Study Title

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

Analytical Laboratory Report Title

Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine, and Feces Samples

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 43201-2693

Serum Analyses

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

Project Identification

3M Medical Department Study: T-6295.12
Argus In-Life Study: #418-013
Analytical Report: FACT TOX-110
3M Laboratory Request No. U2849

Total Number of Pages

354

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine and Feces Samples

Study Identification Numbers:T-6295.12, FACT TOX-110, LRN-U2849

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

Exceptions to GLP compliance:

- Separate study directors were assigned to lead the *in vivo* and analytical portions of the study.
- Some sample receipt data from specimens received on 29 January 1999 are missing the corresponding receipt acknowledgement forms.
- The study files do not contain the raw data documenting sample receipt for the Gestation Day 0 urine samples.
- The original protocol called for study compliance with the FDA final rule 21 CFR Part 58; however, documentation from Advanced Bioanalytical Services, Inc. (the contract lab responsible for serum analyses) cites compliance with the EPA final rule 40 CFR Part 792.
- Dose confirmation analyses were not performed in compliance with GLP regulations.
- Sample Weights/Volumes worksheets for extraction, TOX-110, pages 2, 5, 9 and 11, on 9/13/99, are not completely attributable to the analyst performing the extraction.
- Some of the "Argus Acknowledgement of Tissue Specimen Receipt" forms are missing the date and "reviewed by" signature.
- Sample receipt documents for the 21Dec98 samples are not available.
- The LC/MS instrument run information for the 20Jan99 samples, received on 1/22/99, are not available.
- Not all reagent and solution bottles were labeled with an expiration date.
- The analytical report from Advanced Bioanalytical Services, Inc. does not include the names of all of the participating scientists and professionals.
- The stability of test and control articles under the conditions of administration were not determined.

(Signatures on next page)

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

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

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

GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Serum, Liver, Urine and Feces Samples

Study Identification Numbers: T-6295.12, FACT TOX-110, LRN-U2849

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

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
14 Sept 1999	Extraction	4 Oct 1999	4 Oct 1999
5 Oct 1999	Analytical Set-up	13 Oct 1999	13 Oct 1999
6 Oct 1999	MS Data Reduction	13 Oct 1999	13 Oct 1999
13 Oct 1999 to 16 Dec 1999	First Draft Report	17 Jan 2000	17 Jan 2000
6 Sept 2000 to 8 Sept 2000, 11 Sept 2000 to 12 Sept 2000, 18 Sept 2000, 20 Sept 2000 to 22 Sept 2000, 25 Sept 2000 to 26 Sept 2000	Second Draft Report	27 Sept 2000	27 Sept 2000

See contractor's analytical reports (Appendix G) for contract lab Quality Assurance statements.

Tonya K Rucker — 5/4/01

QAU Representative — Date

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List of Tables

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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 from Battelle Memorial Institute, Centre Analytical Laboratories and Advanced Bioanalytical Services, Inc. (ABS) will be stored at the contract laboratories in such a manner that the control substances will be preserved for use in ensuing studies.

Introduction and Purpose

The purpose of the study is to determine the presence and concentration of perfluorooctanesulfonate (PFOS) in serum, liver, urine and feces samples taken from the Gavage Study of T-6295.12 (Perfluorooctanesulfonate CAS Number: 2795-39-3) in Crl:CD®BR VAF/Plus® Rats. The study was initiated on 10 June 1999 and completed on 8 November 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., permanently identified using Monel® self-piercing ear tags. F0 virgin female rats were at least 60 days of age and weighed approximately 200–225 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.

Five groups consisting of 16 female rats each (total of 80 rats) were used as the test system. All female rats were treated with the control or test article once daily beginning 42 days prior to cohabitation, and continued through day 14 or day 20 of presumed gestation. Table 1 presents the number of female rats in each group. Group I consisted of control F0 female rats that were administered 0.0 mg/kg/day in 0.5% Tween 80 (vehicle). Group II through Group V F0 female rats were administered 0.1, 0.4, 1.6, or 3.2 mg of PFOS per kg of body weight/day in 0.5% Tween 80. At predetermined intervals during and at the end of the in-life phase of the study, sera, liver, urine and feces samples were taken from dams for analyses. Additionally, liver and serum samples were taken from the fetuses. Details of the in-life phase of the present study are presented in the Argus Laboratory final report, "Oral (Gavage) Pharmacokinetic Study of PFOS in Rats, #418-013."

