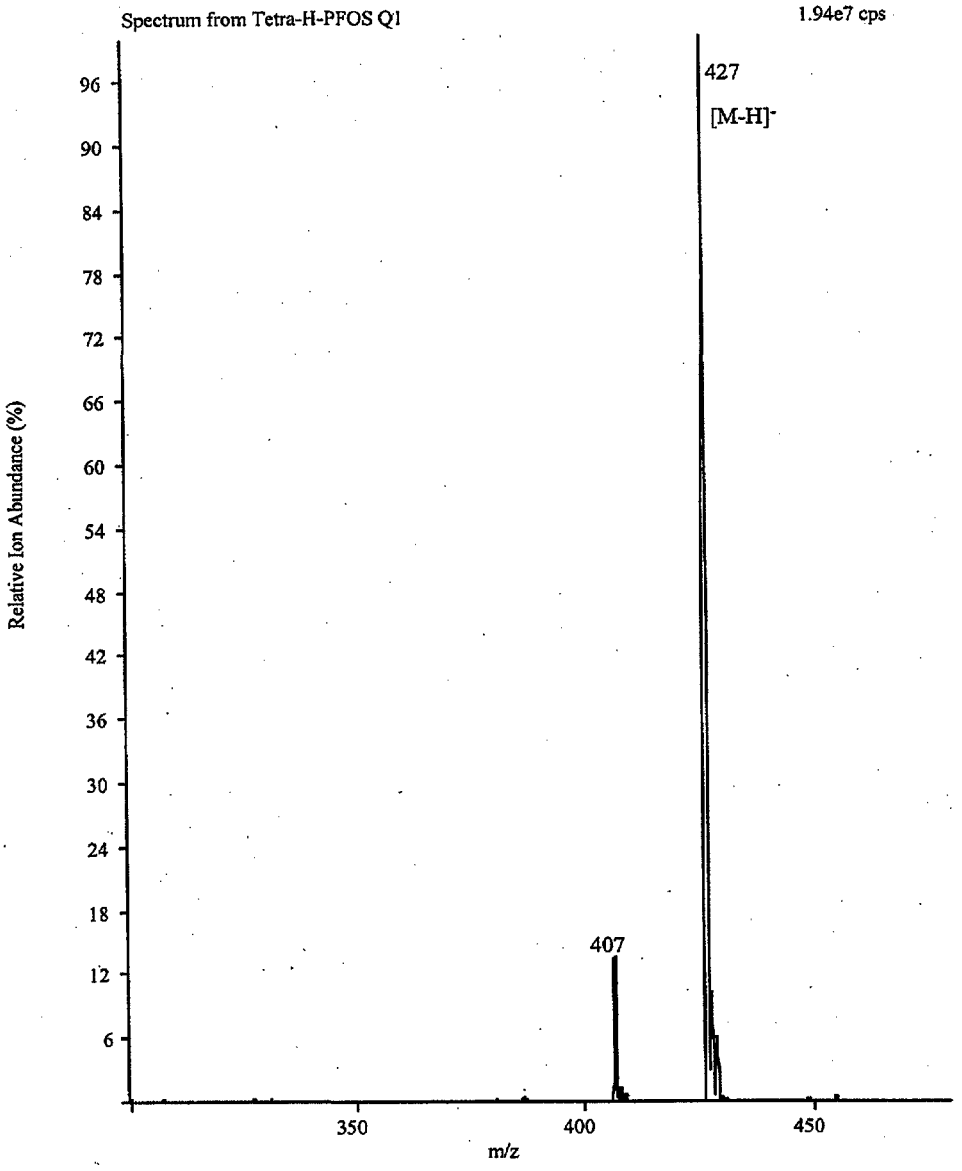
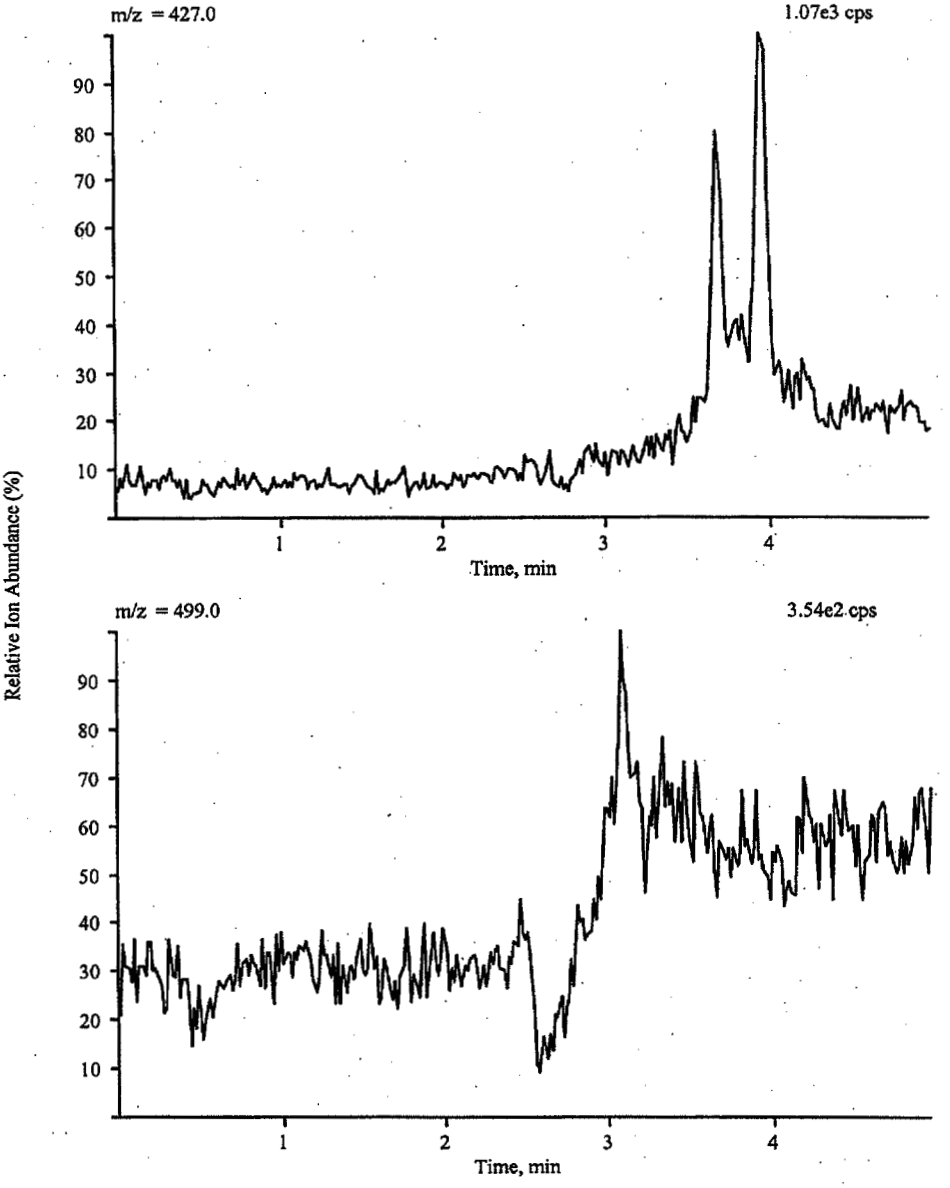


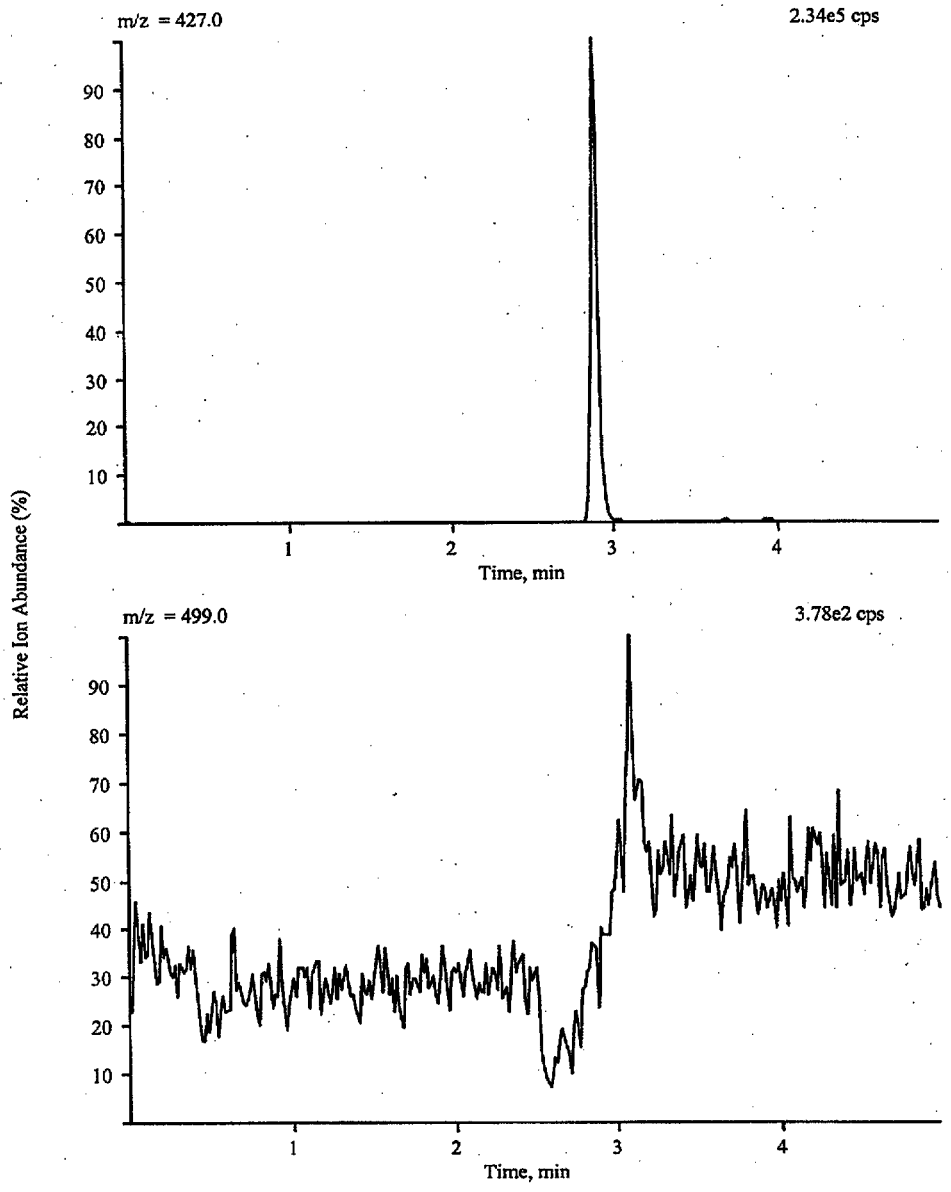
Figure 3: Mass Chromatograms of PFOS and its Internal Standard in Control Blank Rat Serum Extract



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

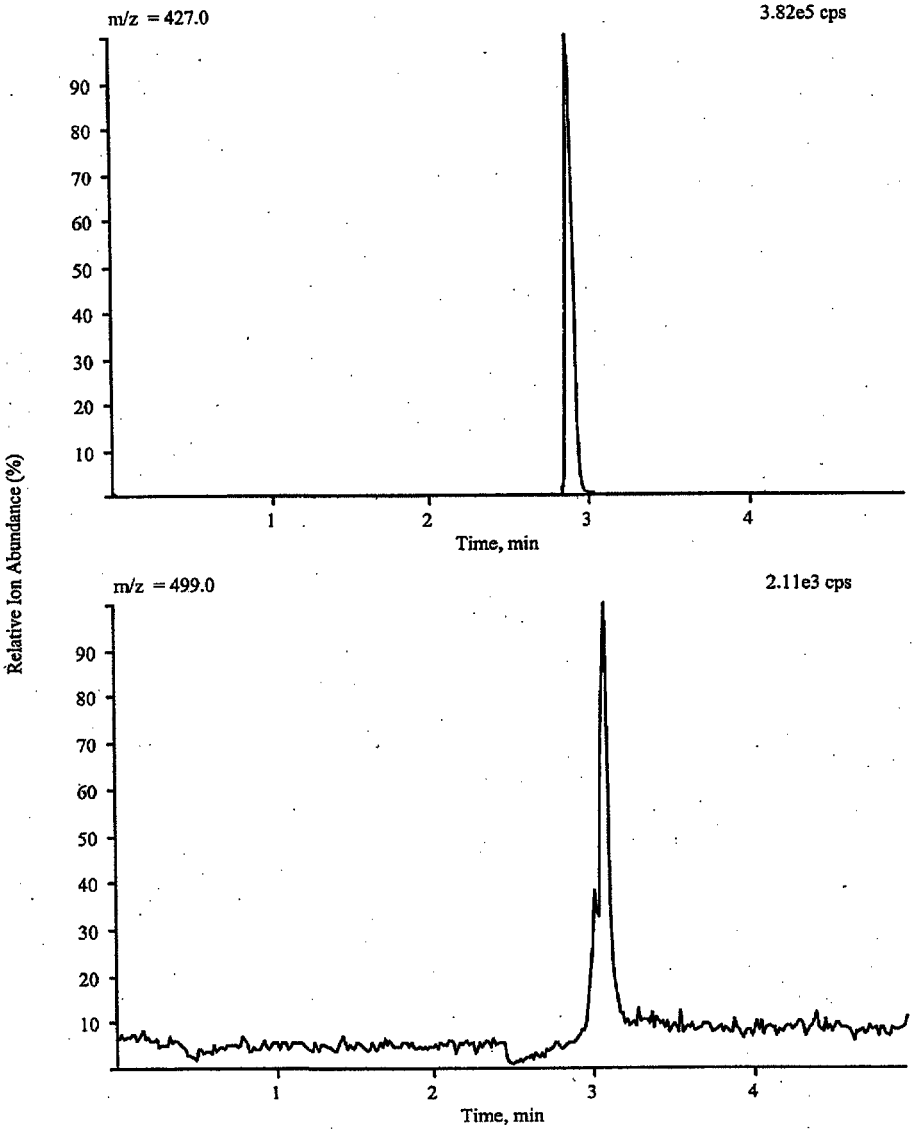
Figure 4: Mass Chromatograms of PFOS in a Rat Serum Extract Sample Containing Internal Standard Only (Zero Sample)



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

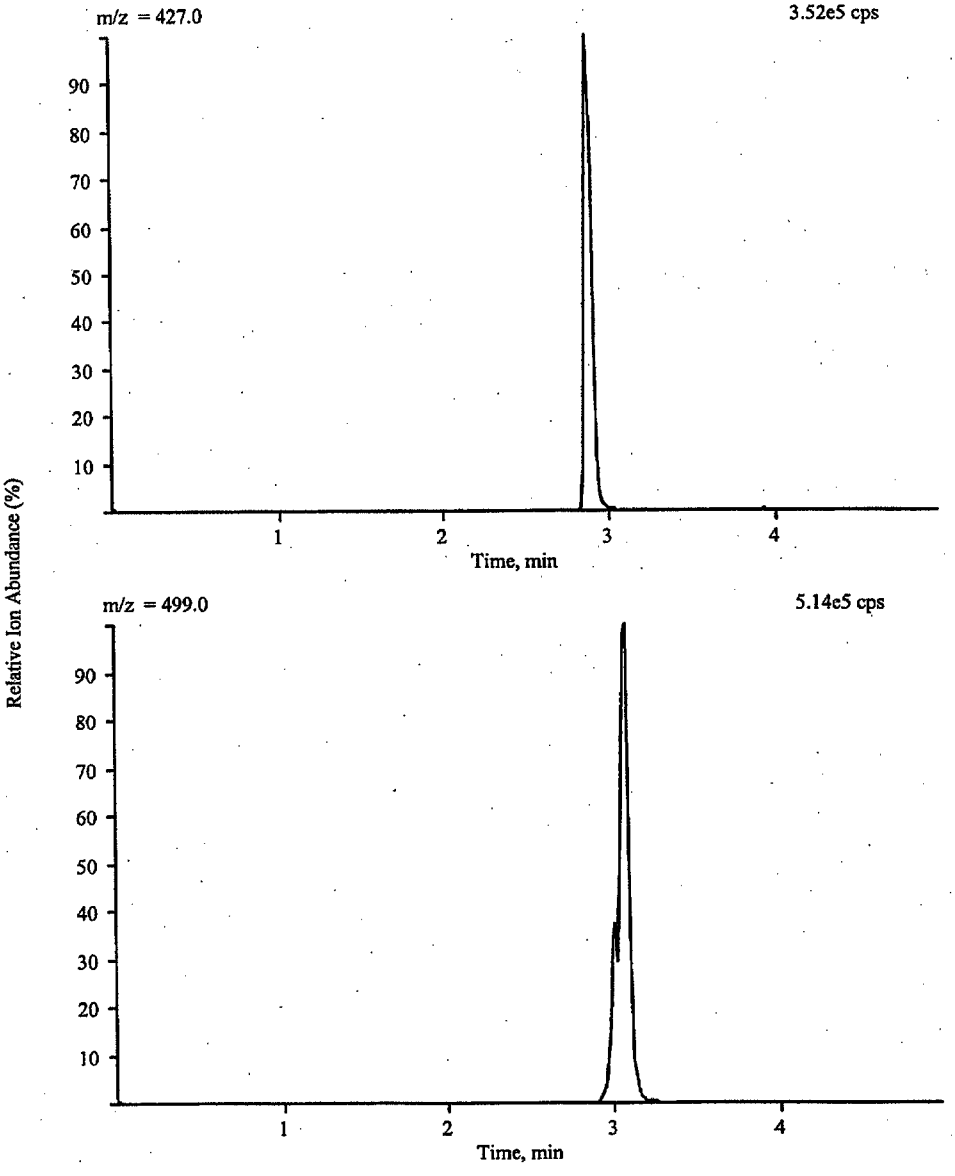
Figure 5: Mass Chromatograms of Calibration Standard 1 in Rat Serum Extract Containing PFOS (0.05 µg/mL) and the Internal Standard



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

Figure 6: Mass Chromatograms of Calibration Standard 10 in Rat Serum Extract Containing PFOS (20 µg/mL) and the Internal Standard



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

9. APPENDIX A: QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

AUTHORS: **David J. Anderson, M.S.**
Amie J. Prince, B.S.
Holly D. Ross, M.S.

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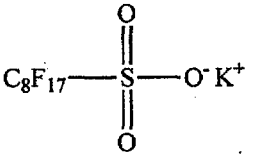
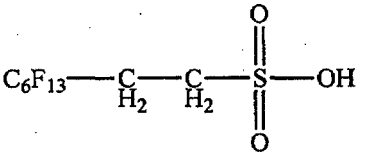
A.1.0 CHEMICAL STRUCTURE(S)



Potassium Perfluorooctanesulfonate
MW = 538



1H, 1H, 2H, 2H-Perfluorooctane Sulfonic Acid
MW = 428, Internal Standard

A.2.0 SPECIMEN(S)

This assay uses 50-µL aliquots of rat serum. Rat serum samples are stored at -20 °C.

A.3.0 ASSAY PRINCIPLE

PFOS and its internal standard, Tetra-H-PFOS, are extracted from rat serum samples (50 µL) using a liquid-liquid extraction procedure. The organic layer is evaporated to dryness and then reconstituted. An aliquot is then analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

A.4.0 COMPOUNDS

PFOS: Lot# 171, 99.976% purity, 3M, Inc., Minneapolis, MN.

Tetra-H-PFOS: Lot# 59909, 90% purity, 3M, Inc., Minneapolis, MN.

A.5.0 CHEMICALS

All chemicals may be substituted with that of an equivalent manufacturer and grade of chemical.

Acetonitrile	Cat# 015-4, Burdick & Jackson, Muskegon, MI 49442
Ammonium Acetate	Cat# 24,019-2, Aldrich, Milwaukee, WI 53201
Ethyl Acetate	Cat# 100-4, Burdick & Jackson, Muskegon, MI 49442
Methanol	Cat# 230-4, Burdick & Jackson, Muskegon, MI 49442
Rhodapex mass calibration solution	A mixture of 0.11 mM trifluoroacetic acid (J.T. Baker, Phillipsburg, NJ 08865), 0.52 mM Rhodapex CO-436, 1 mM ABEX EP-110 and 1 mM ABEX EP-120 (all three supplied by Rhone-Poulenc, Inc., Cranbury, NJ 08512) in 1:1 methanol:water.
Sodium Bicarbonate	Cat# 3509-01, J.T. Baker, Phillipsburg, NJ 08865
Sodium Carbonate, Annhydrous	Cat# 3604-01, J.T. Baker, Phillipsburg, NJ 08865
Tetrabutylammonium Hydrogen Sulfate	HPLC Grade, Cat# J360-07, J.T. Baker, Phillipsburg, NJ 08865
Water	High Purity (NANOpure), Barnstead Model D7331 Ultrapure Water System, Dubuque, IA 52001
Rat Serum	Lampire Biological Laboratories, Piperville, PA 18947
Rat Serum	Harlan Bioproducts for Science, Inc., Indianapolis, IN 46229

A.6.0 MATERIALS AND EQUIPMENT

Mass Spectrometer	PE SCIEX API 365 atmospheric pressure ionization tandem triple quadrupole mass spectrometer equipped with TurboIonSpray™ interface, PE SCIEX, Concord, Ontario L4K 4V8
Data system	API Standard Software, MacQuan v 1.4, LC2Tune v 1.3, MacDAD v 1.3, Bundler v 1.3, Multiview v 1.3,

	and Sample Control v 1.3, on a Power Macintosh, PE SCIEX, Concord, Ontario L4K 4V8
HPLC Pump	Shimadzu LC-10AD pump, Shimadzu Co., Columbia, MD 21046
LC Pump Controller	Shimadzu SCL-10A, Shimadzu Co., Columbia, MD 21046
Autosampler	Waters 717plus, Waters Associates, Millipore Corporation, Milford, MA 01757
HPLC Column	Betasil C_{18}, 5 µm particle size, 2.1 x 50 mm, Cat #852055-701, Keystone Scientific, Inc., Bellefonte, PA 16823
Harvard syringe pump 11	Harvard Apparatus Inc., South Natick, MA 01760
Multi-tube vortexer	Cat# 58816-115, VWR Scientific, West Chester, PA 19380
Balance	Model FX-300, AND Ltd., Tokyo, Japan
pH Meter	Model 340, Corning Inc., Corning, NY 14830
Mechanical shaker	Cat# 6000, Eberbach Corp., Ann Arbor, MI 48106
NANOpure-UV water purification system	Barnstead, Dubuque, IA 52001
Microbalance	Model MT-5, Mettler-Toledo Inc., Hightstown, NJ 08520
Micro weigh boats	Cat# 0219-0041, Perkin-Elmer Corp., Norwalk, CT 06859
Weigh boats	Cat# 12577-025, VWR Scientific, West Chester, PA 19380
Sorvall RT-6000D refrigerated centrifuge	Cat# 83071, DuPont Co., Wilmington, DE 19898
Beckman GS6KR Refrigerated centrifuge	Beckman, Palo Alto, CA 94304

TurboVap LV Evaporator	Cat# 43750, Zymark Instruments, Hopkinton, MA 01748
Pipettes	Cat# P-5000, P-1000, P-200, P-100, Rainin Instrument Co., Woburn, MA 01888
Pipette tips	RT-20, RT-200, C-5000, CP-25, CP-50, CP-250, Rainin Instrument Co., Woburn, MA 01888
Polypropylene tubes	13 mm x 100 mm, Cat# 15070-574, VWR Scientific, West Chester, PA 19380
Screw-capped Polypropylene vials	16.5 x 57 mm, Cat# 60.542, Sarstedt, Inc., Newton, SC 28658
Screw-capped Polypropylene vials	16.5 x 101 mm, Cat# 60.541, Sarstedt, Inc., Newton, SC 28658
Plug Tite Multipurpose Caps	Cat# 78-127-0019-100, Elkay Products, Inc., Worcester, MA 01607
Pyrex volumetric flask	Cat# 28014P-10, 28014P-25, 28014P-100, 28014P-1000, Kimble/Kontes, Vineland, NJ 08360
Solvent Filtration Apparatus	Cat# 953781-0000, 953751-0000, 953753-0000, 953826-0000, 953827-0000, Kimble/Kontes, Vineland, NJ 08360
Nylon Titan Membrane Filters	0.45 µm Scientific Resources Inc., Cat# 74547-NN, Eatontown, NJ 07724
PEEK tubing	0.005" i.d. x 1/16" o.d., Cat# 1535, Upchurch Scientific Inc., Oak Harbor, WA 98277
Liquid Nitrogen	Supplied in-house from bulk tank, BOC Gasses, Buffalo, NY 14210-2005
Gastight Glass Syringes	Cat# 1750, Hamilton Company, Reno, NV 89510

Other general laboratory glassware and supplies were used.

A.7.0 PREPARATION OF SOLUTIONS

NANOpure water (18 megaohm-cm.) or equivalent should be used wherever water is called for. Mix all solutions well.

A.7.1 Preparation of Analytical Standard Stock Solutions

Analytical Standard Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to a polypropylene vial. Dilute with the appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

Standard Spiking Solutions

Prepare Standard Working Solutions A through J in 5-mL class "A" volumetric flasks according to the following dilution scheme. Dilute to the 5-mL mark with water to yield the final concentration. Prepare fresh solutions every three months. Store the solutions at 4 °C and bring to ambient temperature before use.

Standard Working	Volume Spiked (µL)	Solution Used	PFOS Final Conc. (µg/mL)
A	1000	Stock	200
B	800	Stock	160
C	400	Stock	80
D	200	Stock	40
E	100	Stock	20
F	250	200 µg/mL (A)	10
G	125	200 µg/mL (A)	5
H	62.5	200 µg/mL (A)	2.5
I	500	10 µg/mL (F)	1.0
J	250	10 µg/mL (F)	0.5

A.7.2 Preparation of Internal Standard Stock Solutions

Internal Standard Stock Solution, Tetra-H-PFOS (1 mg/mL): Weigh approximately 5 mg of Tetra-H-PFOS on a microbalance and transfer it to a polypropylene vial. Dilute with an appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

A.7.3 Preparation of Internal Standard Working Solution

Internal Standard Working Solution (4 µg/mL Tetra-H-PFOS): Add 400 µL of Internal Standard Stock Solution to a 100-mL class "A" volumetric flask. Dilute to the mark with water to give a 4 µg/mL Tetra-H-PFOS solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare fresh solution as needed.

A.7.4 Preparation of Standard Curve and Control Blank

Prepare fresh calibration standards for each analytical run by combining 360 µL of rat control serum and 40 µL of the analytical standard working solution (see table below) in labeled 1.7-mL microtubes. Prepare control blanks and zero samples by combining 360 µL of rat control serum with 40 µL of water. Vortex each for 60 seconds.

Analytical Standard Dilution Table:

Standard #	Volume Spiked (µL)	Solution to use	µL control serum	Final Conc. (µg/mL)
10	40	A	360	20
9	40	B	360	16
8	40	C	360	8
7	40	D	360	4
6	40	E	360	2
5	40	F	360	1
4	40	G	360	0.5
3	40	H	360	0.25
2	40	I	360	0.1
1	40	J	360	0.05

A.7.5 Preparation of Other Solutions

0.25 M Sodium Carbonate/0.25 M Sodium Bicarbonate: Weigh 26.5 g of sodium carbonate and 21 g of sodium bicarbonate and dissolve in approximately 900 mL of HPLC-grade water. Transfer the solution to a 1000-mL volumetric flask and dilute to the mark with HPLC-grade water. Mix the solution thoroughly. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Ammonium Acetate: Add 7.7 g of ammonium acetate to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

2 mM Ammonium Acetate: Combine 2 mL of 1M ammonium acetate and 900 mL NANOpure water in a 1000-mL volumetric flask; stir until completely mixed. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

10 M Sodium Hydroxide: Add 40 g of sodium hydroxide to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Sodium Hydroxide: Add 10 mL of 10 M sodium hydroxide to a 100-mL volumetric flask. Dilute to the mark with NANOpure water; stir until completely mixed. Store at ambient temperature. Prepare a fresh solution every three months.

0.5 M Tetrabutylammonium Hydrogen Sulfate, pH 10: Weigh 16.98 g of tetrabutylammonium hydrogen sulfate and dissolve in 40 mL of NANOpure water. Adjusted the pH to 10.0 with 10 M and 1 M sodium hydroxide. Transfer the solution to a 100-mL volumetric flask and dilute to the mark with NANOpure water.

10:90 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent A): Add 100 mL of methanol and 900 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

90:10 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent B): Add 900 mL of methanol and 100 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

A.8.0 PREPARATION OF QUALITY CONTROL SAMPLES

A.8.1 Preparation of Quality Control Stock Solutions

QC Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to polypropylene vial. Dilute

with the appropriate volume of methanol to yield a 1 mg/mL solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare a fresh solution every three months.

A.8.2 Preparation of Serum QC Samples

QC4 (Dilution QC, 100 µg/mL PFOS): Add 1000 µL QC Stock Solution to a 10-mL class "A" volumetric flask to yield a 100-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.2 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC3 (18 µg/mL PFOS): Add 450 µL QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 18-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC2 (6 µg/mL PFOS): Add 150 µL of QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 6-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC1 (0.2 µg/mL PFOS): Add 111.1 µL of QC3 to a 10-mL class "A" volumetric flask to yield a 0.2-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

A.9.0 LIQUID-LIQUID EXTRACTION PROCEDURE

1. Prepare fresh calibration standards for each run according to Section A.7.4.
2. Thaw QC samples.
3. Prepare QC4 samples (diluted 1 in 10) in two steps:
   a) Add 180 µL of control serum and 20 µL of QC4 into 1.7-mL microtubes and

vortex.

b) Aliquot 50 µL of diluted QC4 into labeled 16 x 100 mm polypropylene tubes.

4. Aliquot 50 µL of each control blank, zero sample, calibration standard, and quality control samples 1-3 into labeled 16 x 100 mm polypropylene tubes. Standards and blanks are analyzed in duplicate. All QC samples are analyzed in replicates of five.
5. Add 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate, pH 10 to each tube.
6. Add 2 mL of 0.25 M sodium carbonate/0.25 M sodium bicarbonate to each tube.
7. Add 500 µL of the Internal Standard Working Solution to each tube (except control blank, add 500 µL water).
8. Add 5 mL ethyl acetate to each tube.
9. Shake the tubes on a reciprocal shaker at medium speed for 20 minutes.
10. Centrifuge the tubes at 3000 rpm for 20 minutes at 20 °C.
11. Freeze the aqueous phase in an acetone/dry ice bath and keep the tubes in the bath an additional 5 minutes.
12. Transfer the ethyl acetate layer to a fresh, labeled 13 x 100 mm polypropylene tube.
13. Evaporate the ethyl acetate layer to dryness in a TurboVap™ at approximately 20 °C under nitrogen.
14. Reconstitute the dried extracts in two steps:
    Add 500 µL acetonitrile and vortex for 60 seconds.
    Then add 500 µL water and vortex for 60 seconds.
15. Transfer 200 µL of the extract to a labeled polypropylene autosampler vial.

A.10.0 INSTRUMENT CONDITIONS

HPLC Conditions:

Eluent (gradient)	Mobile Phase A: 10:90 methanol:2 mM ammonium acetate (v/v) Mobile Phase B: 90:10 methanol:2 mM ammonium acetate (v/v)

Eluent Gradient Conditions:

Time (min)	%B
0	45
1.0	100
5	100
5.5	45%
8	Stop

Flow Rate	300 µL/min.
Autosampler	Waters 717plus
Autosampler Temperature	Ambient
Injection Volume	3 to 5 µL
HPLC Column	Keystone Betasil C_{18}, 5 µm particle size, 2.1 mm x 50 mm
HPLC Column Temperature	Ambient
Typical Initial Column Pressure	90 bar
Autosampler Run Time	8.0 minutes

Analyte	Representative Retention Time (minutes)
PFOS	3.1
Tetra-H-PFOS (IS)	2.9

Mass Spectrometer Conditions:

Curtain Gas	UHP Nitrogen
Nebulizer Gas	UHP Nitrogen
TurboIonSpray™ Temperature	400 °C
TurboIonSpray™ Auxiliary Gas	UHP Nitrogen at 8 L/min

Ions Monitored:

Analyte	Ion Monitored	Dwell Time
PFOS	m/z = 499.0	500 ms
Tetra-H-PFOS (IS)	m/z = 427.0	500 ms

Ionization Mode	Negative Ion
Ion Spray Voltage	-3000 V
Declustering Potential	-35 V
MS Acquisition Time	5 minutes
Pause Time	2 ms

Calibrate the mass axis of the instrument by infusion of Rhodapex mass calibration solution (Section A.5.0) at a flow rate of 10 µL/min. Optimize the sensitivity of the instrument using an infusion of a 10 µg/mL solution of the analyte at 10 µL/min into a flow of 190 µL/min of mobile phase using the gradient condition at which the analyte elutes from the LC column (100% eluent B). Mass spectral peak widths should be approximately 0.6 amu at half-height.

One set of calibration standards was injected at the beginning and one set of calibration standards was injected at the end of each analytical run (tray).

A.11.0 CALCULATIONS

Calculated concentrations are based on peak area ratios of PFOS to Tetra-H-PFOS. The peak area for the analyte ion is divided by the peak area for the corresponding internal standard ion.

Data generated from samples in this study were acquired and integrated using PE SCIEX software applications Sample Control (v. 1.3) and MacQuan (v. 1.4). The validation data were formatted in the ABS laboratory information management system (Watson, v.5.3.1.01). All data were rounded to no less than three significant figures by ABS prior to reporting.

Acceptance Criteria for Sample Analysis

Calibration standards: The coefficient of determination (r^2) must be ≥0.98. At least three-fourths of the individual calibration standard replicates will have deviations within ±15% from their nominal concentrations.

LLQ acceptance criteria: At least one replicate at the LLQ must exhibit a deviation within ±20% from its nominal concentration. If this criterion is not met, both replicates are rejected and the standards at the next higher level are subjected to the same test.

Quality control samples: At least two-thirds of the individual QC sample replicates will have deviations within ±15% from their nominal concentrations, with at least one replicate at each QC concentration meeting this criterion.

BIOLOGICAL SAMPLE ANALYSIS

Battelle ... Putting Technology To Work

Battelle Study Number: N003296-G

3M Toxicology Services Protocol Number: FACT-TOX-111

FINAL REPORT

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

SPONSOR

3M Toxicology Services-Medical Department
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

Testing Facility

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

Prepared By

Patrick L. South, B.S.

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

FINAL REPORT

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

Jon C. Andre

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

4-24-01
Date

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

4/24/01
Date

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

EXECUTIVE SUMMARY

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

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

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

QUALITY ASSURANCE STATEMENT

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

Phase Inspected	Inspection Date	Date Reported to Battelle Task Leader/ Battelle Management	Date Reported to Offsite Study Director/ Management
Standard Preparation	09/29/1999	10/01/1999	10/14/1999
Sample weights	09/30/1999	10/01/1999	10/14/1999
Sample homogenization	09/30/1999	10/01/1999	10/14/1999
Extraction	10/01/1999	10/11/1999	10/14/1999
Sample analysis	10/01/1999	10/11/1999	10/14/1999
Audit study file	10/11/1999	10/11/1999	10/14/1999
Audit final report	10/11/1999	10/11/1999	10/14/1999
Audit final report	02/19/2001	02/19/2001	03/14/2001

Cynthia M. Gay 4-24-01
Quality Assurance Unit Date
Battelle Memorial Institute

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

Study Title: ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

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

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

4-24-01
Date

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

27 April 2001
Date

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

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Battelle Study Number: N003296-G
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1.0 Introduction

This report presents a description of the method used to analyze PFOS in rat liver samples from 3M Study Number FACT-TOX-111 and the results from this analysis. See Appendix E for a copy of the study protocol, amendments, and deviations.

2.0 Reference Substances

The analytical reference substance for this study was potassium perfluorooctanesulfonate (PFOS) lot number 171, supplied by 3M. Note that based on information supplied to Battelle from 3M, PFOS has two equivalent names. The name appearing on the Material Safety Data Sheet and bottle label is potassium perfluoroalkyl sulfonate. The name more commonly used by 3M in analytical methods and correspondence is potassium perfluorooctanesulfonate. The latter name will be used in this report. See Appendix F for purity data supplied by 3M to Battelle.

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

3.0 Receipt of Samples

Samples were received frozen and intact at Battelle, from 3M Environmental Technology and Services, in one batch on August 20, 1999. Samples were generated by Argus Research under protocol number 418-015. The samples were stored at approximately –20°C.

4.0 Analysis of Samples

4.1 Summary of Method

Samples were analyzed by a previously validated method (Battelle study number N003604-A) in one batch. The current version of the method is attached to this report in Appendix C. Samples were analyzed by LC/MS/MS, and an example of the instrument parameters is listed in Table 1. Note that only PFOS itself (and the surrogate) was quantitated. The other related fluorochemicals were added to the stock solutions but not monitored. Quadratic regressions weighted 1/x were used to construct the calibration curves. The run order consisted of system suitabilities, 3 calibration curves analyzed at the start, near the middle, and at the end of the run and quadruplicate QCs interspersed throughout the run. The various recovery samples were also interspersed throughout the run.

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Table 1. Example of Instrument Parameters Used to Analyze Samples

LC/MS/MS System			
Autosampler	Make: Gilson	Model: 234	
HPLC pumps	Make: Gilson	Models: 305 and 306	
Mass spectrometer	Make: Micromass	Model: Quattro LC with Z-spray source	
Analytical column	Keystone Betasil C18, 5µm, 2 x 50 mm, Part No. 055-701-2		
Mobile phase components	Component A: Ammonium acetate(2mM):methanol, 60:40, v:v Component B: Ammonium acetate(2mM):methanol, 5:95, v:v		
Gradient profile	**Time, min**	**%B**	**Flow, mL/min**
	0	0	0.3
	1	0	0.3
	4.5	100	0.3
	6	100	0.3
	6.1	100	0.6
	8.5	100	0.6
	9	0	0.3
	11	0	0.3
Injection volume	10 µL		
Flow	LC column flow at start split to 20 µL/min into the MS		
Column temp	Ambient		
HPLC pressure	Approximately 850 psi at gradient start		
MS source	Electrospray, Negative Ion		
Desolvation gas	Nitrogen at ~575 L/hr		
Nebulizer gas	Nitrogen at ~80 L/hr		
Source block temp	140°C		
Desolvation temp	250°C		
Cone voltage	70 V		
Collision energy	40 eV		
Collision gas	Argon, gas cell, at ~2.5×10^{-3} mb		
Multiplier	650 V		
Resolution	12.0 for MS1; 10.0 for MS2		
Ions monitored	427>81 MRM transition for SS 499>99 MRM transition for PFOS		
Total run time	11 minutes		
Approximate retention times:	SS: 4 min PFOS: 4.2 min		

4.2 Results

4.2.1 Quality Control

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

4.2.2 Sample Results

The results of the sample analyses are presented in Appendix A. The limit of quantitation is defined as the concentration of the lowest standard which meets acceptance criteria for accuracy (25% RE). The notation BLOQ denotes "Below Limit of Quantitation" for samples that had concentrations lower than the theoretical concentration for the 0.13 µg/g calibration standard. The "Corrected PFOS Conc" presented in the results tables is the concentration found for the diluted sample multiplied by its dilution factor (final volume) sample homogenate volume).

The method detection limit (MDL) of PFOS was calculated in Battelle study N003296-F to be 0.0173 µg/g from the analysis of 7 replicate preparations of 0.13 µg/g calibration standard. The MDL was calculated by multiplying the standard deviation of the found concentrations of the 7 reps by 3.143; the Signal-to-Noise (S/N) ratio was calculated by dividing the mean found concentration of the 7 reps by their standard deviation.

5.0 Conclusions

The analysis met all acceptance criteria except for dilution recovery. The average dilution recovery was 133.3%. See Appendix E for deviation report.

6.0 Acknowledgements

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

Participant	Title
Jon C. Andre, Ph.D.	Battelle Principal Investigator
Richard W. Slauter, Ph.D., D.A.B.T.	Senior Program Director
Patrick L. South, B.S.	Mass spectroscopist
Gerke H. van der Zwaag, M.S.	Chemist

7.0 Specimen Storage and Record Archives

See Appendix E, protocol amendment 2 for records archival information. All residual liver samples, extracts, and unused test article will be disposed of or returned to the Sponsor as directed by the Sponsor.

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

APPENDIX A -RESULTS

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

RAT LIVER SAMPLE RESULTS
STUDY: N003296-G
ANALYSIS DATE AND INSTRUMENT ID: 01Oct99; 9053
DATA ENTERED: Electronically
SPREADSHEET SOFTWARE: Excel 97

FINAL LIVER SAMPLE RESULTS:

Animal numbers ending in "P" indicate pooled fetal liver

Animal Number	Dose Grp mg/kg/day	Corrected PFOS Conc (µg/g)	Note if Sample is a Rerun	Note if Rerun is Needed
Animal 13726	0.0	0.341		
Animal 13726P	0.0	0.425		
Animal 13727	0.0	0.429		
Animal 13727P	0.0	0.158		
Animal 13728	0.0	0.299		
Animal 13728P	0.0	0.141		
Animal 13731	0.0	0.298		
Animal 13734	0.0	0.218		
Animal 13734P	0.0	0.156		
Animal 13735	0.0	0.251		
Animal 13735P	0.0	0.158		
Animal 13736	0.0	0.191		
Animal 13736P	0.0	BLOQ		
Animal 13737	0.0	0.219		
Animal 13737P	0.0	0.171		
Animal 13739	0.1	6.04		
Animal 13739P	0.1	6.35		
Animal 13740	0.1	6.76		
Animal 13740P	0.1	5.92		
Animal 13741	0.1	8.32		
Animal 13741P	0.1	4.85		
Animal 13744	0.1	6.26		
Animal 13744P	0.1	6.33		
Animal 13745	0.1	6.84		
Animal 13745P	0.1	6.14		
Animal 13746	0.1	6.91		
Animal 13746P	0.1	6.06		
Animal 13748	0.1	6.78		
Animal 13748P	0.1	5.02		
Animal 13749	0.1	8.99		
Animal 13749P	0.1	5.67		
Animal 13751	1.6	80.6		
Animal 13751P	1.6	58.7		
Animal 13752	1.6	102		
Animal 13752P	1.6	63.2		
Animal 13753	1.6	50.4		
Animal 13753P	1.6	57.6		
Animal 13754	1.6	49.6		
Animal 13754P	1.6	72.8		
Animal 13756	1.6	65.0		
Animal 13756P	1.6	83.3		
Animal 13758	1.6	56.7		
Animal 13758P	1.6	58.6		
Animal 13759	1.6	79.1		
Animal 13759P	1.6	60.9		

BLOQ = BELOW CONC OF LOWEST STANDARD IN CALIBRATION CURVE

Battelle Study Number: N003296-G
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APPENDIX B- ACCEPTANCE CRITERIA RESULTS, CALIBRATION CURVE

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

PFOS IN RAT LIVER
STUDY: N003296-G
ANALYSIS DATE AND INSTRUMENT: 01Oct99; 8853
DATA ENTERED: ELECTRONICALLY
SPREADSHEET SOFTWARE: EXCEL 97

System Suitability

Filename	PFOS/IS Area Ratio	Ave Ratio	Std Dev	%RSD
01-Oct-01	7.778E+00	7.693	0.252	3.3
01-Oct-02	7.907E+00			
01-Oct-03	7.622E+00			
01-Oct-04	7.970E+00			
01-Oct-05	7.288E+00			

QC 1 Theor Conc (µg/g) = 1.885E+01

Replicate	Det'd. Conc. (µg/g)	Avg. Det'd. Conc. (µg/g)	s (µg/g)	% RSD	Avg. %RE
1	1.282E+01	1.066E+01	1.5	14.3	6.1
2	1.018E+01				
3	9.226E+00				
4	1.043E+01				

QC 2 Theor Conc (µg/g) = 3.321E+00

Replicate	Det'd. Conc. (µg/g)	Avg. Det'd. Conc. (µg/g)	s (µg/g)	%RSD	Avg. %RE
1	3.669E+00	3.663E+00	0.1	3.9	10.3
2	3.615E+00				
3	3.630E+00				
4	3.536E+00				

QC 3 Theor Conc (µg/g) = 7.539E-01

Replicate	Det'd. Conc. (µg/g)	Avg. Det'd. Conc. (µg/g)	s (µg/g)	%RSD	Avg. %RE
1	8.332E-01	7.931E-01	0.0	3.8	5.2
2	7.700E-01				
3	7.702E-01				
4	7.969E-01				

QC 4 Theor Conc (µg/g) = 1.660E-01

Replicate	Det'd. Conc. (µg/g)	Avg. Det'd. Conc. (µg/g)	s (µg/g)	%RSD	Avg. %RE
1	1.849E-01	1.716E-01	0.0	5.2	3.4
2	1.671E-01				
3	1.671E-01				
4	1.672E-01				

Homogenizer Recovery Target Concs

Nominal Conc, µg/g	Mass of Liver, g	Source Conc, ng/mL	Mass TA added, µg	Target Liver Conc, µg/g
4, Rep A	0.5207	2.028E+04	2.028E+00	3.895E+00
4, Rep B	0.5633	2.028E+04	2.028E+00	3.600E+00
0.8, Rep A	0.5141	4.056E+03	4.056E-01	7.890E-01
0.8, Rep B	0.5288	4.056E+03	4.056E-01	7.656E-01
0.4, Rep A	0.4967	1.978E+03	1.978E-01	3.983E-01
0.4, Rep B	0.5249	1.978E+03	1.978E-01	3.769E-01

Target liver conc (µg/g) = Mass TA added (µg) / Mass of liver (g)

Homogenizer Recovery Results

Target Liver Conc, µg/g	Found Liver Conc, µg/g	% Recovery	
3.895E+00	4.107E+00	105.4	
3.600E+00	4.522E+00	125.6	
7.890E-01	8.764E-01	111.1	
7.656E-01	8.323E-01	108.7	Ave % Recov: 117.4
3.983E-01	1.417E+00	355.8	Std Dev: 13.1
3.769E-01	5.136E-01	136.3	%RSD: 11.1

% Recovery = Found Conc / Target Conc x 100%

Rep A of 0.4 ug/g excluded by Q test as outlier:
Q= (355.8 - 136.3)/(355.8-105.4)= 0.877 OK to drop per SOP CHEM IV-015-03 at 95% confidence level

DILUTION TEST CONC

Undiluted liver density (mg/mL), Prep 28Sep99, Study N003296-G= 107.86

TA Source Conc, ng/mL	First Dln Factor	First Dln, ng/mL	First Dln, ug/g	2nd Dln Factor	Conc Extracted, ug/g
0.5070	80	6.450E+03	5.034E+01	10	5.034

TO CONVERT CONCS FROM ng/mL TO ug/g:
CONC (µg/g) = CONC (ng/mL) / Ave Undiluted Liver Density (mg/mL) x 1000 mg/g x 0.001 µg/ng

Dilution Recovery Results

Target Liver Conc, µg/g	Found Liver Conc, µg/g	% Recovery	
5.034E+00	6.661E+00	132.3	Ave % Recov: 133.3
5.034E+00	6.496E+00	129.0	Std Dev: 4.8
5.034E+00	6.979E+00	138.6	%RSD: 3.7

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Quantify Calibration Report
PFOS in Rat Liver: N003296-G

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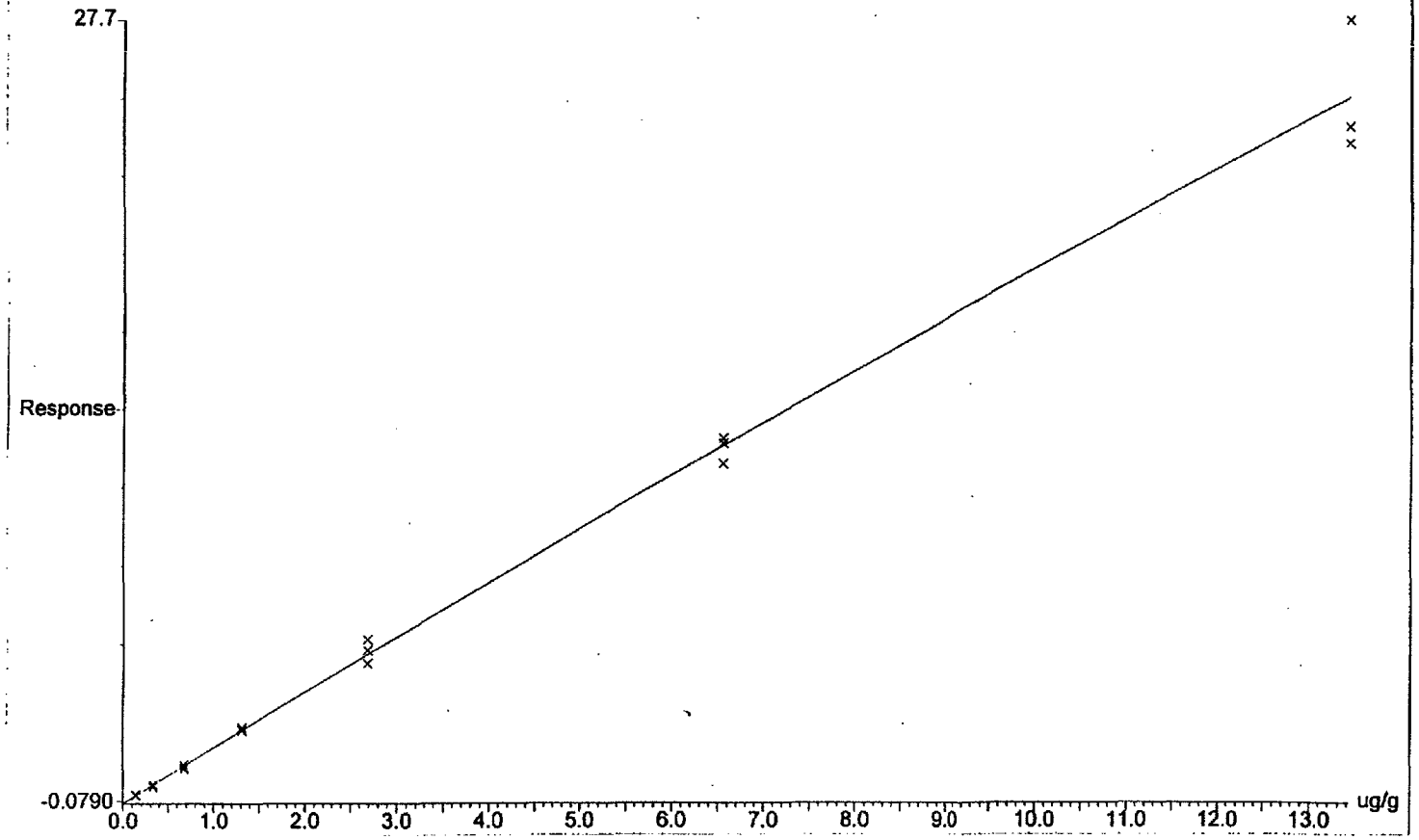
Calibration: C:\MASSLYNX\N003296G.PRO\CurveDB\01Oct
Last modified: Sat Oct 02 14:33:44 1999
Printed: Sat Oct 02 14:50:01 1999

Compound 2 name: PFOS
Coefficient of Determination: 0.992024
Calibration curve: -0.00950557 * x^2 + 1.99272 * x + -0.0790111
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: None



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Battelle Study Number: N003296-G
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APPENDIX C-METHOD

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

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

Version 1.0

Study No.: _____
Analyst/Date: _____

Revisions to the method

Date of Revision	Revised by	Approved by

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

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

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

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

Version 1.0

Study No.: _____

Analyst/Date: _____

I. SUMMARY

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

II. PURPOSE

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

III. SAMPLES

See Chain of Custody records if applicable.

IV. GENERAL INSTRUCTIONS

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

V. MATERIALS

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

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

Version 1.0

Study No.: _____
Analyst/Date: _____

Table 1. Materials

Materials	Use	Supplier	Grade or Purity	Storage Temp	Lot or ID
Potassium Perfluorooctane-sulfonate (PFOS)	Analytical Standard	3M		Room Temp	
1H,1H,2H,2H-Perfluorooctane Sulphonic Acid	Surrogate Standard	ICN		Room Temp	
M-556	Analytical Standard	3M		Room Temp	
M570	Analytical Standard	3M		Room Temp	
PFOSAA	Analytical Standard	3M		Room Temp	
PFOSA	Analytical Standard	3M		Room Temp	
PFOSEA	Analytical Standard	3M		Room Temp	
Rat Liver	Matrix	Harlan	Sprague-Dawley	~-20°C	
Ammonium Acetate, $NH_4C_2H_3O_2$	Mobile Phase			RT	
Sodium Hydroxide, NaOH	Reagent Prep			RT	
Tetrabutylammonium Hydrogensulfate (TBA), $[CH_3(CH_2)_3]_4N(HSO_4)$	Extract Prep			RT	
Sodium Carbonate, Na_2CO_3	Extract Prep			RT	
Sodium Bicarbonate, $NaHCO_3$	Extract Prep			RT	
Ethyl Acetate	Extract Prep			RT	
Methanol	Mobile Phase, Stocks, WS			RT	
Milli-Q Water	Reagent Prep, Mobile Phase	Millipore	ASTM Type I	RT	
pH 7 Buffer	pH meter calibration			RT	
pH 10 Buffer	pH meter calibration			RT	

RT means Room Temperature.

VI. EQUIPMENT

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

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

Version 1.0

Study No.: _____
Analyst/Date: _____

Table 2. Equipment

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

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

Version 1.0

Study No.: _____
Analyst/Date: _____

VII. PROCEDURE

A. Preparation of 2 mM Ammonium Acetate

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

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

B. Preparation of ~29% Sodium Hydroxide Solution

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

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

C. Preparation of ~2.9% Sodium Hydroxide Solution

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

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

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

pH Meter Calibration

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

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

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

Study No.: _____
Analyst/Date: _____

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

E. Preparation of 0.25 M Carbonate Buffer

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

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

F. Preparation of Mobile Phase

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

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

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

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

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

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

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

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

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Study No.: _____
Analyst/Date: _____

2. WSS (1000 ng/mL):

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

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

H. Preparation of Calibration Solvent Stocks and Working Standards

1. Solvent Stocks:

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

2. Mixed Solvent Stocks:

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

Date of preparation: _____
Study No: _____

3. Working Standards (WS):

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

Date of preparation: _____

Table 3. Calibration Solvent Stocks and Working Standards

Standard	Source	Target Amount	Actual Amount Analytical Std. Stock, or WS	Target Final Vol (mL)	Actual Final Vol. (mL)	Nominal Conc (ng/mL)
Stock 1A	PFOS	50 ± 1 mg	mg*	10		5,000,000
Stock 1B	PFOS	25 ± 0.5 mg	mg*	10		2,500,000
Stock 2A	M-556	50 ± 1 mg	mg*	10		5,000,000
Stock 2B	M-556	25 ± 0.5 mg	mg*	10		2,500,000
Stock 3A	M570	50 ± 1 mg	mg*	10		5,000,000
Stock 3B	M570	25 ± 0.5 mg	mg*	10		2,500,000
Stock 4A	PFOSAA	93 ± 1 mg	mg*	10		5,000,000
Stock 4B	PFOSAA	46 ± 0.5 mg	mg*	10		2,500,000
Stock 5A	PFOSA	50 ± 1 mg	mg*	10		5,000,000
Stock 5B	PFOSA	25 ± 0.5 mg	mg*	10		2,500,000
Stock 6A	PFOSEA	50 ± 1 mg	mg*	10		5,000,000

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Study No.: _____
Analyst/Date: _____

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

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

I. Preparation of Calibration Standards and Blanks

1. Liver homogenate

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

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

Determine density of calibration/QC matrix:

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

Table 4. Calibration Stds/QCs Matrix Density

Replicate #	1	2	3	4	5	6	7	8	9	10
Mass (mg)										

2. Liver Calibration Standards

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

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Study No.: _____
Analyst/Date: _____

Table 5. Calibration Standards and Blanks

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

Date of preparation of cal stds/blank: _____

J. Preparation of Quality Control Liver Samples (QCs)

1. Quality Control Working Standards

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

Table 6. QC WS Preparation

Soln ID	Source	Vol Source, mL	Final Vol, mL	Conc, ng/mL
QC WS 1	Mixed Stock A	3 ( )	100 ( )	15,000
QC WS 2	Mixed Stock B	1 ( )	50 ( )	5000
QC WS 3	QC WS 1	7.5 ( )	100 ( )	1125
QC WS 4	QC WS 2	2.5 ( )	50 ( )	250

2. Preparation of Quality Control Liver Samples

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

Table 7. QC Preparation

QC	Source	Vol Source, mL	Final Vol, mL	Conc, ng/mL	Conc, µg/g
1	QC WS 1	2.5 ( )	25 ( )	1500	10
2	QC WS 2	2.5 ( )	25 ( )	500	3.3
3	QC WS 3	2.5 ( )	25 ( )	112.5	0.7
4	QC WS 4	2.5 ( )	25 ( )	25	0.16

Date of QC prep: _____ Study: _____

K. Preparation of MS Check Standard for System Suitability

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

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

Version 1.0

Study No.: _____

Analyst/Date: _____

L. Preparation of homogenizer recovery liver samples

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

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

M. Preparation of Dilution Check Sample

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

N. Homogenization of study samples

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

O. Analysis Standards, Blanks, QCs, and Samples

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

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

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

P. LC/MS/MS Analysis

② WN KCP 8/28/99

1. Use the system conditions specified in Table 8 ②. The conditions which are designated may be modified by the analyst to produce acceptable peak shape.

Table 8 - LC/MS/MS Conditions

LC/MS/MS System			
Autosampler	Make: _____	Model: _____	ID: _____
HPLC Pumps	Make: _____	Model: _____	ID: _____
Mass Spectrometer	Make:	Model:	ID:
Analytical column	Keystone Betasil C18, 5µ, 2 x 50 mm, Part No. 055-701-2, S/N: ; Lot:		
Mobile Phase Components	Component A: Ammonium acetate:methanol, 60:40, v:v Component B: Ammonium acetate:methanol, 5:95, v:v		
Gradient profile	**Time, min**	**%B**	**Flow, mL/min**
	0	0	0.3
	1	0	0.3
	4.5	100	0.3
	6	100	0.3
	6.1	100	0.6
	8.5	100	0.6
	9	0	0.3
	11	0	0.3
Injection volume	10 µL (µL)		
Flow split	LC column flow split to *30 µL/min (µL/min) into the MS at run start		
Column Temp	Ambient		
HPLC Pressure	1000 psi at gradient start (psi)		
MS Source	Electrospray, Negative Ion		
Desolvation gas	*Nitrogen at 575 L/hr (L/hr)		
Nebulizer gas	*Nitrogen at 80 L/hr (L/hr)		
Source Block Temp	*140°C (°C)		
Desolvation Temp	*250°C (°C)		
Cone voltage	*70 V (V) PFOS, ~~IS~~ ① *20 V (V) PFOSA, PFOSAA, PFOSEA, M-556, M570		
Collision energy	*40 eV (eV) PFOS, IS, PFOSA, PFOSAA, M-556, M570 *30 eV (eV) PFOSEA ①		
Collision gas	Argon at *2.5 x 10^{-3} mb gas cell (mb)		
Multiplier	*650 V (V)		
Resolution	*12.0 for MS1 ( ); *10.0 for MS2 ( )		

① SHOULD READ "55"; LE KCP 8/28/99

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

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

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

VIII. CALCULATIONS

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

Undiluted liver density (mg/mL) = (g of liver x average density of homogenate)/(g of liver + g of water)
where g of liver and g of water are masses used to prepare bulk homogenate; density of water is assumed to be 1 g/mL.

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

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

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

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

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

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

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

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

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

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

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

IX. ACCEPTANCE CRITERIA

A. MS Check Standard (System Suitability)

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

B. Calibration Standards

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

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Table 9. Calibration Standard Acceptance limits

ANALYTE	% Error
PFOS	20 (25% at LOQ)
M-556	20 (25% at LOQ)
M570	20 (25% at LOQ)
PFOSAA	20 (25% at LOQ)
PFOSA	20 (25% at LOQ)
PFOSEA	25 (30% at LOQ)

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

C. QCs

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

Table 10. QC Acceptance limits

ANALYTE	%
PFOS	20
M-556	20
M570	20
PFOSAA	20
PFOSA	20
PFOSEA	25

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

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

D. Homogenizer Recovery and Dilution Check Samples

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

E. Sensitivity (LOQs)

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

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

F. Specificity

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

G. General

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

X. RESULTS

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

XI. COMMENTS

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Analyst/Date: _____

XII. CONCLUSIONS

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XIII. SIGNATURES

Analysts

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

Technical Review

_____ Date: _____
_____ Date: _____

QC Review

_____ Date: _____
_____ Date: _____

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APPENDIX D-REPRESENTATIVE CHROMATOGRAMS

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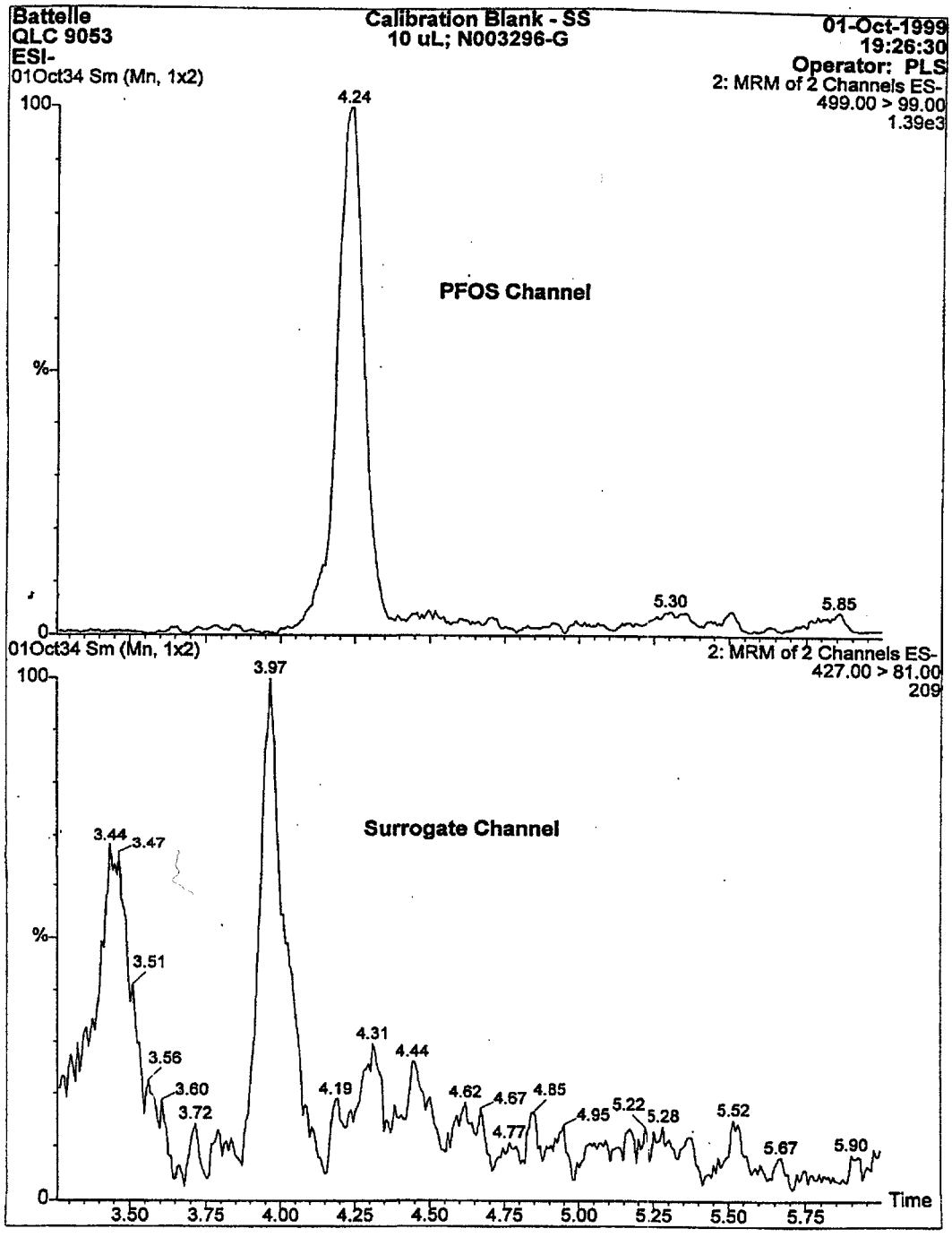

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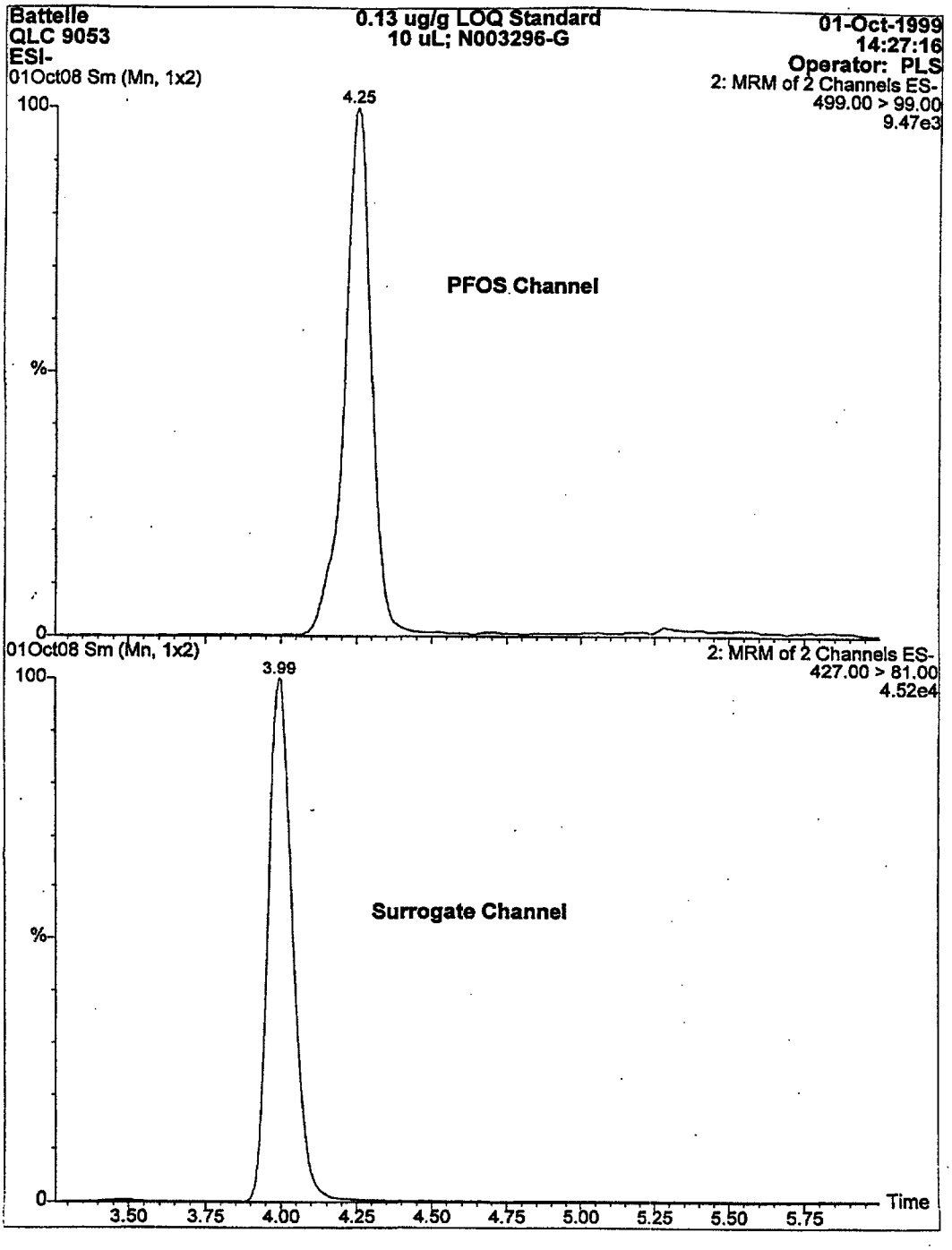

Battelle Study Number: N003296-G
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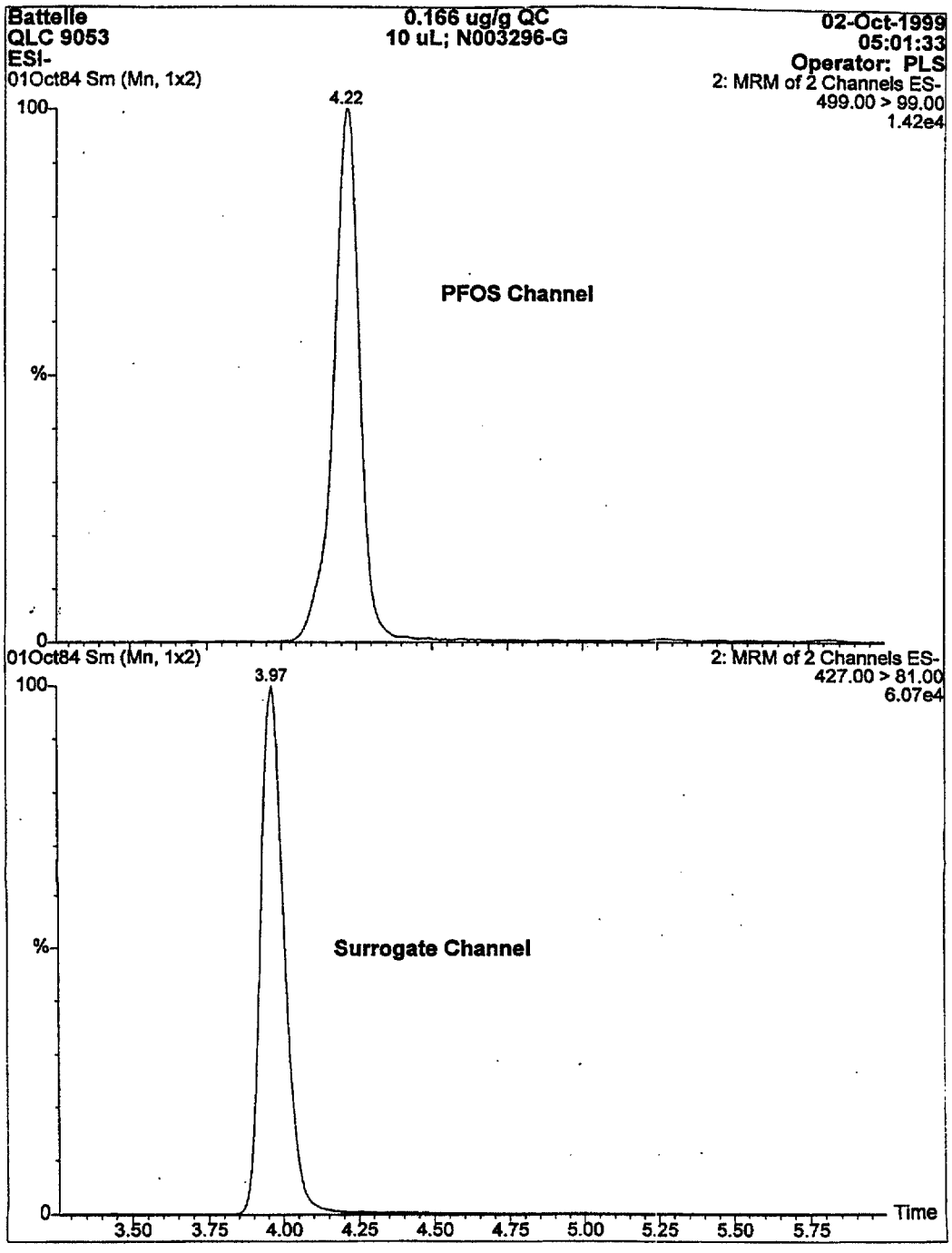

Battelle Study Number: N003296-G
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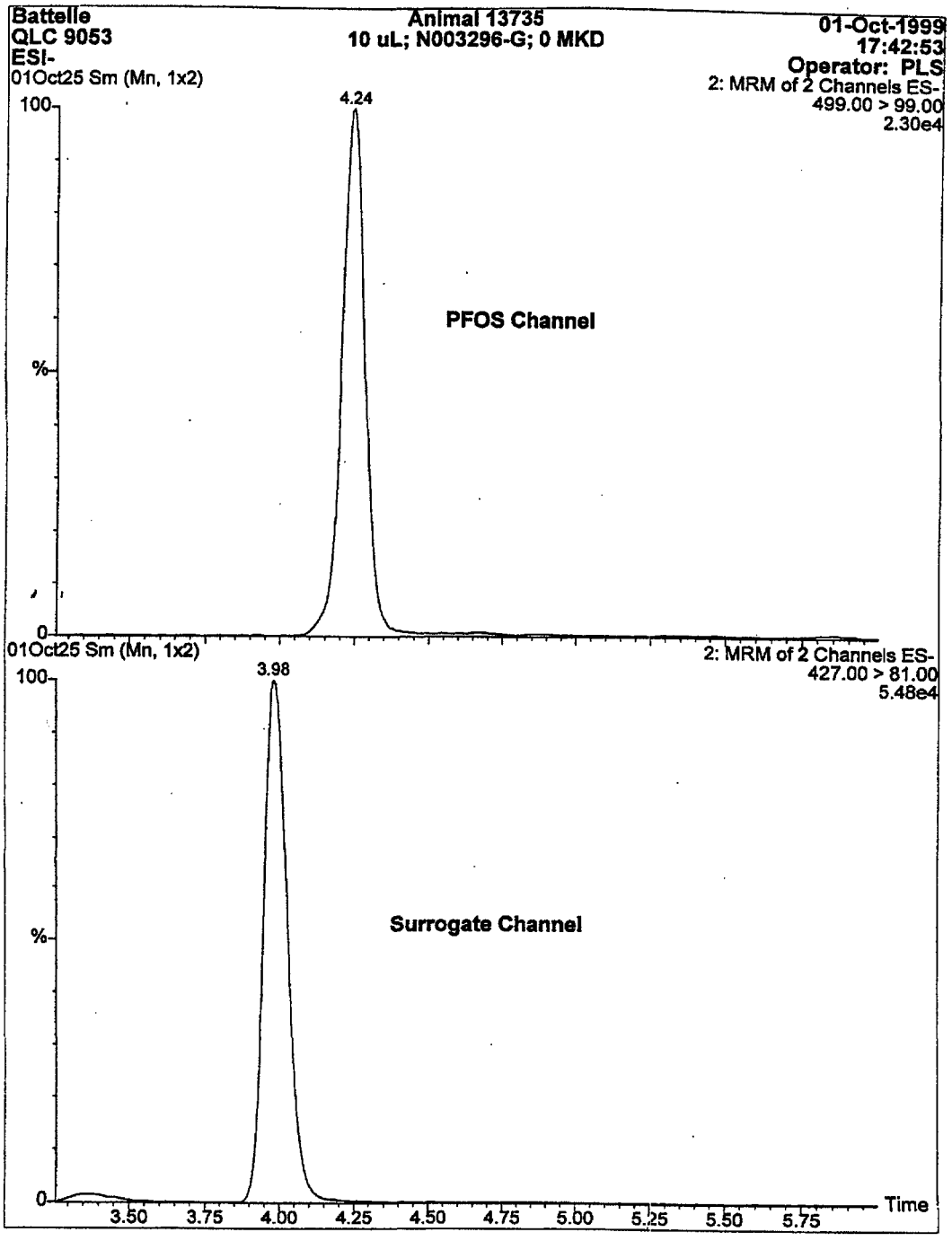

Battelle Study Number: N003296-G
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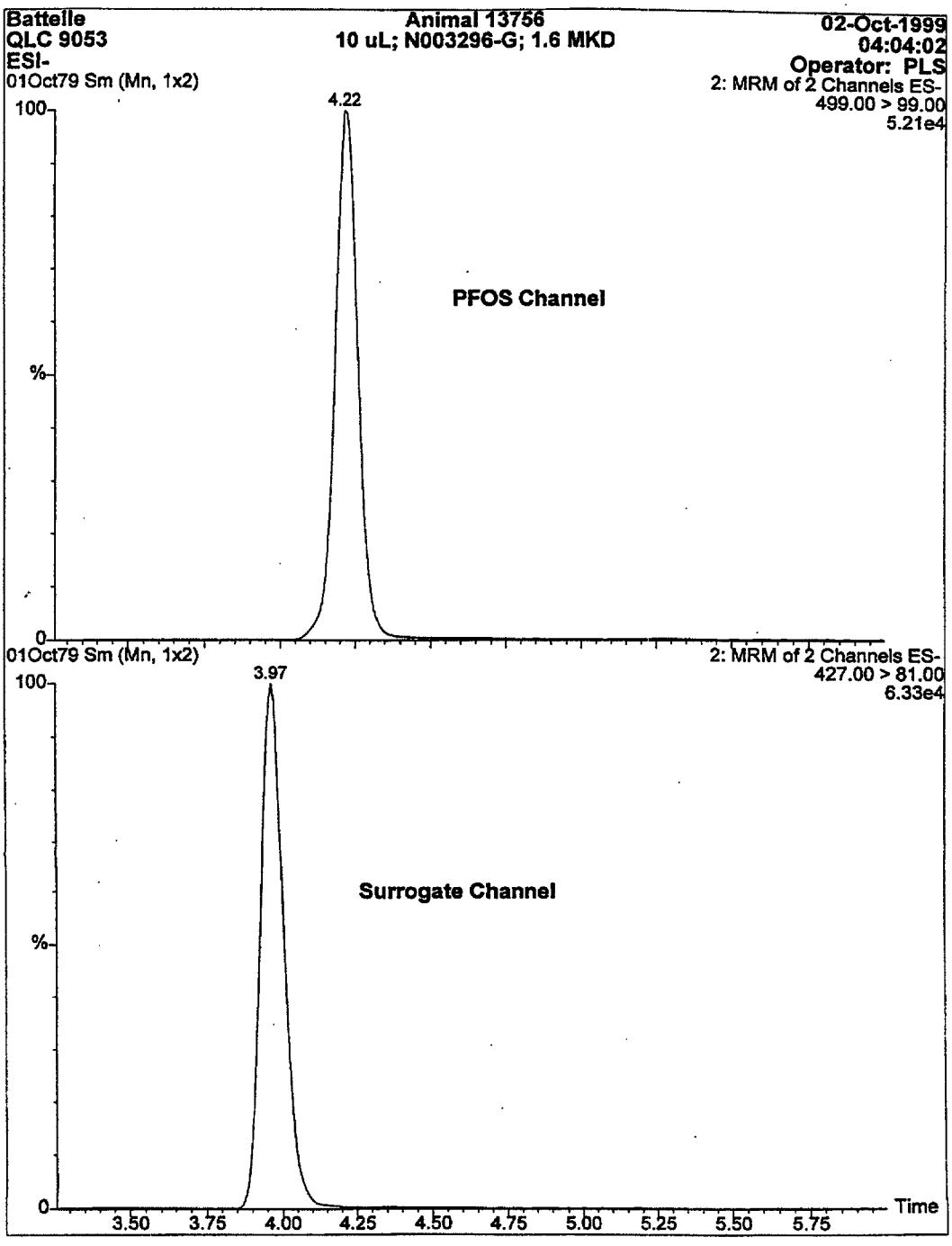

Battelle Study Number: N003296-G
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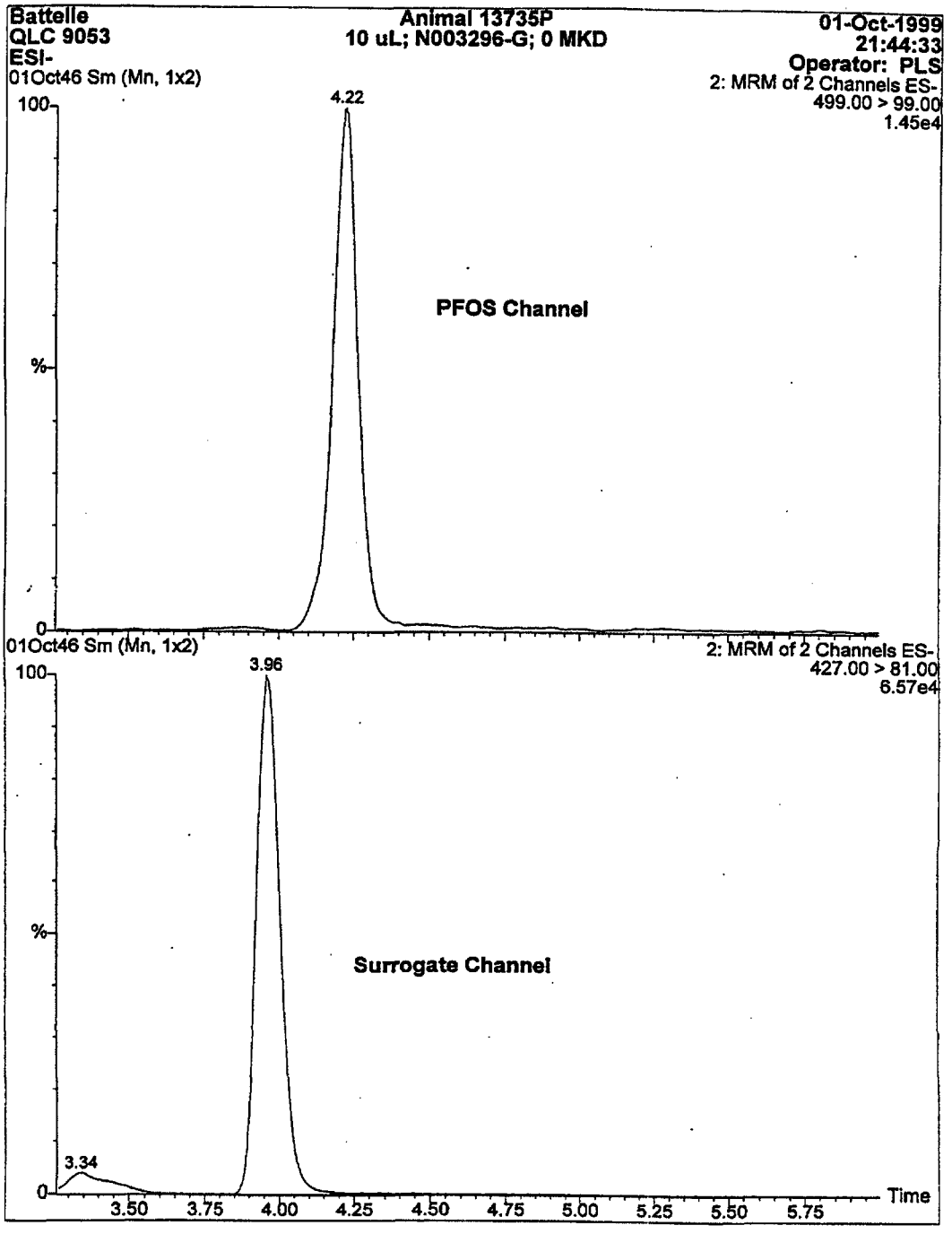

Battelle Study Number: N003296-G
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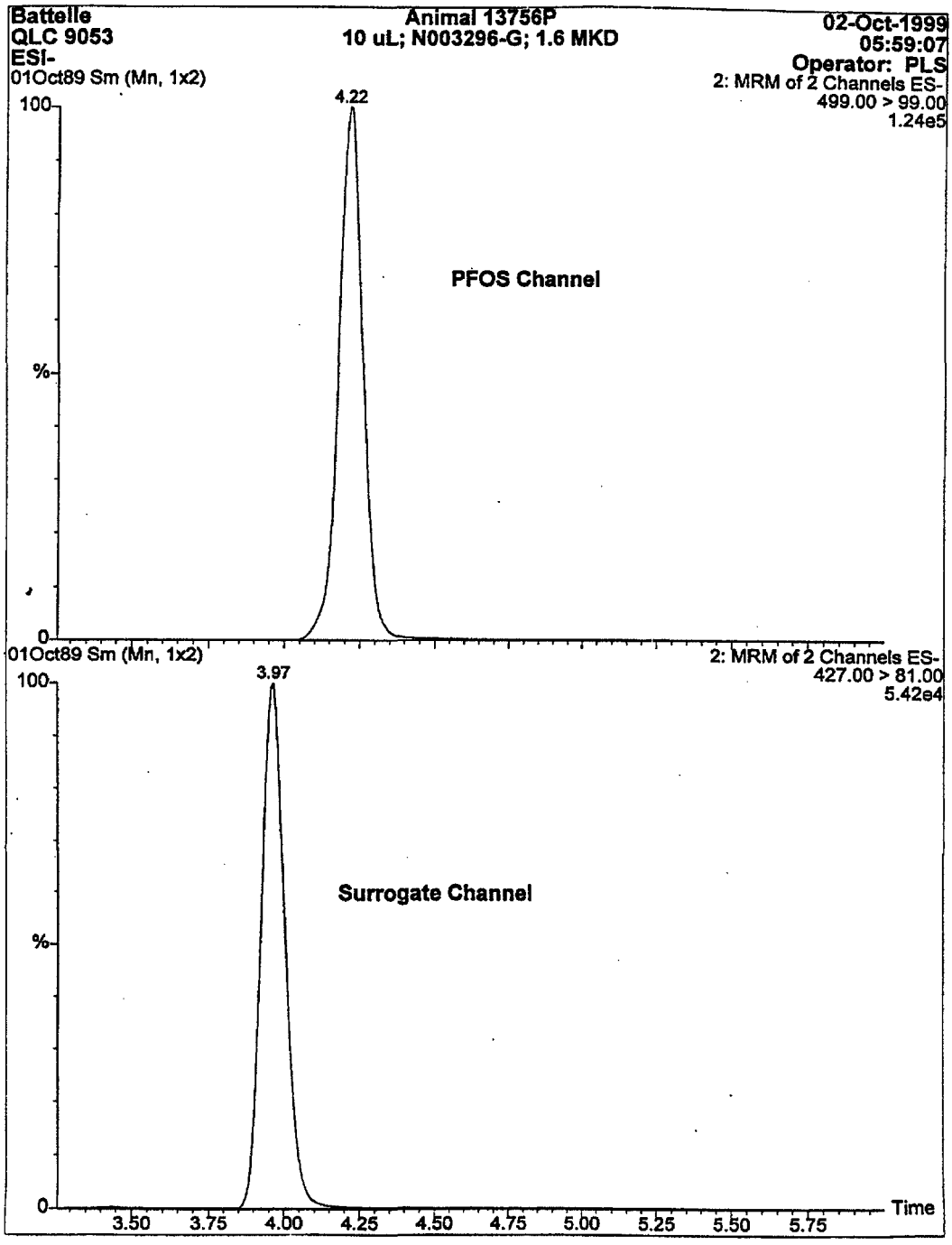

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APPENDIX E-PROTOCOL, AMENDMENTS, AND DEVIATIONS

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3M Toxicology Services Protocol Number: FACT-TOX-111

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

Protocol #FACT-TOX-111

3M

Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

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

Laboratory Project Identification
FACT-TOX-111
U2994

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Study Identification

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

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

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Sub-Contract Laboratories

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

Battelle Memorial Institute
505 King Avenue
Columbus Ohio 43201-2693

Proposed Study Timetable

Study Initiation Date	June 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic recovery study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-015. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. . If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

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

4. QUALITY ASSURANCE

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

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

5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-015.

6. CONTROL MATRICES

6.1 *Identification* Rat liver and serum, and/or rabbit liver and serum traceability numbers will be recorded in the raw data and included in the final report

6.2 *Source* Argus Research and/or Sigma Chemical

6.3 *Physical Description* Rat liver and serum, and/or rabbit liver and serum

6.4 *Purity and Stability* Not applicable

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

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

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

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

7. REFERENCE MATERIAL

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

7.2 *Source* 3M Specialty Chemicals

7.3 *Physical Description* White powder

7.4 *Purity and Stability* Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 *Storage Conditions* Room temperature

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

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

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

8. Test System

Rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-015. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation until day 0 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	8	0	0	5
2	8	0.1	0.02	5
3	8	0.6	0.32	5

9. Specimen and Sample Receipt

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

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	Dam-Predose, Days 7, 14, 15, 21, and 22 Pup-Day 21	144 Dam 24 Pup (pooled)
Urine and Feces – Dam and Pup animals	Predose, Days 7, 15, 21, and 22	120 Urine and 120 Feces
Liver – Dam and Pup animals	Dam-At the termination of the study Pup-Day21	24 Dam 24 Pup (pooled)

Total number of expected specimens: 456
Total number of test animals: 16
Total number of control animals: 8

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

10. Preparatory Methods

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

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

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

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

11. Analytical Methods

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

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

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

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

12. Data Quality Objectives

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

12.1 ***Linearity*** $r^2 \geq 0.98$

12.2 ***Limits of detection / quantitation***

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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

12.3 ***Duplicate acceptable precision*** < 30% for the method

12.4 ***Spike acceptable recoveries*** 70% - 130%

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

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

13. Sub-Contracted Analysis

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

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

14. Statistical Analysis

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

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

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

15. Report

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

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

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

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

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

16. Location of Raw Data, Records, and Final Report

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

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

- ***16.1.1*** Approved protocol and amendments
- ***16.1.2*** Study correspondence
- ***16.1.3*** Shipping records
- ***16.1.4*** Raw data
- ***16.1.5*** Approved final report (original signed copy)
- ***16.1.6*** Electronic copies of data

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

- ***16.2.1*** Training records
- ***16.2.2*** Calibration records
- ***16.2.3*** Instrument maintenance logs
- ***16.2.4*** Standard Operating Procedures, Equipment Procedures, and Methods

17. Specimen Retention

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

18. Protocol Amendments and Deviations

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

19. Attachments

19.1 Attachment A Preparatory and analytical methods

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

20. SIGNATURES

Marvin T Case 7 June 1999
Marvin T. Case, D.V.M., Ph.D., Sponsor Representative Date

Kristen Hansen 6/9/99
Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director Date

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 1

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

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

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

AMEND TO READ: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to:

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"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

The extraction and analytical method to follow at Battelle Memorial Institute is:

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

REASON: The analytical methods at the sub-contract laboratories were not included in the original protocol.

D REVIEW 9/27/99
kjk 9/27/99

3M Environmental Laboratory

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 1

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

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

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

Amendment Approval

Marvin T Case — 17 August 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kris J. Hansen — 8/18/99
Kris J. Hansen, Ph.D., Study Director — Date

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 30, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 2

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

1. ***PROTOCOL READS:***
Section 2 states the study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and sera in rats.

AMEND TO READ:
The study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver, sera, and urine in rats.

REASON:
The urine analytical methods were validated and approved after approval of the original protocol.

2. ***PROTOCOL READS:***
Section 10.0 and 11.0 list the following methods to use for extraction and analysis:
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"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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"

AMEND TO READ::
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"
ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

REASON: The extraction and analytical methods ETS-8-96.0 and ETS-8-97.0, were approved after approval of the original protocol.

3M Environmental Laboratory

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 2

3. ***PROTOCOL READS:***
Section 6.1 lists control matrices as rat liver and serum or rabbit liver and serum. Source Argus and/or Sigma Chemical.

AMEND TO READ:
Control matrices identification rat liver and serum, rabbit liver and serum, human urine and/or rat urine. Source Argus, Sigma Chemical, Lampire Biologicals, Biological Specialty Corp. and/or Golden West Biologicals.

REASON:
Addition of control urine specifications.

4. ***PROTOCOL READS:***
Section 12.2.1 a) lists sera method detection limit as 1.75 ppb and b) lists liver method detection limit as 15 ppb.

AMEND TO READ:
The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

REASON:
The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

5. ***PROTOCOL READS:***
Section 16 states that 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, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that 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:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

6. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

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

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 2

Amendment Approval

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

Kristen Hz
Kristen J. Hansen, Ph.D., Study Director — 5 Oct 1999 Date

3M Environmental Laboratory

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number 2759-39-3) in the Serum, Liver, and Urine of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS Via Gavage

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

Performing Laboratories

Performing Laboratories

Urine Analyses	Liver Analyses	Serum Analyses
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

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

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

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

Protocol LRN-U2994
Amendment Number 3

Amendment Approval

John L. Butenhoff — February 10, 2000

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

Kristen Hansen — 11-Feb-2000

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

Marvin T Case — 10 February 2000

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

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

DEVIATON REPORT

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

TYPE OF DEVIATION: PROTOCOL

DATE OF DEVIATION: September 28, 1999

NATURE OF DEVIATION: The source of control matrix will not be either Argus Research or Sigma Chemical as specified in section 6.2 of the protocol.

CAUSE OF DEVIATION: Harlan will be the supplier of control rat livers used to prepare blanks, any standards, and QCs for the analytical portion of the study.

IMPACT OF DEVIATION ON STUDY: Harlan was used as the control matrix supplier for Battelle's validation of the analytical method (Battelle study number N003604-A). This supplier provided matrix that allowed achievement of the reported method acceptance criteria so that there is not impact on the study.

CORRECTIVE ACTION: This protocol deviation report was prepared.

APPROVED BY:

Jon C Andre — 4-24-01

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

Marvin T Case — 27 April 2001

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

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

DEVIATON REPORT

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

TYPE OF DEVIATION: PROTOCOL

DATE OF DEVIATION: October 4, 1999

NATURE OF DEVIATION: The dilution recovery results for rat liver did not meet the method acceptance criteria of 70-130%. This is required in section IX. D. of the method and section 12.4 of the study protocol.

CAUSE OF DEVIATION: The actual dilution recovery was 133%.

IMPACT OF DEVIATION ON STUDY: The remaining method performance tests (calibration curve, QCs, and homogenization recoveries) met their respective acceptance criteria. Approximately 2/3 of the study samples were diluted before extraction, and therefore potentially affected. The dilution recovery was not considered to be exceedingly high enough, at only approximately 3% above the normal acceptance level, to have significantly impacted the data.

CORRECTIVE ACTION: The dilution recovery data will be presented in the final report.

APPROVED BY:

Jon C Andre — 4-24-01
Jon C. Andre, Ph.D. — Date
Battelle Principal Investigator

Marvin T Case — 27 April 2001
Marvin T. Case, D.V.M., Ph.D. — Date
Study Director

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

DEVIATON REPORT

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

ORAL (GAVAGE) PHARMACOKINETIC RECOVERY STUDY OF PFOS IN RATS

TYPE OF DEVIATION: Protocol

DATE OF DEVIATION: August 20, 1999

NATURE OF DEVIATION: Protocol states Battelle will analyze 24 dam and 24 pup (pooled). Chain of Custody Record indicates only 45 samples were received.

IMPACT OF DEVIATION ON STUDY: The impact of this is minimal to the interpretation of the study outcome.

CORRECTIVE ACTION: This protocol deviation report was prepared.

APPROVED BY:

Jon C. Andre — 4-24-01

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

Marvin T Case — 27 April 2001

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

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

APPENDIX F - PFOS PURITY REPORT

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

ETSS 2 3W ☎ 651 778 4226 04/26/99 09:56 :02/03 NO:341

3M SPECIALTY ADHESIVES & CHEMICALS ANALYTICAL LABORATORY

Request # 57830

To:	Lisa Clemen - (8-5568) – ET&SS- 2-03-09
From:	Tom Kestner - (3-5633) - SA&C Analytical Lab - 236-2B-11
Subject:	***Chemical Characterization of POSF-Based Fluorochemicals by ^{1}H-NMR & ^{19}F-NMR Spectroscopy***
Date:	March 24, 1999: Preliminary report for FC-95 (PFOS), lot 171

SAMPLE DESCRIPTION:

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

INTRODUCTION:

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

EXPERIMENTAL:

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

RESULTS:

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

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

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

Tom Kestner

c: Rick Payfer – SA&C Analytical Lab – 236-2B-11 File Reference: LC57830.DOC/61

Battelle Study Number: N003296-G
3M Toxicology Services Protocol Number: FACT-TOX-111

March 24, 1999

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

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

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

RE-ISSUED ANALYTICAL REPORT

STUDY TITLE

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

DATA REQUIREMENTS

Analytical Method Requirements

STUDY DIRECTOR

Marvin T. Case, 3M

PRINCIPAL INVESTIGATOR

Enaksha Wickremesinhe, Centre

RE-ISSUED ANALYTICAL REPORT COMPLETION DATE

November 3, 2000

PERFORMING LABORATORY / TESTING FACILITY

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

STUDY SPONSOR

3M Toxicology Services – Medical Department
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

PROJECT IDENTIFICATION

Sponsor Protocol Number: FACT-TOX-111
Centre Study Number: 023-017

Total Pages: 92

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-017, entitled "Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats," conducted for 3M Toxicology Services – Medical Department, was performed in compliance with US FDA Good Laboratory Practice Standards (21 CFR 58) by Centre Analytical Laboratories, Inc. with the following exceptions:

1. The reference substances (analytical standards) used in this study (PFOS and THPFOS) have not been characterized under 21 CFR 58.105

2. The automated data collection systems used in this study are not fully compliant with 21 CFR 58.130(e).

Enaksha Wickremesinhe, Ph.D*
Principal Investigator
Centre Analytical Laboratories, Inc.

3-NOV-00
Date

*Enaksha Wickremesinhe is no longer employed at Centre, therefore Centre Management, Rick Grazzini will be signing in his stead.

Marvin T Case, D.V.M., Ph.D.
Study Director
3M Toxicology Services

21 Nov 2000
Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-017, entitled, "Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats". All phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Centre Management	Date Reported to Study Director and Sponsor Management
1. Protocol Review	5/17/00	6/1/00	7/14/00
2. Extraction & Fortification	7/7/00	7/11/00	7/14/00
3. Raw Data & Summary Table Review	7/28,31/00	8/15/00	8/25/00
4. Draft Report Review	8/15/00	8/17/00	8/25/00
5. Final Report Review	9/11/00	9/11/00	9/11/00

Naomi Lovallo
Quality Assurance Auditor

11/3/00
Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

CERTIFICATION OF AUTHENTICITY

This report, for Centre Study Number 023-017, is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801
(814) 231-8032

Principal Investigator, Centre:

Enaksha Wickremesinhe, Ph.D.* — Date: 3-NOV-00
Group/Team Leader
Centre Analytical Laboratories, Inc.

*Enaksha Wickremesinhe is no longer employed at Centre, therefore Centre Management, Rick Grazzini will be signing in his stead.

Centre Analytical Laboratories, Inc. Facility Management:

John Flaherty — Date: 11/3/00
Laboratory Manager
Centre Analytical Laboratories, Inc.

Study Director, 3M:

Marvin T. Case, D.V.M., Ph.D. — Date: 30 Nov 2000
3M Toxicology Services

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

STUDY IDENTIFICATION

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

SPONSOR PROTOCOL NUMBER: FACT-TOX-111

TYPE OF STUDY:	Oral (Gavage) Pharmacokinetic Recovery
TEST SYSTEM:	Rat Feces
TEST MATERIAL:	Perfluorooctane sulfonic acid potassium salt (T-6295)
SPONSOR:	3M Toxicology Services – Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
STUDY DIRECTOR:	From 6/9/99 to 2/10/00 Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Phone: (651) 778-6018 From 2/10/00 to Present Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Phone: (651) 733-5180
TESTING FACILITY:	Centre Analytical Laboratories, Inc. 3048 Research Drive State College, PA 16801
PRINCIPAL INVESTIGATOR	Enaksha Wickremesinhe, Ph.D. Centre Analytical Laboratories, Inc. Phone: (814) 231-8032

ANALYTICAL PHASE TIMETABLE:

Study Initiation Date:	06/09/99
Study Assignment Date:	04/19/00
Analytical Start Date:	07/06/00
Analytical Termination Date:	07/20/00

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

PROJECT PERSONNEL

The Study Director for this project was Marvin T. Case at 3M Toxicology Services and the Principal Investigator for this project at Centre Analytical Laboratories, Inc. was Enaksha Wickremesinhe. The following personnel from Centre Analytical Laboratories, Inc., were associated with various analytical phases of the study:

Name	Title
Enaksha Wickremesinhe	Group/Team Leader
Emily Stauffer	Scientist
Karen Smith	Scientist
Tiffany Proctor	Technician
Rickey Keller	Sample Custodian
Lawrence Ord	Sample Custodian

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1.0 SUMMARY

The purpose of this study was to analyze perfluorooctanesulfonate (PFOS) in rat feces as specified in 3M Protocol FACT-TOX-111. The analytical method used for this study was, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2", Centre method number: 00M-023-003, revision 2.

The limit of quantification for PFOS in rat feces established during the method validation was 10 ppb (0.0100 µg/g). The method validation did not determine a method detection limit.

Residues (corrected for purity) ranging from non-detected levels to 13.5 µg/g were found in the rat feces samples.

Fortification recoveries ranged from 73-136% with an average of 92% and relative standard deviation of 18%.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of feces of rats using the analytical method "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2", Centre method number 00M-023-003, revision 2.

3.0 INTRODUCTION

The study was initiated on June 9, 1999, when the study director signed the protocol FACT-TOX-111. The study was assigned to Centre on April 14, 2000 when the study director signed Amendment #4. The complete protocol and amendments and deviations can be found in **Appendix A.** The analytical start date was July 6, 2000, and the analytical termination date was July 20, 2000.

This report details the results of the residues of PFOS detected in rat feces, using the analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (revision 2)", Centre method number 00M-023-003, revision 2. Complete details of the analytical methodology can be found in **Appendix B**

4.0 TEST SYSTEM

The 117 incurred rat feces samples analyzed in this study were received frozen on dry ice from 3M Environmental Laboratory St. Paul, MN on June 28, 2000. Samples were logged in and stored frozen (< -10°C) on June 28, 2000 by Centre personnel.

The control rat feces used for standards and blanks were purchased from Lampire Biological Laboratories, Inc., Piperesville, PA and received frozen on dry ice at Centre on June 22, 2000, logged in by Centre personnel and placed in frozen storage (<-10°C).

Sample login and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc. and a true copy of the storage records can be found in the raw data package associated with this study.

5.0 REFERENCE MATERIAL

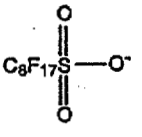
The analytical standard for PFOS determination (potassium perfluorooctane sulfonate) was received at Centre on November 12, 1999 and the surrogate standard THPFOS was received on December 3, 1999 from 3M Environmental Technology and Services. Characterization of the reference material PFOS was performed at Centre on August 31, 2000 and documentation can be found in the raw data package associated with this report.

The available information for the reference materials is listed below. The reference materials were stored at room temperature.

Compound	Centre Control No.	Batch No.	Purity (%)	Expiration Date
PFOS	99-023-002	217	86.9	08/31/01
THPFOS	99-023-011	53406	NA	01/01/10

Molecular structures of PFOS and THPFOS are given below.

PFOS
Chemical Name: Perfluorooctane sulfonate
Molecular weight: 499 ($C_8F_{17}SO_3^-$)



Note: The neutral molecule and standard form from which PFOS (anion) is derived, is potassium perfluorooctane sulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

THPFOS
Chemical Name: 4-H, perfluorooctane sulfonic acid
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, $C_8F_{13}SO_3H$

6.0 EXPERIMENTAL DESIGN

Samples were extracted according to group number and sampling day. Each set of samples contained two matrix blanks, two matrix blanks spiked with the surrogate standard, two matrix spikes, and 7 – 16 samples.

The extracts were analyzed by LC/MS/MS. Samples with residues outside the linear range of the calibration curve were diluted and re-analyzed.

7.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)," Centre method number 00M-023-003, revision 2 **(Appendix B)** was used for this study.

7.1 Extraction Procedure

One gram (± 0.05 g) of sample was weighed into 20 mL polyethylene scintillation vials and fortified (if necessary) using disposable micropipettes. Ten mL of acetonitrile was added to the vial, capped tightly, and placed on a wrist-action shaker for ~ 30 min. The samples were filtered through a glass acrodisc filter. The filtered extract was passed through a conditioned carbon SPE column and collected. The columns were then eluted with ~10 mL acetonitrile and ~20 mL 90:10 acetonitrile:2% ascorbic acid in methanol. The combined extracts were evaporated down to near dryness with a rotary evaporator and then re-constituted with 2 mL methanol. The samples were analyzed using electrospray LC/MS/MS.

7.2 Preparation of Standards and Fortification Solutions

Standard solutions were prepared on May 3, 2000 as specified in Centre Analytical Laboratories' analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, (Revision 2)," Centre method number 00M-023-003, revision 2. An individual stock standard solution of PFOS was prepared at a concentration of 100 µg/mL by dissolving 10 mg of the standard (corrected for salt purity only) in methanol. From this solution, a 10 µg/mL fortification standard solution was prepared by taking 10 mL of the stock and bringing the volume up to 100 mL with methanol. Also, a stock standard of THPFOS was prepared at 100 µg/mL by dissolving 10 mg of the standard in methanol. An individual 10 µg/mL solution of THPFOS was prepared in the same fashion described above.

A 2.5 µg/mL fortification standard of PFOS was prepared by taking 25 mL of the 10 µg/mL fortification standard of PFOS and bringing the volume up to 100 mL with methanol. An individual fortification solution of THPFOS was prepared in the same manner. A 0.5 µg/mL fortification standard of PFOS was prepared by taking 20 mL of

the 2.5 µg/mL standard of PFOS and bringing the volume up to 100 mL with methanol. To make the 0.1 µg/mL fortification standard, 20 mL of the 0.5 µg/mL was brought up to 100 mL with methanol.

Calibration standards were processed through the extraction procedure, identically to the samples. The fortification of the standards before extraction was done according to the following table:

Conc. of PFOS Fortification Solution (µg/ml)	Fortification Volume (µL)	Weight of Control Sample (g) (± 0.05 g)	Fort. Level of Extracted Calibration Standard (ppb)	Vol. of Surrogate Standard* added (µL)
0.1	100	1.0	10	200
0.1	200	1.0	20	200
0.5	100	1.0	50	200
0.5	200	1.0	100	200
2.5	100	1.0	250	200
2.5	200	1.0	500	200

* 2.5 µg/ml THPFOS fortification solution.

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

7.3 Chromatography

Quantification of PFOS and THPFOS was accomplished by electrospray LC/MS/MS analysis. The retention times of PFOS and THPFOS were ~ 5.2 min. and ~ 5.0 min., respectively, with no significant interfering peaks in the control matrices corresponding to either of the analyte retention times.

7.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run was equivalent to 5 ng/mL of PFOS and 250 ng/mL of THPFOS in the rat feces matrix.

7.5 Description of Instrument and Operating Conditions

A Micromass Quattro Ultima LC/MS/MS coupled to a Hewlet Packard HPLC system was used. Data acquisition and processing were performed using Masslynx 3.4 software. Detailed operating conditions are listed below:

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Instrument: Micromass Quattro Ultima

ELECTROSPRAY ION SOURCE:

Capillary: 3.0 kV	Hexapole 2: 0.3 V
Hexapole 1: 0.1 V	Source Block Temp.: 100°C
Aperture 1: 0.2 V	Desolvation Temp.: 350°C

ANALYZER:

LM Res 1: 10.5 V	LM Res 2: 13.0 V
HM Res 1: 10.5 V	HM Res 2: 13.0 V
IEnergy 1: 1.0 V	IEnergy 2: 2.0 V
Entrance: -2 V	Multiplier: 650 V
Exit: 2 V	

GAS FLOWS AND PRESSURE:

Desolvation N_2 Flow Rate: ~650 L/hr
Nebuliser N_2 Flow Rate: ~150 L/hr
Gas Cell Pressure: ~0.003 mbar

Computer: COMPAQ Professional Workstation AP200

Software: Microsoft Windows NT: Version 4 Build 1381: Service Pack 5
Micromass Limited: Masslynx 3.4 Build 004

HPLC Equipment: Hewlett Packard (HP) Series 1100

HP Binary Pump	HP Vacuum Degasser
HP Autosampler	HP Column Oven

HPLC Column: Genesis C-8, 5 cm x 2.1 mm i.d. x 4 µ
Column Temperature: 35°C
Mobile Phase (A) : 2 mM Ammonium Acetate in Type I Water
Mobile Phase (B) : Methanol

Gradient:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	60.0	40.0	0.3
0.4	60.0	40.0	0.3
1.0	10.0	90.0	0.3
7.0	10.0	90.0	0.3
7.5	0.0	100.0	0.3
10.5	0.0	100.0	0.4
11.0	60.0	40.0	0.4
14.5	60.0	40.0	0.4
15.0	60.0	40.0	0.3

Injected Volume: 10 µL
Ions monitored :

Analyte	Transition Monitored	Dwell (secs)	Coll Energy (eV)	Cone (V)
PFOS	499 → 99	0.2	43	76
THPFOS	427 → 80	0.2	35	34

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7.6 Quantitation and Example Calculation

Ten microliters of sample or extracted calibration standard was injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x weighted linear regression) by Masslynx software using six concentrations of standards. The surrogate standard, THPFOS, was only used to monitor the efficiency of the extraction procedure and was not used for quantitation of PFOS. The residue concentration for rat feces was determined from the following equations:

Use Equations 1 and 2 to calculate the amount of analyte found (in ppb, based on peak area) using the standard curve (linear regression parameters) generated by the Masslynx software program.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

Equation 2:

$$\text{Analyte found (ppb)} = \frac{\text{(analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF)}}{\text{sample wt. (g)}}$$

where DF = dilution factor.

For samples fortified with known amounts of analytes prior to extraction, use Equation 3 to calculate the percent recovery.

Equation 3:

$$\text{Recovery (\%)} = \frac{[\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF}]}{\text{analyte added (ng)}} \times 100$$

Equation 4:

This calculation was only performed on actual samples and not QC recoveries.

Corrected analyte found (ppb) = analyte found (ppb) × % purity

% purity for PFOS lot 217 = 86.9% (0.869)

An example of a calculation using an actual sample analyzed with extracted standards follows:

Rat feces sample Centre ID 0005803 Spk A (Set: 071000A), fortified at 50 ng with PFOS.

Where:

peak area	=	19051
intercept	=	2021.14
slope	=	673.327
dilution factor	=	1

ng added (fort level) = 50
final vol. = 2 mL
sample wt. = 1.01 g

From equation 1:

Analyte found (ng/mL) = $\frac{[19051 - 2021.14]}{673.327}$

= 25.3 ng/mL

From equation 2:

Analyte found (ppb) = $\frac{[25.3 \times 2\ mL \times 1]}{1.01\ g}$

= 50.1 ppb

From equation 3:

% Recovery = $\frac{(25.3\ ng/mL \times 2\ mL \times 1)}{50\ ng} \times 100$

= 101%

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the RAW DATA.

The amount of surrogate standard THPFOS found was calculated by dividing the peak area for each sample by the mean response factor.

Analyte found (ng/mL): $\frac{\text{peak area}}{\text{mean response factor}}$

Other statistical methods used in analyzing this data were:

$$\textit{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad \textit{Mean} = \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Relative Standard Deviation (RSD or Coefficient of Variation (CV)) =

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100\%$$

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8.0 RESULTS AND DISCUSSION

Although, the majority of the control samples did not contain interferences, a couple of samples did contain residues. However these interferences were not significant enough to adversely affect the data. A summary of residues found in all of the matrix blanks and matrix zero blanks is detailed in **Table I.**

Residues (corrected for purity) ranging from non-detected levels to 13.5 µg/g were found in the rat feces samples. The residues found in all of the samples plus the averages and standard deviations for each group at each interval are detailed in **Tables II-XVI**

For this report, the residues found in Tables I-XVI were corrected for the purity of the PFOS standard lot 217 based on the certificate of analysis finalized on September 7, 2000.

Fortification recoveries ranged from 73-136% with an average of 92% and relative standard deviation of 18%. A summary of all of the fortification recoveries can be found in **Table XVII.**

Typical calibration curves and chromatograms representing standards, controls, fortifications, and samples are depicted in **Figures 1-7.**

9.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

Electronic records are not fully compliant with 21 CFR 11, "Electronic Records: Electronic Signature." However, fully approved SOP's were in place and all chromatograph instrumentation used in this study was fully validated (IQ, PQ, OQ), calibrated and operational. All original data were printed as hard copies and fully audited by quality assurance. Verified exact copies and the electronic data have been stored in the archives at Centre Analytical Laboratories, Inc. Original raw data have been returned to the sponsor.

10.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 21 CFR 58. Retained samples of reference substances are archived by the sponsor.

11.0 TABLES

Centre Study No.: 023-017
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Table I. Summary of PFOS residues in Matrix and Matrix Zero Blanks

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
na	0005738 Blank A	070600AR	7/6/00	ND	ND
na	0005738 Blank B	070600AR	7/6/00	ND	ND
na	0005738 Zero Blank C	070600AR	7/6/00	ND	ND
na	0005738 Zero Blank D	070600AR	7/6/00	ND	ND
na	0005738 Blank A	070700A	7/7/00	NQ	NQ
na	0005738 Blank B	070700A	7/7/00	ND	ND
na	0005738 Zero Blank C	070700A	7/7/00	ND	ND
na	0005738 Zero Blank D	070700A	7/7/00	ND	ND
na	0005738 Blank A	071000A	7/10/00	ND	ND
na	0005738 Blank B	071000A	7/10/00	ND	ND
na	0005738 Zero Blank C	071000A	7/10/00	ND	ND
na	0005738 Zero Blank D	071000A	7/10/00	ND	ND
na	0005738 Blank A	071100A	7/11/00	< 0.0100	< 0.00869
na	0005738 Blank B	071100A	7/11/00	NQ	NQ
na	0005738 Zero Blank C	071100A	7/11/00	< 0.0100	< 0.00869
na	0005738 Zero Blank D	071100A	7/11/00	NQ	NQ
na	0005738 Blank A	071200A	7/12/00	< 0.0100	< 0.00869
na	0005738 Blank B	071200A	7/12/00	0.0156	0.0136
na	0005738 Zero Blank C	071200A	7/12/00	< 0.0100	< 0.00869
na	0005738 Zero Blank D	071200A	7/12/00	< 0.0100	< 0.00869
na	0005738 Blank A	071300A	7/13/00	NQ	NQ
na	0005738 Blank B	071300A	7/13/00	NQ	NQ
na	0005738 Zero Blank C	071300A	7/13/00	NQ	NQ
na	0005738 Zero Blank D	071300A	7/13/00	NQ	NQ
na	0005738 Blank A	071700AR	7/17/00	ND	ND
na	0005738 Blank B	071700AR	7/17/00	ND	ND
na	0005738 Zero Blank C	071700AR	7/17/00	ND	ND
na	0005738 Zero Blank D	071700AR	7/17/00	ND	ND
na	0005738 Blank A	071800A	7/18/00	ND	ND
na	0005738 Blank B	071800A	7/18/00	ND	ND
na	0005738 Zero Blank C	071800A	7/18/00	ND	ND
Na	0005738 Zero Blank D	071800A	7/18/00	ND	ND

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

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Table I (continued). Summary of PFOS residues in Matrix and Matrix Zero Blanks

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
na	0005738 Blank A	072000A	7/20/00	NQ	NQ
na	0005738 Blank B	072000A	7/20/00	NQ	NQ
na	0005738 Zero Blank C	072000A	7/20/00	NQ	NQ
na	0005738 Zero Blank D	072000A	7/20/00	NQ	NQ
			AVERAGE:	**< 0.0100**	**< 0.00869**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

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Sponsor Protocol No.: FACT-TOX-111

Table II. Summary of PFOS residues in G1 Prior to Cohabitation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13726F G1 Prior to Cohabitation	0005799	070600AR	7/6/00	ND	ND
13727F G1 Prior to Cohabitation	0005800	070600AR	7/6/00	ND	ND
13728F G1 Prior to Cohabitation	0005801	070600AR	7/6/00	ND	ND
13731F G1 Prior to Cohabitation	0005802	070600AR	7/6/00	ND	ND
13734F G1 Prior to Cohabitation	0005803	070600AR	7/6/00	ND	ND
13735F G1 Prior to Cohabitation	0005804	070600AR	7/6/00	ND	ND
13736F G1 Prior to Cohabitation	0005805	070600AR	7/6/00	ND	ND
13737F G1 Prior to Cohabitation	0005806	070600AR	7/6/00	ND	ND
			AVERAGE:	**ND**	**ND**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Table III. Summary of PFOS residues in G2 Prior to Cohabitation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13739F G2 Prior to Cohabitation	0005807	0706-0700AD	7/6/00	0.933	0.810
13740F G2 Prior to Cohabitation	0005808	0706-0700AD	7/6/00	0.764	0.664
13741F G2 Prior to Cohabitation	0005809	070600AR	7/6/00	0.453	0.394
13744F G2 Prior to Cohabitation	0005810	070600AR	7/6/00	0.463	0.402
13745F G2 Prior to Cohabitation	0005811	0706-0700AD	7/6/00	3.13	2.72
13745F G2 Prior to Cohabitation	0005811	0717-1800AD	7/17/00	1.04	0.904
13746F G2 Prior to Cohabitation	0005812	0706-0700AD	7/6/00	0.791	0.688
13748F G2 Prior to Cohabitation	0005813	0706-0700AD	7/6/00	0.809	0.703
13749F G2 Prior to Cohabitation	0005814	0706-0700AD	7/6/00	0.628	0.546
			AVERAGE:	**1.00**	**0.870**
			STANDARD DEVIATION:	**0.820**	**0.713**

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Table IV. Summary of PFOS residues in G3 Prior to Cohabitation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13751F G3 Prior to Cohabitation	0005815	0706-0700AD	7/7/00	12.4	10.8
13752F G3 Prior to Cohabitation	0005816	0706-0700AD	7/7/00	12.5	10.8
13753F G3 Prior to Cohabitation	0005817	0706-0700AD	7/7/00	14.1	12.3
13754F G3 Prior to Cohabitation	0005818	0706-0700AD	7/7/00	15.6	13.5
13755F G3 Prior to Cohabitation	0005819	0706-0700AD	7/7/00	9.86	8.57
13756F G3 Prior to Cohabitation	0005820	0706-0700AD	7/7/00	12.4	10.8
13758F G3 Prior to Cohabitation	0005821	0706-0700AD	7/7/00	10.8	9.41
13759F G3 Prior to Cohabitation	0005822	0706-0700AD	7/7/00	12.5	10.9
			AVERAGE:	**12.5**	**10.9**
			STANDARD DEVIATION:	**1.77**	**1.54**

Table V. Summary of PFOS residues in G1 Day 6/7 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13726F G1 Day 6/7 Gestation	0005823	070700A	7/7/00	ND	ND
13727F G1 Day 6/7 Gestation	0005824	070700A	7/7/00	ND	ND
13728F G1 Day 6/7 Gestation	0005825	070700A	7/7/00	ND	ND
13731F G1 Day 6/7 Gestation	0005826	070700A	7/7/00	ND	ND
13734F G1 Day 6/7 Gestation	0005827	070700A	7/7/00	ND	ND
13735F G1 Day 6/7 Gestation	0005828	070700A	7/7/00	ND	ND
13736F G1 Day 6/7 Gestation	0005829	070700A	7/7/00	ND	ND
13737F G1 Day 6/7 Gestation	0005830	070700A	7/7/00	ND	ND
			AVERAGE:	**ND**	**ND**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

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Table VI. Summary of PFOS residues in G2 Day 6/7 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13739F G2 Day 6/7 Gestation	0005831	071000AD	7/10/00	0.591	0.514
13740F G2 Day 6/7 Gestation	0005832	071000AD	7/10/00	0.618	0.537
13741F G2 Day 6/7 Gestation	0005833	071000A	7/10/00	0.366	0,318
13744F G2 Day 6/7 Gestation	0005834	071000A	7/10/00	0.356	0.309
13745F G2 Day 6/7 Gestation	0005835	071000A	7/10/00	0.440	0.382
13746F G2 Day 6/7 Gestation	0005836	071000A	7/10/00	0.448	0.389
13748F G2 Day 6/7 Gestation	0005837	071000A	7/10/00	0.371	0.322
13749F G2 Day 6/7 Gestation	0005838	071000A	7/10/00	0.483	0.420
			AVERAGE:	**0.459**	**0.399**
			STANDARD DEVIATION:	**0.101**	**0.0876**

Table VII. Summary of PFOS residues in G3 Day 6/7 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13751F G3 Day 6/7 Gestation	0005839	071000AD	7/10/00	9.80	8.51
13752F G3 Day 6/7 Gestation	0005840	071000AD	7/10/00	11.1	9.61
13753F G3 Day 6/7 Gestation	0005841	071000AD	7/10/00	10.4	9.03
13754F G3 Day 6/7 Gestation	0005842	071000AD	7/10/00	10.2	8.86
13755F G3 Day 6/7 Gestation	0005843	071000AD	7/10/00	14.1	12.2
13756F G3 Day 6/7 Gestation	0005844	071000AD	7/10/00	7.71	6.70
13758F G3 Day 6/7 Gestation	0005845	071000AD	7/10/00	8.80	7.65
13759F G3 Day 6/7 Gestation	0005846	071000AD	7/10/00	5.15	4.47
			AVERAGE:	**9.65**	**8.39**
			STANDARD DEVIATION:	**2.60**	**2.26**

Centre Study No.: 023-017
Sponsor Protocol No.: FACT-TOX-111

Table VIII. Summary of PFOS residues in G1 Day 14/15 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13726F G1 Day 14/15 Gestation	0005847	071100A	7/11/00	ND	ND
13727F G1 Day 14/15 Gestation	0005848	071100A	7/11/00	ND	ND
13728F G1 Day 14/15 Gestation	0005849	071100A	7/11/00	ND	ND
13731F G1 Day 14/15 Gestation	0005850	071100A	7/11/00	ND	ND
13734F G1 Day 14/15 Gestation	0005851	071100A	7/11/00	ND	ND
13735F G1 Day 14/15 Gestation	0005852	071100A	7/11/00	ND	ND
13736F G1 Day 14/15 Gestation	0005853	071100A	7/11/00	ND	ND
13737F G1 Day 14/15 Gestation	0005854	071100A	7/11/00	ND	ND
			AVERAGE:	**ND**	**ND**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Table IX. Summary of PFOS residues in G2 Day 14/15 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13739F G2 Day 14/15 Gestation	0005855	071100A	7/11/00	0.343	0.298
13740F G2 Day 14/15 Gestation	0005856	071100A	7/11/00	0.358	0.311
13741F G2 Day 14/15 Gestation	0005857	071100A	7/11/00	0.354	0.308
13744F G2 Day 14/15 Gestation	0005858	071100A	7/11/00	0.478	0.416
13745F G2 Day 14/15 Gestation	0005859	071100A	7/11/00	0.338	0.294
13746F G2 Day 14/15 Gestation	0005860	071100A	7/11/00	0.397	0.345
13748F G2 Day 14/15 Gestation	0005861	071100A	7/11/00	0.235	0.204
13749F G2 Day 14/15 Gestation	0005862	071100A	7/11/00	0.203	0.176
			AVERAGE:	**0.338**	**0.294**
			STANDARD DEVIATION:	**0.0869**	**0.0755**

Centre Study No.: 023-017
Sponsor Protocol No.: FACT-TOX-111

Table X. Summary of PFOS residues in G3 Day 14/15 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13751F G3 Day 14/15 Gestation	0005863	071200A	7/12/00	5.75	5.00
13752F G3 Day 14/15 Gestation	0005864	071200A	7/12/00	7.26	6.31
13753F G3 Day 14/15 Gestation	0005865	071200A	7/12/00	4.63	4.02
13754F G3 Day 14/15 Gestation	0005866	071200A	7/12/00	4.37	3.80
13755F G3 Day 14/15 Gestation	0005867	071200A	7/12/00	6.50	5.65
13756F G3 Day 14/15 Gestation	0005868	071200A	7/12/00	5.90	5.13
13758F G3 Day 14/15 Gestation	0005869	071200A	7/12/00	6.10	5.30
13759F G3 Day 14/15 Gestation	0005870	071200A	7/12/00	3.92	3.41
			AVERAGE:	**5.55**	**4.83**
			STANDARD DEVIATION:	**1.15**	**1.00**

Table XI. Summary of PFOS residues in G1 Day 20/21 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13726F G1 Day 20/21 Gestation	0005871	071200A	7/12/00	ND	ND
13727F G1 Day 20/21 Gestation	0005872	071200A	7/12/00	ND	ND
13728F G1 Day 20/21 Gestation	0005873	071200A	7/12/00	ND	ND
13734F G1 Day 20/21 Gestation	0005874	071200A	7/12/00	ND	ND
13735F G1 Day 20/21 Gestation	0005875	071200A	7/12/00	ND	ND
13736F G1 Day 20/21 Gestation	0005876	071200A	7/12/00	ND	ND
13737F G1 Day 20/21 Gestation	0005877	071200A	7/12/00	ND	ND
			AVERAGE:	**ND**	**ND**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Centre Study No.: 023-017
Sponsor Protocol No.: FACT-TOX-111

Table XII. Summary of PFOS residues in G2 Day 20/21 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13739F G2 Day 20/21 Gestation	0005878	072000A	7/20/00	0.153	0.133
13740F G2 Day 20/21 Gestation	0005879	072000A	7/20/00	0.0769	0.0668
13741F G2 Day 20/21 Gestation	0005880	072000A	7/20/00	0.190	0.165
13744F G2 Day 20/21 Gestation	0005881	072000A	7/20/00	0.102	0.0883
13745F G2 Day 20/21 Gestation	0005882	072000A	7/20/00	0.0748	0.0650
13746F G2 Day 20/21 Gestation	0005883	072000A	7/20/00	0.130	0.113
13748F G2 Day 20/21 Gestation	0005884	072000A	7/20/00	0.181	0.157
13749F G2 Day 20/21 Gestation	0005885	072000A	7/20/00	0.188	0.163
			AVERAGE:	**0.137**	**0.119**
			STANDARD DEVIATION:	**0.0483**	**0.0420**

Table XIII. Summary of PFOS residues in G3 Day 20/21 Gestation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13751F G3 Day 20/21 Gestation	0005886	0717-1800AD	7/17/00	1.86	1.61
13752F G3 Day 20/21 Gestation	0005887	0717-1800AD	7/17/00	2.39	2.08
13753F G3 Day 20/21 Gestation	0005888	0717-1800AD	7/17/00	1.63	1.42
13754F G3 Day 20/21 Gestation	0005889	0717-1800AD	7/17/00	1.94	1.69
13755F G3 Day 20/21 Gestation	0005890	0717-1800AD	7/17/00	4.93	4.29
13756F G3 Day 20/21 Gestation	0005891	0717-1800AD	7/17/00	2.32	2.02
13758F G3 Day 20/21 Gestation	0005892	0717-1800AD	7/17/00	2.27	1.97
13759F G3 Day 20/21 Gestation	0005893	0717-1800AD	7/17/00	1.59	1.38
			AVERAGE:	**2.37**	**2.06**
			STANDARD DEVIATION:	**1.08**	**0.940**

Centre Study No.: 023-017
Sponsor Protocol No.: FACT-TOX-111

Table XIV. Summary of PFOS residues in G1 Day 21/22 Lactation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13726F G1 Day 21/22 Lactation	0005894	071300A	7/13/00	0.0235	0.0204
13727F G1 Day 21/22 Lactation	0005895	071300A	7/13/00	ND	ND
13728F G1 Day 21/22 Lactation	0005896	071300A	7/13/00	ND	ND
13734F G1 Day 21/22 Lactation	0005897	071300A	7/13/00	ND	ND
13735F G1 Day 21/22 Lactation	0005898	071300A	7/13/00	ND	ND
13736F G1 Day 21/22 Lactation	0005899	071300A	7/13/00	ND	ND
13737F G1 Day 21/22 Lactation	0005900	071300A	7/13/00	ND	ND
			AVERAGE:	**< 0.0100**	**< 0.00869**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Table XV. Summary of PFOS residues in G2 Day 21/22 Lactation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13739F G2 Day 21/22 Lactation	0005901	071800A	7/18/00	0.0776	0.0674
13740F G2 Day 21/22 Lactation	0005902	071800A	7/18/00	0.0797	0.0693
13741F G2 Day 21/22 Lactation	0005903	071800A	7/18/00	0.0448	0.0389
13744F G2 Day 21/22 Lactation	0005904	071800A	7/18/00	0.0839	0.0729
13745F G2 Day 21/22 Lactation	0005905	071800A	7/18/00	0.0390	0.0339
13746F G2 Day 21/22 Lactation	0005906	071800A	7/18/00	0.0701	0.0609
13748F G2 Day 21/22 Lactation	0005907	071800A	7/18/00	0.0585	0.0508
13749F G2 Day 21/22 Lactation	0005908	071800A	7/18/00	0.0266	0.0231
			AVERAGE:	**0.0600**	**0.0522**
			STANDARD DEVIATION:	**0.0212**	**0.0185**

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Table XVI. Summary of PFOS residues in G3 Day 21/22 Lactation

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
13751F G3 Day 21/22 Lactation	0005909	0717-1800AD	7/18/00	0.590	0.513
13752F G3 Day 21/22 Lactation	0005910	0717-1800AD	7/18/00	0.842	0.731
13753F G3 Day 21/22 Lactation	0005911	071800A	7/18/00	0.376	0.327
13754F G3 Day 21/22 Lactation	0005912	071800A	7/18/00	0.340	0.295
13756F G3 Day 21/22 Lactation	0005913	0717-1800AD	7/18/00	0.386	0.336
13758F G3 Day 21/22 Lactation	0005914	071800A	7/18/00	0.243	0.211
13759F G3 Day 21/22 Lactation	0005915	071800A	7/18/00	0.341	0.296
			AVERAGE:	**0.445**	**0.387**
			STANDARD DEVIATION:	**0.204**	**0.177**

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Sponsor Protocol No: FACT-TOX-111

Table XVII. Summary of PFOS recoveries in Fortified Samples

Sponsor ID	Centre ID	Set Number	Date Extracted	Amt. Added (ng)	% Recovery
13726F G1 Prior to Cohabitation	0005799 Spk A	070600AR	7/6/00	50	118
13727F G1 Prior to Cohabitation	0005800 Spk B	070600AR	7/6/00	250	101
13728F G1 Prior to Cohabitation	0005801 Spk A	070700A	7/7/00	50	88
13731F G1 Prior to Cohabitation	0005802 Spk B	070700A	7/7/00	250	86
13734F G1 Prior to Cohabitation	0005803 Spk A	071000A	7/10/00	50	101
13735F G1 Prior to Cohabitation	0005804 Spk B	071000A	7/10/00	2000	94
13736F G1 Prior to Cohabitation	0005805 Spk A	071100A	7/11/00	50	85
13737F G1 Prior to Cohabitation	0005806 Spk B	071100A	7/11/00	20000	84
13726F G1 Prior to Cohabitation	0005799 Spk A	071200A	7/12/00	50	136
13728F G1 Prior to Cohabitation	0005801 Spk B	071200A	7/12/00	20000	87
13734F G1 Prior to Cohabitation	0005803 Spk A	071300A	7/13/00	50	92
13735F G1 Prior to Cohabitation	0005804 Spk B	071300A	7/13/00	250	102
13726F G1 Prior to Cohabitation	0005799 Spk A	071700AR	7/17/00	50	79
13737F G1 Prior to Cohabitation	0005806 Spk B	071700AR	7/17/00	20000	73
13735F G1 Prior to Cohabitation	0005804 Spk A	071800A	7/18/00	50	105
13737F G1 Prior to Cohabitation	0005806 Spk B	071800A	7/18/00	20000	83
13727F G1 Prior to Cohabitation	0005800 Spk A	072000A	7/20/00	50	74
13734F G1 Prior to Cohabitation	0005803 Spk B	072000A	7/20/00	250	74
				AVERAGE:	**92**
				STANDARD DEVIATION:	**16**
				RELATIVE STANDARD DEVIATION:	**18**

12.0 FIGURES

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Figure 1. Typical Calibration Curve for PFOS

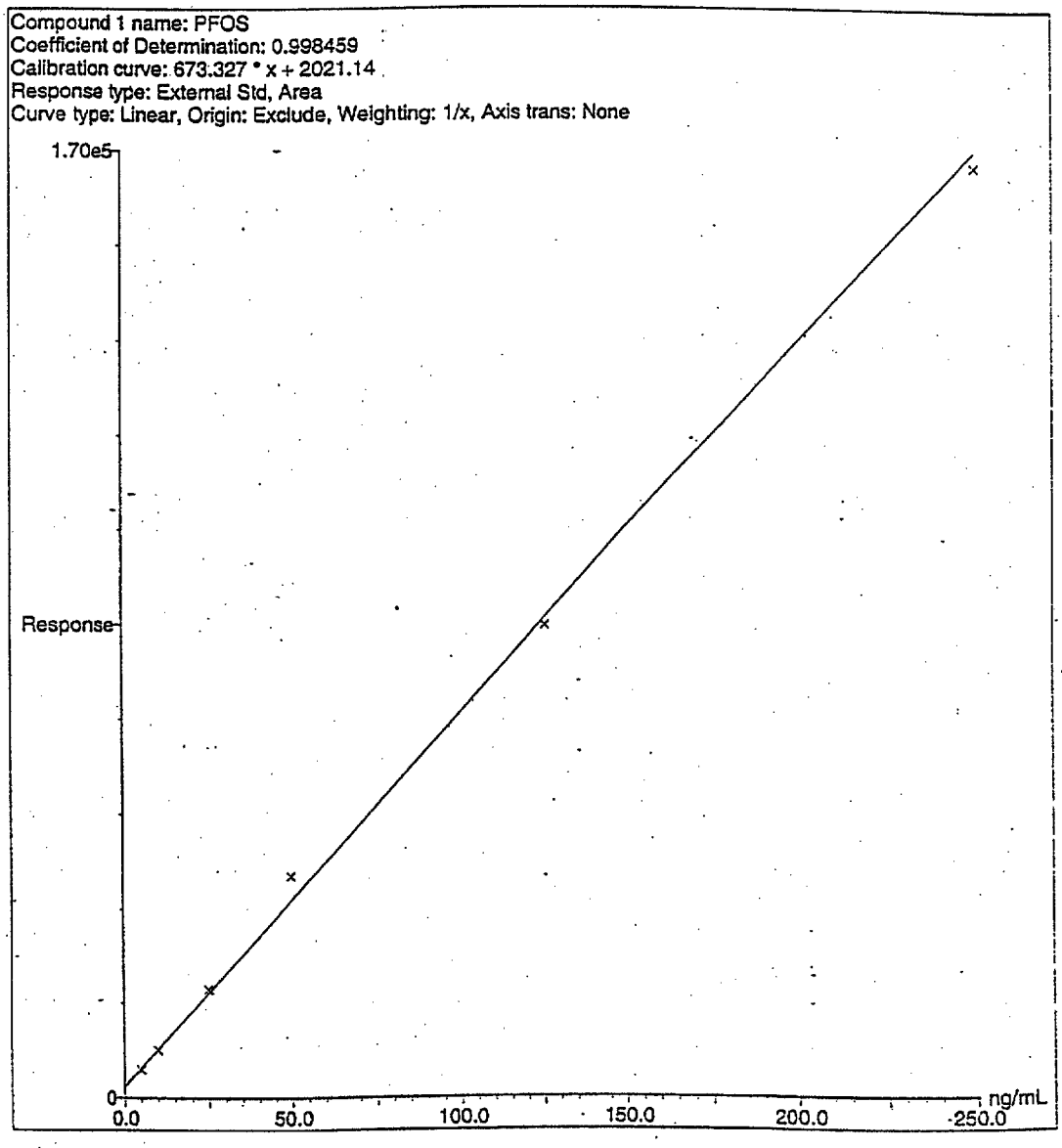

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Figure 2. Typical Mean Response Factor for THPFOS

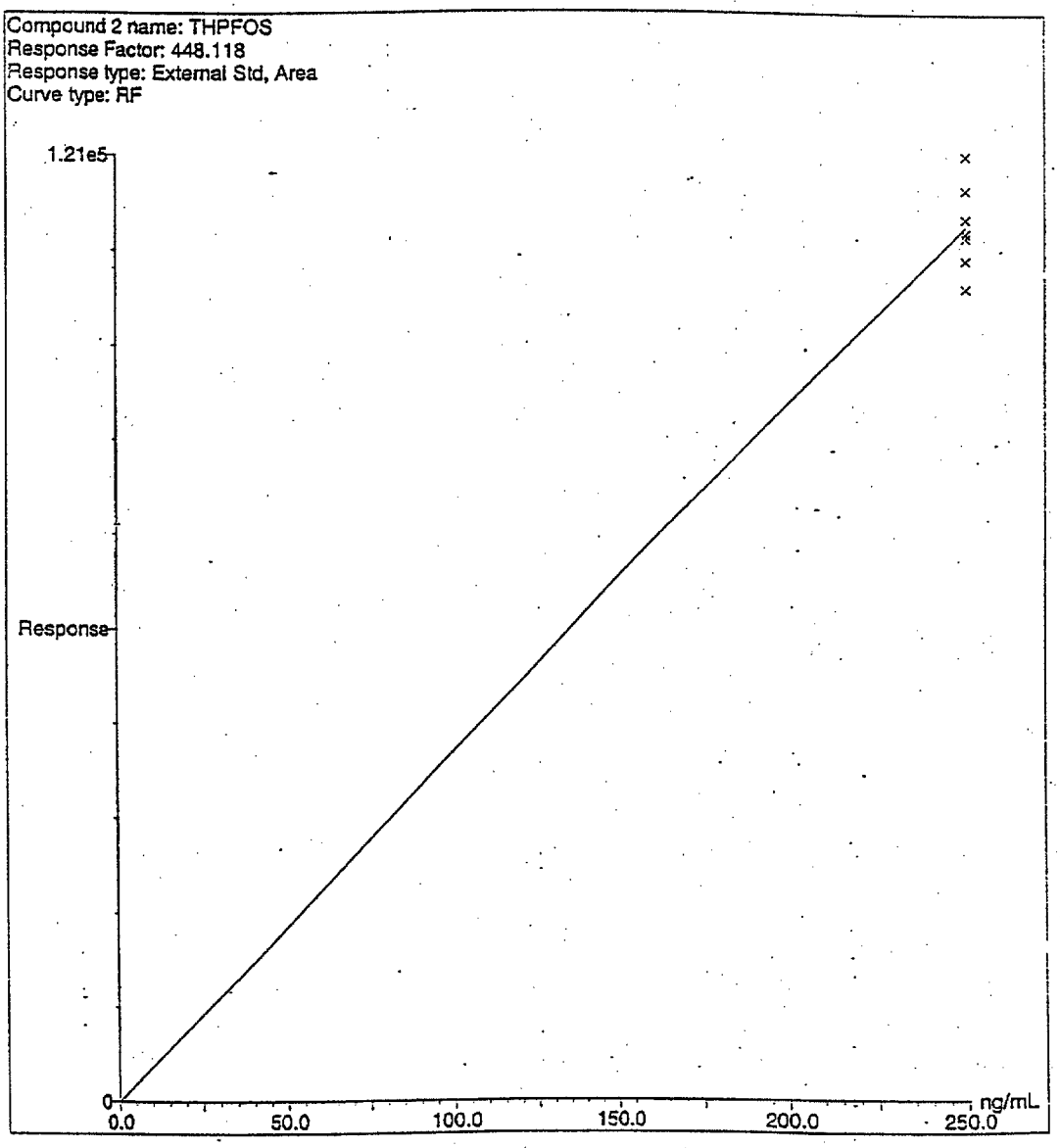

Figure 3. Chromatogram Representing a 5 ng/mL extracted standard for PFOS and 250 ng/mL fortification of THPFOS

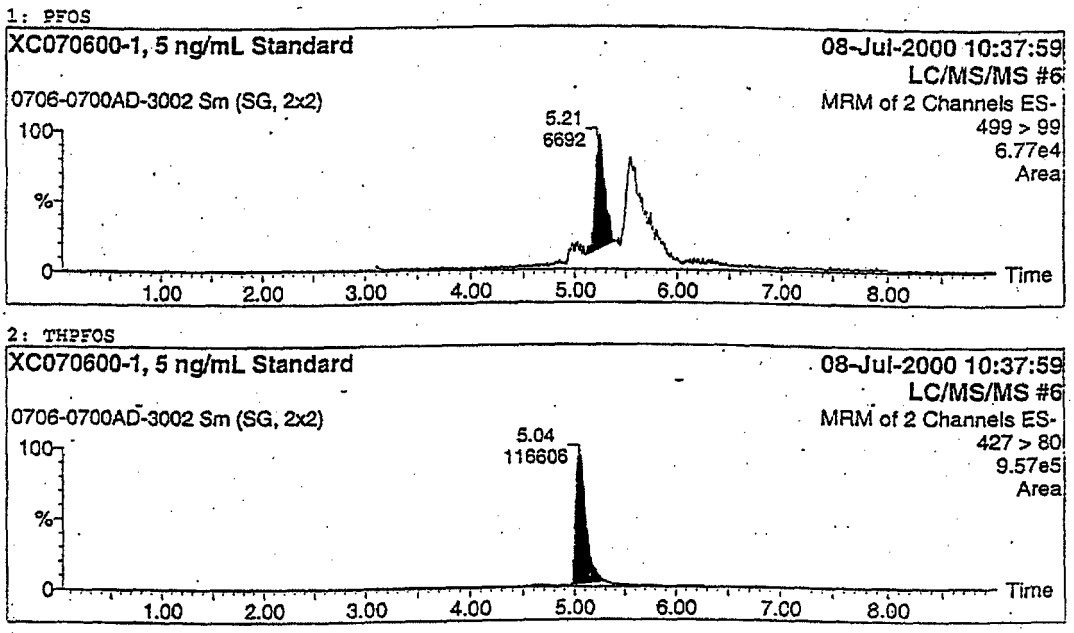

Figure 4. Chromatogram Representing a 125 ng/mL extracted standard for PFOS and 250 ng/mL fortification of THPFOS

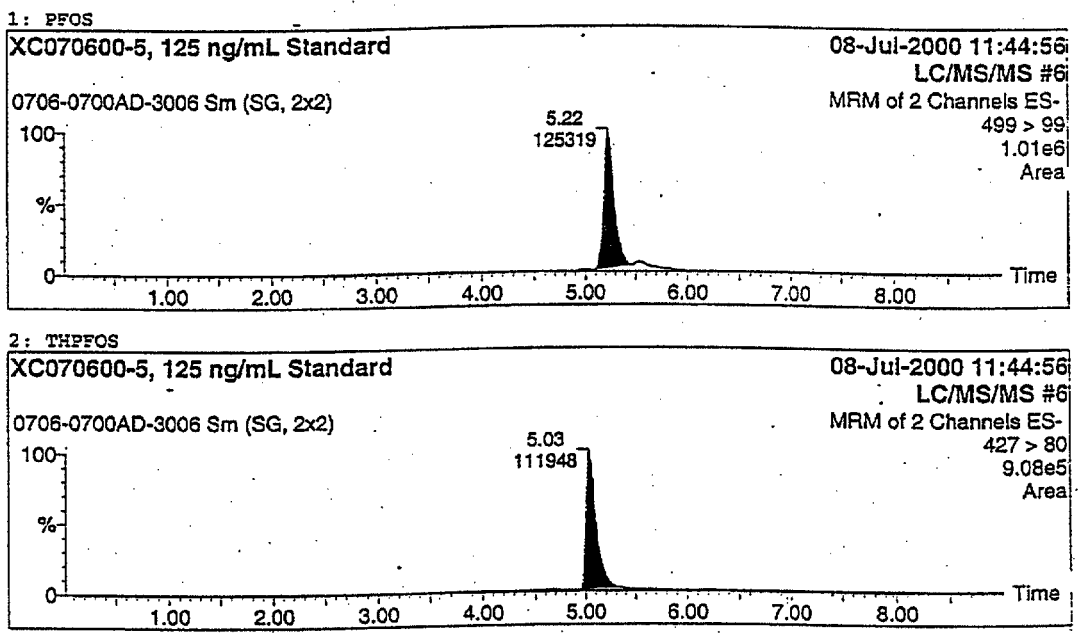

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Figure 6. Chromatogram Representing Control Rat Feces Fortified with 50 ng of PFOS and 500 ng of THPFOS (Centre ID: 0005803 Spk A, Set: 071000A)

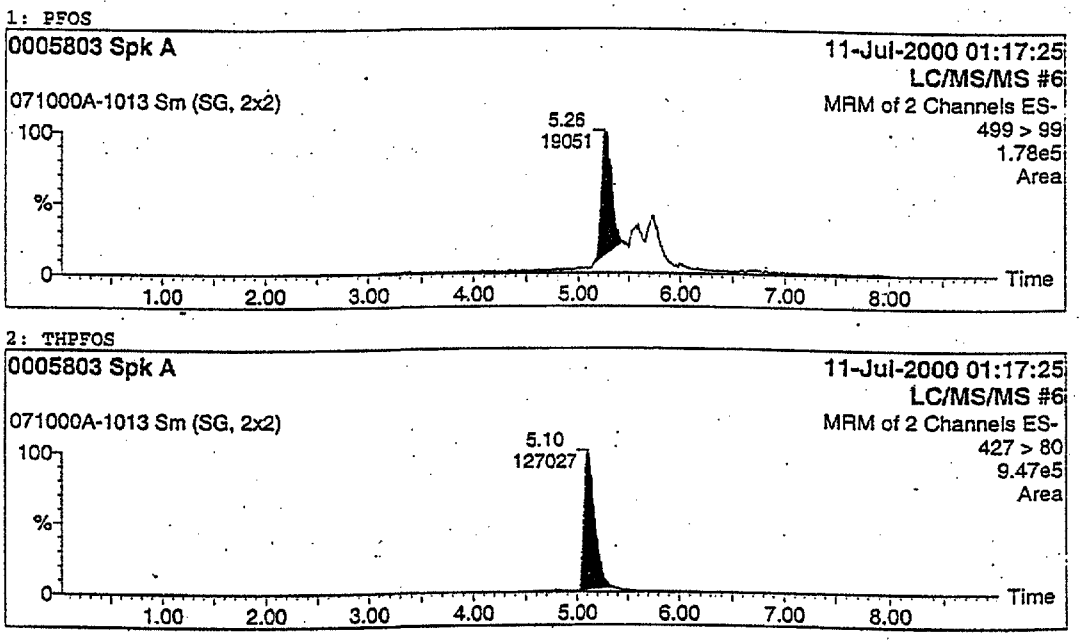
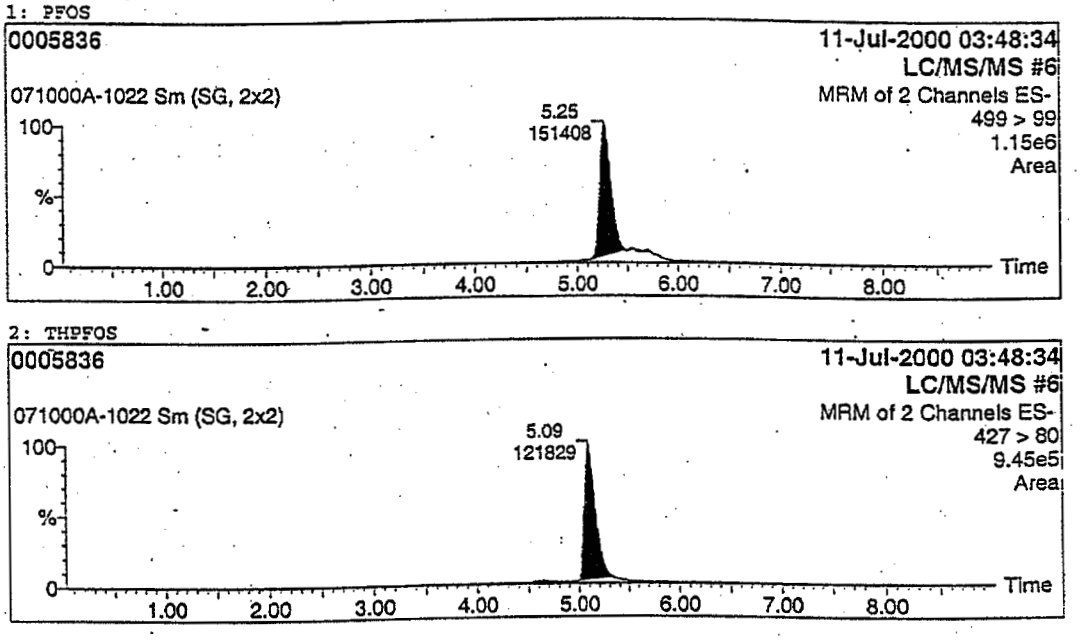


Figure 7. Chromatogram of Rat Feces Sample from G2 Day 6/7 Gestation (Centre ID: 0005836, Set: 071000A)



13.0 APPENDICES

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

APPENDIX A

Study Protocol
FACT-TOX-111
(Centre Study No. 023-017)
and
Amendments and Deviations

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

3M Environmental Technology and Services

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

Protocol #FACT-TOX-111

3M

Study Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

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

Laboratory Project Identification
FACT-TOX-111
U2994

Exact Copy of Original
LAC 04/21/00
Initial Date

Centre Study # 023-017
EW 5-11-00.

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Study Identification

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

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

Centre Study No.: 023-017
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Sub-Contract Laboratories	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850 Battelle Memorial Institute 505 King Avenue Columbus Ohio 43201-2693
Proposed Study Timetable	
Study Initiation Date	June 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic recovery study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-015. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. . If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

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

4. QUALITY ASSURANCE

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

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

5. *TEST MATERIAL*

   5.1 Refer to Argus Research Laboratory protocol for study #418-015.

6. *CONTROL MATRICES*

   6.1 ***Identification*** Rat liver and serum, and/or rabbit liver and serum traceability numbers will be recorded in the raw data and included in the final report

   6.2 ***Source*** Argus Research and/or Sigma Chemical

   6.3 ***Physical Description*** Rat liver and serum, and/or rabbit liver and serum

   6.4 ***Purity and Stability*** Not applicable

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

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

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

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

7. *REFERENCE MATERIAL*

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

   7.2 ***Source*** 3M Specialty Chemicals

   7.3 ***Physical Description*** White powder

   7.4 ***Purity and Stability*** Purity of PFOS is 99% or greater. Stability has not been determined.

   7.5 ***Storage Conditions*** Room temperature

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

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

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

8. TEST SYSTEM

Rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-015. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation until day 0 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	8	0	0	5
2	8	0.1	0.02	5
3	8	~~0.6~~	0.32	5

(F.C.) 1.6 kjh 9/22/99

9. SPECIMEN AND SAMPLE RECEIPT

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

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	Dam-Predose, Days 7, 14, 15, 21, and 22 Pup-Day 21	144 Dam 24 Pup (pooled)
Urine and Feces – Dam and Pup animals	Predose, Days 7, 15, 21, and 22	120 Urine and 120 Feces
Liver – Dam and Pup animals	Dam-At the termination of the study Pup-Day21	24 Dam 24 Pup (pooled)

Total number of expected specimens: 456
Total number of test animals: 16
Total number of control animals: 8

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

10. Preparatory Methods

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

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

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

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

11. Analytical Methods

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

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

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

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

12. Data Quality Objectives

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

12.1 ***Linearity*** $r^2 \geq 0.98$

12.2 ***Limits of detection / quantitation***

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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

12.3 ***Duplicate acceptable precision*** < 30% for the method

12.4 ***Spike acceptable recoveries*** 70% - 130%

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

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

13. Sub-Contracted Analysis

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

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

14. Statistical Analysis

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

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

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

15. Report

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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

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

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

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

16. Location of Raw Data, Records, and Final Report

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

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

- *16.1.1* Approved protocol and amendments
- *16.1.2* Study correspondence
- *16.1.3* Shipping records
- *16.1.4* Raw data
- *16.1.5* Approved final report (original signed copy)
- *16.1.6* Electronic copies of data

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

- *16.2.1* Training records
- *16.2.2* Calibration records
- *16.2.3* Instrument maintenance logs
- *16.2.4* Standard Operating Procedures, Equipment Procedures, and Methods

17. Specimen Retention

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

18. Protocol Amendments and Deviations

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

19. Attachments

19.1 Attachment A Preparatory and analytical methods

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

20. SIGNATURES

Marvin T. Case, D.V.M., Ph.D., Sponsor Representative — 9 June 1999 — Date

Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — 6/9/99 — Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:
August 12, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Exact Copy of Original
LAL 04/21/00
Initial Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 1

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

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

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

AMEND TO READ: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to:

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"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. *PROTOCOL READS:* Section 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

The extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat ~~Sera~~ Liver by LC/MS/MS, Version 1.0".

REASON: The analytical methods at the sub-contract laboratories were not included in the original protocol.

D RELAC 9/27/99
kh 9/27/99

3M Environmental Laboratory

Protocol FACT-TOX111
Amendment No. 1

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

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

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

Amendment Approval

Marvin T Case — 17 August 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kris J. Hansen — 8/18/99
Kris J. Hansen, Ph.D., Study Director — Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 30, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX111
LIRN U2994

Exact Copy of Original
LAC 04/20/00
Initial Date

3M Environmental Laboratory

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 2

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

1. ***PROTOCOL READS:***
Section 2 states the study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and sera in rats.

AMEND TO READ:
The study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver, sera, and urine in rats.

REASON:
The urine analytical methods were validated and approved after approval of the original protocol.

2. ***PROTOCOL READS:***
Section 10.0 and 11.0 list the following methods to use for extraction and analysis:
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"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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".

AMEND TO READ:
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"
ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Serum Extracts 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-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

REASON: The extraction and analytical methods ETS-8-96.0 and ETS-8-97.0, were approved after approval of the original protocol.

3M Environmental Laboratory

3. ***PROTOCOL READS:***
Section 6.1 lists control matrices as rat liver and serum or rabbit liver and serum. Source Argus and/or Sigma Chemical.

AMEND TO READ:
Control matrices identification rat liver and serum, rabbit liver and serum, human urine and/or rat urine. Source Argus, Sigma Chemical, Lampire Biologicals, Biological Specialty Corp. and/or Golden West Biologicals.

REASON:
Addition of control urine specifications.

4. ***PROTOCOL READS:***
Section 12.2.1 a) lists sera method detection limit as 1.75 ppb and b) lists liver method detection limit as 15 ppb.

AMEND TO READ:
The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

REASON:
The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

5. ***PROTOCOL READS:***
Section 16 states that 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, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that 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:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

6. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

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

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol FACT-TOX111
Amendment No. 2

Amendment Approval

Marvin Case, D.V.M., Ph.D., Sponsor Representative — 4 Oct 1999 Date

Kristen J. Hansen, Ph.D., Study Director — 5 Oct 1999 Date

3M Environmental Laboratory

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Potassium Perfluorooctanesulfonate (CAS Number 2759-39-3) in the Serum, Liver, and Urine of Crl:CD®BR VAF/Plus® Rats Exposed to PFOS Via Gavage

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

Performing Laboratories

Performing Laboratories

Urine Analyses
3M Environmental Technology and Safety Services
Fluorine Analytical Chemistry Team
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Liver Analyses
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

Serum Analyses
Advanced Bioanalytical Services, Inc.
15 Catherwood Road
Ithaca, NY 14850

Laboratory Project Identification

ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

Exact Copy of Original
UAC 04/26/00
Initial Date

3M Environmental Laboratory

Protocol LRN-U2994
Amendment Number 3

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

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

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

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol LRN-U2994
Amendment Number 3

Amendment Approval

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

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

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

3M Environmental Laboratory

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Study Title
Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:
20 April 2000

Performing Laboratories

URINE ANALYSES
3M Environmental Technology
and Safety Services
Fluorine Analytical Chemistry Team
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

FECES ANALYSES
Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801

LIVER ANALYSES
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

SERUM ANALYSES
Advanced Bioanalytical Services, Inc.
15 Catherwood Road
Ithaca, NY 14850

Laboratory Project Identification
ET&SS LRN-U2994
FACT TOX-111
Argus Study: 418-015
3M Medical Department Study: T-6295.14

Exact Copy of Original
LAC 04/26/00
Initial Date

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol LRN-U2994
Amendment Number 4

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

1. ***PROTOCOL READS:***
The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
AMEND TO READ:
This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
REASON:
The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:***
The amended section 6.0 lists rat or rabbit liver, serum, and urine.
AMEND TO READ:
Add: rat or rabbit feces with a physical description of rat or rabbit feces.
REASON:
Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. ***PROTOCOL READS:***
Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
AMEND TO READ:
Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
REASON:
Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. ***PROTOCOL READS:***
Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
AMEND TO READ:
The feces extraction and analytical method used by Centre Analytical Laboratories will be; 00M-023-003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
REASON:
The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Protocol LRN-U2994
Amendment Number 4

Amendment Approval

John L. Butenhoff April 19, 2000

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

Marvin T. Case 19 April 2000

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

3M Environmental Laboratory

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

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

Page 1 of 1

PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 7/12-13/00

Centre Study Number: 023-017 Centre Protocol Number: FACT-TOX-111

DESCRIPTION OF DEVIATION

1. § 12.4 Page 7: Accepted recovery of 136% for PFOS for 0005799 Spk A in Set 071200A.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 8/1/00

IMPACT ON THE STUDY

1. No negative impact on the study because 0005801 Spk B in Set 071200A was acceptable.

[signature] 8/16/00
Principal Investigator Signature Date

[signature] 7/1/2000
Sponsor Representative Signature Date

CAL QAU Review NCL 7/4/00

Marvin Rice, Med Director 23 Aug 2000 February 12, 1998/2

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

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

Page 1 of 1

PROTOCOL DEVIATION

Deviation Number: 2

Date of Occurrence: (1 & 2) entire study

Centre Study Number: 023-017 Centre Protocol Number: FACT-TOX-111

DESCRIPTION OF DEVIATION

1. § 12.3 Page 7: Did not perform duplicate extractions on any of the samples for this study.
2. § Amendment 2 Item #4: A method detection limit was not established for rat feces during the method validation.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1&2. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 8/4/10

IMPACT ON THE STUDY

1 & 2. No negative impact.

RATIONALE

1. Although none of the study samples were duplicated, at least one of the matrix fortifications (QC's) in each set was prepared to match one of the extracted calibration standards and a precision of < 30% was observed.
2. Any sample that contained residue with ~ 3:1 signal:noise was integrated and any number that was greater than 0 was reported. The limit of quantitation was established at 10 ppb during the method validation.

Principal Investigator Signature — Date 8/11/00

Sponsor Representative Signature — Date 9/1/2000

CAL QAU Review ncc 8/4/10

February 12, 1998/2

APPENDIX B

Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) (Method #00M-023-003, revision 2), Deviations and Modifications

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

TITLE

Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS
(Revision 2)

AUTHORS

Enaksha Wickremesinhe, Shaozhi Zheng, and John Flaherty

DATE ISSUED

June 02, 2000

SPONSOR

3M Environmental Technology and Safety Services
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

PERFORMING LABORATORY

Centre Analytical Laboratories, Inc. (Centre)
3048 Research Drive
State College, PA 16801

CENTRE STUDY NUMBER

023-003

CENTRE METHOD NUMBER

00M-023-003, revision 2

TOTAL NUMBER OF PAGES

20

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Centre Method No. : 00M-023-003, revision 2

MANAGEMENT APPROVAL

The work cited here was performed in conformance with applicable standard operating procedures and general GLP's.

6-02-00
Enaksha Wickremesinhe, Ph.D. Date
Principal Investigator
Centre Analytical Laboratories, Inc

6-02-00
Shaozhi Zheng Date
Principal Investigator
Centre Analytical Laboratories, Inc.

6/9/00
John Flaherty Date
Laboratory Manager
Centre Analytical Laboratories, Inc.

2-JUNE-00
2-JUNE-00
Richard A. Grazzini, Ph.D. Date
President
Centre Analytical Laboratories, Inc.

06/06/00
Kris Hansen, Ph.D. Date
Group Leader
3M Environmental Laboratory

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

Centre Method No. : 00M-023-003, revision 2

TABLE OF CONTENTS

1. SUMMARY

This document details a method of analysis for residues of perfluorooctane sulfonate (PFOS), perfluorooctane sulfonylamide (PFOSA), perfluorooctane sulfonylamidoethyl-acetate (PFOSAA), perfluorooctanesulfonylethylamide (PFOSEA), perfluorooctanoate (POAA), 2(*N*-ethylperfluorooctanesulfonamido)-ethyl alcohol (Et-FOSE-OH), M570, and M556 in monkey feces. The chemical formulas of the analytes are given in Section 2 of this method.

This method is being re-issued as method 00M-023-003 revision 2. It is being revised to add the rat feces as a matrix, to document the use of 20-mL vials instead of 15 mL tubes for sample extraction, to add the option of homogenizing feces samples before weighing, to clarify the preparation of QC recovery samples and to correct some typographical errors in method 00M-023-003 revision 1. Rat feces is being added to the method, however, it was validated for the analysis of PFOS only. Therefore, this method should be used for the analysis of PFOS only, in rat feces.

Residues of these fluorochemicals are extracted from feces with acetonitrile (ACN). The ACN extract is filtered and passed through a carbon solid-phase extraction (SPE) column. Approximately 3-4 drops of 1-octanol are added to the extract and evaporated to near dryness using rotary evaporation. Each extract is then reconstituted with methanol. Quantification of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA is accomplished by liquid chromatography/tandem mass spectrometry. (LC/MS/MS) analysis using selected reaction monitoring (SRM).

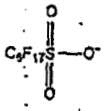
The proposed limit of quantitation (LOQ) for this method is 10 ppb each for PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA. This is based on studies conducted during the development of this method using control monkey feces.

2. FLUOROCHEMICAL STANDARDS

Molecular structures of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA are given below.

PFOS

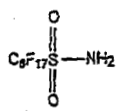
Molecular weight: 499 ($C_8F_{17}SO_3^-$)



Chemical Name = Perfluorooctane sulfonate

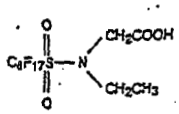
Note: The neutral molecule and standard form that the PFOS (anion) is derived from is potassium perfluorooctane sulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

PFOSA
Molecular weight: 499



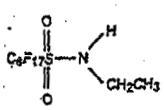
Chemical Name = Perfluorooctane sulfonylamide

PFOSAA
Molecular weight: 585



Chemical Name = perfluorooctane sulfonylamidoethylacetate

PFOSEA
Molecular weight: 527



Chemical Name = Perfluorooctanesulfonylethylamide

POAA
Molecular weight: 413

$C_7F_{15}COO^-$

Chemical Name = Perfluorooctanoate

Note: The neutral molecule and standard form that the POAA (anion) is derived from is ammonium perfluoroctanoate [$C_7F_{15}COONH_4$], molecular weight 431.

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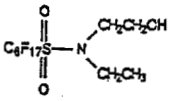
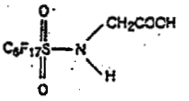
Et-FOSE-OH
Molecular weight: 571



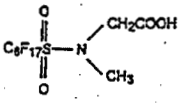
Chemical Name = 2(N-ethylperfluorooctanesulfonamido)-ethyl alcohol

M 556
Molecular weight: 557



Chemical Name = M556

M 570
Molecular weight: 571



Chemical Name = M570

THPFOS
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, $C_8F_{13}SO_3H$

Chemical Name = 4-H, perfluorooctane sulfonic acid

3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source	Catalog No.
Acetonitrile (ACN)	HPLC	(VWR) J. T. Baker	JT9017-3
Carbon 120/400 mesh	-	Supelco	57210-U
Methanol (MeOH)	HPLC	(VWR) J. T. Baker	JT9093-2
1-Octanol	Reagent	Aldrich Chemical	111-87-5
Ammonium Acetate	Reagent	Sigma-Aldrich	A-7330
L-Ascorbic acid	Reagent	Sigma-Aldrich	L-5960
Toluene	Reagent	(VWR) J. T. Baker	JT9460-3
Dimetyldichlorosilane	Reagent	Supelco	3-3009
Water	Type I	in house	-

(Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25°C, from a Labconco waterpro workstation)

3.2. FLUOROCHEMICAL STANDARDS

Standard	Source
PFOS	3M
PFOSA	3M
PFOSAA	3M
PFOSEA	3M
Et-FOSE-OH	3M
M556	3M
M570	3M
POAA	3M
THPFOS	3M

3.3. EQUIPMENT AND SUPPLIES

Equipment	Source
Balance, analytical, (display at least 0.0001g)	Mettler
Balance, top loading, (display at least 0.01 g)	Mettler
Rotary evaporator	Buchi
Rotary evaporator trap	Buchi
Pear-shaped flasks (125 mL)	Pyrex
20-mL SPE tubes (cat. no. N057177) with frits	Supelco
Vacuum pump	Buchi
Visiprep vacuum manifold	Supelco

Wrist-action shaker	Burrell
20-mL polyethylene scintillation vials with lids	Wheaton (VWR)
20-mL syringe	VWR
25-mm diameter glass fibre acrodisc (cat # 4523)	Gelman
Disposable pipets, test tubes etc.	VWR
Disposable micropipets, 100 – 200 µL.	VWR
125-mL LDPE narrow-mouth bottles	Nalgene
2-mL clear HPLC vial kit (cat # 5181-3400)	HP
Standard lab equipment (class A pipets and volumetric flask, graduated cylinders, etc.)	various
LC/MS/MS and HPLC systems	As described in Section 4.5.1

Notes:

1. In order to avoid contamination, the use of disposable containers/tubes/pipets etc. is highly recommended.
2. Teflon or teflon-lined containers or equipment, including teflon-lined HPLC vial caps should not be used.
3. Pear-shaped flasks are silanized before use.
4. It may be necessary to check the solvents (methanol) for the presence of contaminants (especially POAA) by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
5. Disposable micropipets or pipets should be used to aliquot standard solutions, when preparing standards and samples for extraction.
6. Equivalent materials may be substituted for those specified in this method. However, the use of carbon from Supelco is strongly recommended.

3.4. SOLUTIONS

1. 50 mM Ammonium Acetate: Dissolve 3.85 g of ammonium acetate in 1 L of type I water.
2. 2 mM Ammonium Acetate: Dilute 40 mL of the 100 mM ammonium acetate solution in a liter of type I water, for mobile phase A.
3. Saturated Ascorbic Acid in Methanol: Dissolve ~ 10 g ascorbic acid in 100 mL methanol.
4. The 2% Ascorbic Acid in Methanol: Dissolve 2 g ascorbic acid in 100 mL methanol.

Note: The volumes shown are provided for guidance; alternative volumes may be prepared.

3.5. Preparation of Stock, Fortification, and Calibration Solutions

Analytical standards are used for two purposes: (1) to fortify control matrix samples designated to be extracted and processed as calibration standards that will be then used to calibrate the response of the detector used in the analysis; and (2) to fortify other control matrix samples to determine analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained. The solutions cited below are given as an example; alternative concentrations may be prepared if needed.

3.5.1. Stock solutions

To prepare stock solutions of 100 µg/mL each PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, POAA, and the surrogate standard THPFOS, weigh out 10 mg of analytical standard (corrected for purity and percent salt, if necessary) and dilute to 100 mL with methanol in a 100-mL volumetric flask. Prepare a separate solution for each analyte. These stock solutions (in 125-mL LDPE bottles) are to be stored in a refrigerator at 2°C to 6°C and are stable for a maximum period of 6 months from the date of preparation.

3.5.2. Fortification Solutions

a. 10 µg/mL Mixed Fortification Solution – Pipet 10.0 mL each of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA stock solution to a 100 mL volumetric flask. Bring up to volume with methanol.

b. 2.5 µg/mL Mixed Fortification Solution – Pipet 25.0 mL of the 10 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

c. 0.5 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 2.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

d. 0.1 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 0.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

Follow Steps a and b above to make a 2.5 µg/mL solution of the surrogate standard (THPFOS) from the stock solution.

Store all fortification standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

3.5.3. Calibration Standards

Prepare LC/MS/MS calibration standards by fortifying weighed out aliquots of the same control feces sample (or a combined "bulk" control feces sample) before they are processed through the extraction procedure.

The following is a typical example; additional concentrations may be prepared as needed. It is recommended to prepare the fortification solutions so that the volume of fortification is between 50 and 200 µL and to use disposable micropipets for aliquoting fortification volumes.

Conc. of Mixed Fortification Solution (µg/ml)	Fortification Volume (µL)	Weight of Control Sample (g)	Conc. of Extracted Calibration Standard (ppb)	Vol. of Surrogate Standard* added (µL)
NA	NA	1.0	NA	200
0.1	100	1.0	10	200
0.1	200	1.0	20	200
0.5	100	1.0	50	200
0.5	200	1.0	100	200
2.5	100	1.0	250	200
2.5	200	1.0	500	200
10	100	1.0	1000	200

* 2.5 µg/ml THPFOS fortification solution.

Calibration standard preparations may be stored in a refrigerator at 2°C to 6°C, if needed, and used over a period of time as long as its stability has been verified.

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Weigh 1.0 g of sample
(fortify samples designated as calibration standards and QC recoveries)
↓
Extract with ACN and filter
↓
Carbon Solid-Phase Extraction
↓
Concentrate to almost dryness
↓
Adjust final volume (2.0 mL)
↓
LC/MS/MS

4.2. SAMPLE PROCESSING

All samples are received frozen and will be kept frozen (below -10°C) until time of extraction. The rat feces do not need any processing, however, the monkey feces may need to be homogenized in order to be able to weigh out the specified amounts.

4.3. SAMPLE PREPARATION

Prepare the following blank and calibration samples (Sections 4.3.1 to 4.3.4) from same control feces sample (or a combined "bulk" control feces sample).

4.3.1. Matrix Blanks

Prepare two blank samples without any fortifications, per set of samples analyzed. These will be used as the matrix blank.

4.3.2. Matrix Zero Blanks

Prepare two matrix blank samples fortified with the surrogate, per set of samples analyzed. These will be used as the matrix zero blanks.

4.3.3. Calibration Standards

Prepare calibration standards as described in Section 3.5.3.

4.3.4. Continuing Calibration Verification Standards

Two of the extracted calibration standards, a low-mid and a mid-high level standard, will be used as continuing calibration verification (CCV) standards.

4.3.5. QC Recovery Samples

Prepare QC recovery samples to represent every control matrix included in the study. Prepare at least one QC recovery sample in the low-range, and one in the high-range, to approximately bracket the expected range of analyte in the samples. For sets containing more than 20 samples, prepare an additional QC recovery sample for every group of 10 additional samples (for example, if one set has 24 samples, then 3 levels of QC recovery samples will be prepared). Prepare these additional QC recovery samples to bracket the range of analyte found in the samples, as appropriate.

Note: An example of a sample extraction worksheet is attached, as Attachment 1.

4.4. EXTRACTION

Note: A. Preparation/conditioning of SPE columns is described in Section 4.4.1.
B. Silanization of pear-shaped flasks is described in Section 4.4.2.
C. Evaluation/standardization of SPE columns (when a different supplier is used etc.) is described in Section 4.4.3.

a. Weigh approximately 1.0 g (± 0.05 g) of sample in to 20 mL polyethylene scintillation vial (Note: designated samples need to be fortified with appropriate aliquots of fortification standards).

b. Add 10 mL of ACN (note: break-up any clumps using a spatula or glass rod), shake on a wrist-action shaker for 30 minutes.

c. Let the vials sit for ~ 5 minutes, carefully decant, directly on to a 20 mL disposable syringe barrel connected to a glass acrodisc (Gelman) filter.

d. Transfer the filtrate to a conditioned SPE column (see Section 4.4.1). Collect the elute in a 125-mL "silanized"(see Section 4.4.2) pear-shaped flask.

Note: The 20 mL SPE column fits well inside the mouth (sleeve) of the pear-shaped flask. Make sure that the pear-shaped flask is well supported and will not topple. No vacuum is needed for this step.

e. Add 10 mL of ACN and then 20 ml of 90:10 ACN : 2% ascorbic acid in MeOH to the SPE column and collect the eluate in to the same pear shaped flask, combining the eluates.

f. Add 3-4 drop of 1-octanol to the pear shaped flask and rotary evaporate to near dryness (a minute amount of 1-octanol will remain) at a reduced pressure of ~ 40°C.

g. Reconstitute by adding 2.0 mL methanol (final volume = 2 mL) and swirl/mix to dissolve. Transfer the extract to HPLC vials using disposable pipets.

4.4.1 SPE Column Preparation

Pack 20-mL SPE tubes with 2 g carbon. Condition columns with 10 mL of saturated ascorbic acid in MeOH followed by 10 mL ACN. Discard all washes. Do not allow the column to dry.

Note: The SPE columns may be packed and conditioned at any point. Use the vacuum manifold to condition the columns. Do not let the SPE columns to run dry at any time.

4.4.2 Silanization of Pear-Shaped Flasks

Silanize the pear-shaped flasks before use, by rinsing with a 30% dimethyl-dichlorosilane in toluene solution followed by a toluene rinse and finally a methanol rinse.

4.4.3 Standardization of SPE Columns

When using carbon from a different supplier, SPE columns should be standardized in the following manner, prior to analyzing samples:

a. Using a mixed standard with a concentration between 100 and 500 ng/mL (each of all eight analytes) follow the elution schemes as outlined in steps 2 and 3. Collect all eluting fractions.

b. Collect a post-elution fraction, after step 3, eluting with an additional 20 mL of 90:10 (ACN : 2% ascorbic acid in MeOH).
c. Adjust the final volume of all the fractions to 10 mL with methanol.
d. Analyze all the fractions by LC/MS/MS.
e. If the target fraction contains a minimum of 85% of the respective analytes, it may be considered acceptable.
f. If the "post-elution" fraction contains significant amount of analytes, the target elution volume may be increased.

4.5. ANALYSIS BY LC/MS/MS

4.5.1. LC/MS/MS System and Operating Conditions (Electrospray)

Mass Spec: Micromass Quattro Ultima (Micromass)
Interface: Electrospray (Micromass)
Harvard infusion pump
Computer: COMPAQ Professional Workstation AP200
Software: Windows NT, MassLynx 3.3

HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

Note: A 4 x 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_4 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in Type I water
Mobile Phase (B): Methanol

Time	% A	% B	Flow Rate (mL/min)
0	60	40	0.300
0.4	60	40	0.300
1.0	10	90	0.300
7.0	10	90	0.300
7.5	0	100	0.300
9.0	0	100	0.400
9.5	60	40	0.400
13.5	60	40	0.400
14.0	60	40	0.300

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 mm x 30 mm) and also columns from different manufacturers (Keystone Betasil C_{18} etc.) can be used provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Parent Ion	Product Ion	Approximate Retention Time (min)
PFOS	negative	499	99	5.3
PFOSA	negative	498	78	6.0
PFOSAA	negative	584	526	5.7
PFOSEA	negative	526	169	6.7
Et-FOSE-OH	negative	630	59	6.7
POAA	negative	413	369	5.1
M556	negative	556	498	5.4
M570	negative	570	419	5.6
THPFOS	negative	427	80	5.1

Note that the retention times may vary slightly, on a day-to-day basis, depending on the batch of mobile phase, etc.

4.5.2. Example Tune File Parameters

The following values are provided as an example. Actual values may vary from instrument to instrument. Also these values may be changed from time to time in order to optimize for greatest sensitivity.

The mass spectrometer is tuned using a solution of each analyte at ~0.5 µg/mL, prepared via dilution of the stock solution in methanol. The solution is infused (using a "T" connector) at 10 µL/min into a 0.2 mL/min stream of mobile phase consisting of 40% methanol and 60% 2 mM ammonium acetate. The analytes are initially tuned for the parent ion and then tuned for the product ion. The optimized parameters are saved as a "tune file". This tune file is then used during routine analysis.

Analyte	Dwell (s)	Collision Energy (eV)	Cone (V)
PFOS	0.2	43	76
PFOSA	0.2	28	34
PFOSAA	0.2	20	37
PFOSEA	0.2	28	49
Et-FOSE-OH	0.2	31	30
POAA	0.2	11	25
M556	0.2	28	50
M570	0.2	20	60
THPFOS	0.2	35	34

Source	Set
Capillary	2.56 kV
Hexapole 1	0.5 V
Aperture 1	0.2 V
Hexapole 2	0.8 V
Source Block Temp.	100°C
Desolvation Temp.	400°C

Analyzer	Set
LM Res 1	13.0 V
HM Res 1	13.0 V
IEnergy 1	0.7 V
Entrance	-2 V
Exit	1 V
LM Res 2	11.0 V
HM Res 2	11.0 V
IEnergy 2	1.0 V
Multiplier	650 V

Gas Flows	Set
Cone Gas	~150 L/hr
Desolvation	~700 L/hr

Pressures	Read back
Gas Cell	~3.0e-3 mbar

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

4.5.3. Calibration Procedures

a. Inject an aliquot (10 µL) of each calibration standard into the LC/MS/MS system. Include standards corresponding to six or more concentration levels (ranging from the LOQ to the highest concentration level to be included in the analysis) in an analytical set.

b. Use a linear y = mx + b function for quantification. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using the Windows NT, MassLynx 3.3 (or equivalent) software system. Any extracted standards that fall outside the 70 to 130%, based on the average response factor of the surrogate standard, must be excluded from the calibration curve.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.98 (R^2≥0.96). If calibration results fall outside these limits, take appropriate steps to adjust instrument operation, and reanalyze the relevant sets of samples.

4.5.4. Sample Analysis

a. Each set of samples analyzed must include matrix blanks (without surrogate standard), martrix zero blanks (with the surrogate standard), a set of extracted standards, and QC recovery samples.

b. Inject an aliquot (10 µL) of each standard/sample/QC recovery/control into the LC/MS/MS system. Begin a set of samples with an entire set of calibrations. Include two continuing calibration verification (CCV) standards (one from the low-mid and one from the mid-high range) after every 5 to 10 samples, at the most.

c. Determine the concentration of each sample/QC recovery/control from the standard curve, based on the peak area of each analyte. The standard responses must bracket responses of the residue found in each sample set. Samples that fall outside the range of the standard curve must be diluted appropriately and re-analyzed.

d. Evaluate the ongoing acceptability of instrumental analysis based on the CCV results. Samples analyzed will be acceptable as long as both CCV's fall within ± 30% of their true value. Calculate the CCV concentration found using the linear calibration curve. If the CCV recovery is outside 70% to 130%, re-analyze samples analyzed after the last acceptable check.

e. Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The response is calculated using the "average response factor" function using MassLynx 3.3 (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The average response factor for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% to 140% (± 40%). Those deviating by greater than 30% (outside 70% to 130% range) must be checked for possible errors and may need to be re-extracted. Any responses deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.

f. If analysis is delayed, samples must be stored refrigerated at approximately 2°C to 6°C until analysis, and analyzed preferably within a week.

4.6. Performance Criteria

The following two criteria should be met before the initial analysis of samples, especially when using different instrumentation set-ups than those cited in this method.

First Criterion - Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (the 10 ppb extracted standard) and obtain a signal to noise ratio of at least 9:1 for all analytes. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion - Run a set of standards of six or more concentration levels, ranging from the proposed LOQ up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.98 for each analyte.

4.7. Time Required for Analysis

A set of 14 samples can be taken through the extraction procedure in approximately six hours by one person. The LC/MS/MS analysis (12-14 standards and 14 samples) will take approximately seven hours.

5. CALCULATIONS

5.1. Analyte Found

Calculate the amount of analyte found (in ppb) using the standard curve generated by the Mass Lynx software program using Equation 1. Correction for sample weight (1 g) and final volume (2 mL) is not required since the samples and standards are extracted the same way.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{(\text{peak area} - \text{intercept}) \times DF}{\text{slope}}$$

Where DF = dilution factor, if samples were diluted.

5.2. QC RECOVERY

For samples fortified with known amounts of analytes prior to extraction, calculate the percent recovery from Equation 2.

Equation 2:

% Recovery =

$$\frac{\text{analyte found (ppb)} - \text{analyte found in matrix blank (ppb)}}{\text{amount analyte added (ppb)}} \times 100$$

5.3. MEAN RESPONSE FACTOR

The mean response factor (MRF) is calculated by the Mass Lynx software program by averaging the mean response for all calibration standards (response/amount added) and dividing by the amount added. The actual concentration from each injection is then calculated by dividing the individual response by the MRF. The recovery for each injection is the percent of the actual concentration to the amount added. This calculation is performed by the MassLynx 3.3 (or equivalent) software.

6. SAFETY

All samples must be treated as potential biohazards and appropriate universal precautions must be taken (follow standard operating procedures for the handling of biofluids). These include, but are not limited to, the use of double layers of latex or vinyl gloves, goggles, dust mask, and lab coat. All disposable materials must be treated as biohazards and handled accordingly.

Acetonitrile, methanol, dimethyldichlorosilane and toluene are highly flammable. Open and use under fume hood only.

All organic waste and solvent waste must be segregated and disposed of accordingly.

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ATTACHMENT 1.

Centre Analytical Laboratories, Inc.

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3048 Research Drive, State College, PA 16801. Phone: (814)231-6022 Fax: (814)231-1253

PREPARATION OF SAMPLES FOR EXTRACTION AND ANALYSIS

Protocol No.: NA Centre Study No.: 023-003 Matrix: Feces

Method No.: NA Analytes: PFOS, PFOSA, POAA, Et-FOSE-OH, PFOSEA, PFOSAA, M 570, M 556, THPFOS

Sponsor Sample ID	Centre Sample ID	Sample Description	Sample Weight (g)	Surrogate Fort. Vol. (µL)	Fortification Standard ID	Fort. Conc. (µg/mL)	Fort. Vol. (µL)	Fort. Level (ppb)
		Matrix blank	1.0	-	-	-	-	-
		Matrix zero blank	1.0	200		-	-	-
		Std 1	1.0	200		0.1	100	10
		Std 2	1.0	200		0.1	200	20
		Std 3	1.0	200		0.5	100	50
		Std 4	1.0	200		0.5	200	100
		Std 5	1.0	200		2.5	100	250
		Std 6	1.0	200		2.5	200	500
		Std 7	1.0	200		10.0	100	1000
				Fortified with:_____ Capacity:_____µL				
				Initials/Date: ____/____				

If a sample is not fortified, draw a line through the fortification section. Vertical arrows in a column indicate identical values.

Samples removed from freezer # _____ Time:_____ Initials/Date: ____/____

Samples were weighed on Balance # _____ Time:_____ Initials/Date: ____/____

Samples returned to freezer # _____ Time:_____ Initials/Date: ____/____

* Surrogate Std ID: _____ Initials/Date: ____/____

Samples extracted with ACN and filtered: Initials/Date: ____/____

Carbon SPE clean-up and rotovap: Initials/Date: ____/____

Final volume adjusted to 2 mL with methanol: Initials/Date: ____/____

Extracts placed in refrigerator # _____ Initials/Date: ____/____

Reagent	Lot #
Acetonitrile	
Glass Aerosolve	
Carbon 120/400	
Ascorbic Acid	
Methanol	
1-Octanol	
$C_8H_{17}Cl_3Si$	
Toluene	

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METHOD DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 7/7/00

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.4.a : Only 0.78 g was weighed for Centre sample ID 0005822 because that was all of the sample available.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: [signature] R Stauffer 8/1/00

IMPACT ON THE STUDY

1. No negative impact

[signature] 8/16/00
Principal Investigator Signature Date

Centre QAU Review [signature] 8/4/00

February 12, 1998/2

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

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METHOD DEVIATION

Deviation Number: 2

Date of Occurrence: (1) 7/19/00

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.5.4.c : Quantitated Centre sample ID 0005890 residue outside the range of the standard curve in Set 0717-1800AD.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: [signature] R Stauffer 8/11/00

IMPACT ON THE STUDY

1. No negative impact because sample response only ~1.1% higher than response of highest standard.

[signature] 8/16/00
Principal Investigator Signature Date

Centre QAU Review [signature] 8/14/00

February 12, 1998/2

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

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Page 1 of 1

METHOD DEVIATION

Deviation Number: 3

Date of Occurrence: (1) 7/10-20/00

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.3.5: Did not fortify any of the control samples from intervals Day 6/7 Gestation, Day 14/15 Gestation, Day 20/21 Gestation, or Day 21/22 Lactation.

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: Emily R Stauffer 8/16/00

IMPACT ON THE STUDY

1. No negative impact.

Principal Investigator Signature 8/17/00 Date

Centre QAU Review MLL 8/17/00

February 12, 1998/2

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Page 1 of 1

METHOD MODIFICATION FORM

Modification No.: 1
Study Director: Marvin T. Case

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

MODIFICATION:

Section 3.4 Solutions

2. The 100 mM ammonium acetate solution should be 50 mM ammounium acetate.

JUSTIFICATION:

To correct for a typographical error.

EFFECT ON STUDY:

No negative impact.

Originator: [signature] 7/7/00
Date

Principal Investigator: [signature] 7/7/00
Date

Sponsor Management: [signature] 23 Aug 2000 [signature] 7/24/00
Date

February 12, 1998/3

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METHOD MODIFICATION FORM

Modification No.: 2
Study Director: Marvin T. Case

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

MODIFICATION:

1. Section 4.5.4. Sample Analysis (Item e.)
Should read as follows:
Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The concentration is calculated using the "average response factor" function from MassLynx 3.3 software (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The percent recovery for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% and 140%. Those deviating by greater than 30% must be checked for possible errors and may need re-extracted. Any recoveries deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.

2. Section 5.1 Analyte Found
Remove second sentence.

3. Section 5.1 Equations
Modify Equation 1 to:

$$\text{Analyte found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

Change Equation 2 to:

$$\text{Analyte found (ppb)} = \frac{\text{(analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF)}}{\text{sample wt. (g)}}$$

Add Equation 3:
Recovery (%) =

$$\frac{[\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF}]}{\text{analyte added (ng)}} \times 100$$

4. Section 5.3 Mean Response Factor
Modify first sentence to read:
The mean response factor (MRF) is calculated by the MassLynx software program by averaging the response for all calibration standards and dividing by the amount added.

Centre Study No.: 023-017
Sponsor Protocol No: FACT-TOX-111

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Page 2 of 2

METHOD MODIFICATION FORM

Modification No.: 2
Study Director: Marvin T. Case

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

JUSTIFICATION:

1. To clarify SS acceptable criteria.

2. Remove incorrect statement because calculations did include sample weight and final volume.

3. Modify calculations to correspond with those used in study.

4. Clarify calculation of mean response factor

EFFECT ON STUDY:

1-4. No negative impact.

Originator: [signature] 9/10/00 Date

Principal Investigator: [signature] 9/11/00 Date

Sponsor Management: [signature] 22 Sep 2000 Date

February 12, 1998/3

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12 Amended pages + report amendment

ANALYTICAL PHASE REPORT AMENDMENT RE-ISSUED

Amendment Number: 1

Effective Date: 9/20/00

Centre Study Number: 023-017 Sponsor Protocol Number: FACT-TOX-111

Report Title

Oral (Gavage) Pharmacokinetic Recovery Study of PFOS in Rats

Amended Section

Section 1.0 Summary: 13548.2 ppb was changed to 13.5 µg/g in the third paragraph (See attached page 11).

Section 7.6 Quantitation and Example Calculation:
Equation 5: Residue Found (µg/g) = Residue Found (ng/g) * (1 µg / 1000 ng)

Equation 6: Corrected Residue Found (µg/g) = Residue Found (µg/g) * 0.869

These equations were only applied to the tables in the report.

Section 8.0 Results: 13548.2 was changed to 13.5 µg/g in the second paragraph (See attached page 18).

Section 11.0: Tables I – XVI (See attached pages 20 – 28)

Reason for Amendment

The units reported in the tables and text were changed from ng/g to µg/g, the amount of significant figures represented in the tables and text was changed to three, the zeros reported in Table I were changed to ND's, and any value less than 0.0100 µg/g (LOQ) was reported as < 0.0100.

Impact on the Study

No negative impact

FOR ENAKSHA WICKREMESINHE — 10-DEC-00
Principal Investigator Signature* Date

Mann T Case — 15 Dec 2000
Study Director Signature Date

* Rick Grazzini signing for Enaksha Wickremesinhe who is no longer employed at Centre.

CAL QAU Review WEC 12/14/00

November 1995/0

Appendix H: Certificate of Analysis

Centre Analytical Laboratories, Inc.
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INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

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

Test Name	Specifications	Result
Purity[1]		**86.4%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.017 wt./wt.% 2. 0.007 wt./wt.% 3. 1.355 wt./wt.% 4. 6.552 wt./wt.% 5. 0.003 wt./wt.% 6. 0.004 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.00 wt./wt.%
Total % Impurity (LC/MS)		10.60 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.30 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.27 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.82 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.08 wt./wt.% 2. 0.794 wt./wt.% 3. 1.61 wt./wt.% 4. 10.1 wt./wt.% 5. 50.4 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

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

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

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

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

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

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

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

Test Name	Specifications	Result
Purity[1]		**86.9%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.93 wt./wt.%
Total % Impurity (LC/MS)		8.41 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		0.33 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		Not Applicable[3]
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

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

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

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

[4]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[5]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

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

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

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

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

Appendix I: Report Signature Page

Marvin T. Case, D.M.V., Ph.D., *Study Director* — 4 May 2001 Date

William K. Reagen, Ph.D., *Laboratory Manager* — 05/03/01 Date

John L. Butenhoff, Ph.D., *Sponsor Representative* — 5/4/01 Date

Kris J. Hansen, Ph.D., *Analytical Investigator* — 05/04/01 Date