Table 1. Female Rat Population Demographics for Study #418-013

Population	Total	Selected for Study
Initial Quantity Acclimated (Total)	120	80
Control Group I 0 mg/kg/day	16	16
Treatment Group (total)	64	64
Group II 0.1 mg/kg/day	16	16
Group III 0.4 mg/kg/day	16	16
Group IV 1.6 mg/kg/day	16	16
Group V 3.2 mg/kg/day	16	16

Specimen Collection

In the analytical study reported here, 822 serum, urine, liver and feces specimens were collected from adult female rats before mating and at gestation day (GD) 7, GD 15 and GD 21 and sent to the 3M Environmental Laboratory and the contract labs to be analyzed for PFOS. A total of 54 pooled serum and liver specimens were collected from fetuses. Specimens of pooled embryo/placenta, pooled liver/lung, pooled liver/placenta/liver-lungs, amniotic fluid, and carcasses from fetuses, as well as milk secreting glands and placentas from adult females, were collected by and received from Argus In-life Study #418-013, but were not a part of the scope of this study as determined by the study director. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

Specimens Collected from Study Groups 1 through 5 (through 1/22/99):
Serum Specimens—277 specimens
Liver Specimens—100 specimens
Urine Specimens—249 specimens
Feces Specimens—250 specimens

Blood specimens were separated by refrigerated centrifugation. Serum specimens were then harvested, immediately frozen on dry ice and maintained frozen at -70°C or below until being shipped to the 3M Environmental Laboratory. Urine and feces samples were collected into centrifuge tubes, placed on dry ice and stored frozen at -70°C or below until being shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were collected, frozen and then stored at -70°C or below on dry ice until being shipped to the 3M Environmental Laboratory. All specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice. Specimens were then shipped to the contract analytical laboratories for analysis.

Urine samples were extracted beginning on 13 September 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.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received serum, liver, urine and feces specimens that were collected during premating, GD7, GD15 and GD21 of the in-life phase of this study #418-013 from Argus. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C. Specimens that were analyzed at Battelle 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-6295.12 via Gavage

Tissue/Sample	Generation	Number of Samples Received	Date Received	Date Shipped to Lab
Liver	Dam	73	3 February 1999	19 August 1999
Serum	Dam	250	2 February 1999	15 June 1999
Urine	Dam	249	29 January 1999 30 January 1999 2 February 1999	—[a]
Feces	Dam	250	29 January 1999 2 February 1999	25 May 2000
Liver	Fetal (pooled)	27	3 February 1999	19 August 1999
Serum	Fetal (pooled)	27	2 February 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 and are presented in Appendix A (see Table 6).

Chemical Characterization

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

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

Chemical characterization information on potassium perfluorooctanesulfonate, CAS #2795-39-3, is presented in Table 3.

Table 3. Characterization of Test Article

	Test Article[a]
Chemical Name	**KPFOS** Potassium Perfluorooctanesulfonate
Source	3M Toxicology Services, Medical Department
Expiration Date	8/31/01
Storage Conditions	Frozen ≤ -10°C
Chemical Lot #	217
Physical Description	White crystalline powder
Purity	86.9%

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

See Interim Certificate of Analysis in Appendix H for complete characterization information.

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 mg/mL to 0.64 mg/mL) collected on 15 November 1998 during the in-life phase of the study. The results are presented in Appendix A (see Table 8). The dose confirmation data were collected according to a method that was not fully validated.

Dose confirmation was performed by diluting the dose samples with methanol into the linear range of the instrument. In all cases, samples were analyzed versus an unextracted 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 labs. The methods used in this study are located in Appendix 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 lab 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 HPLS-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-cc 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, selected as the primary ion for PFOS ($C_8F_{17}SO_3$-) analysis, was fragmented further to produce ion 99 (FSO_3-). The characteristic product ion 99 was monitored for quantitative analysis.

- **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 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*
0.0	40%B
1.0	40%B
4.5	95%B
6.5	95%B
7.0	40%B

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.1
Cone Voltage: 50–70 V
Collision Gas Energy: 30–50 eV
Mode: Electrospray Negative
Source Block Temperature: 125°C–150°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 4. Negative Ions Monitored in 3M Laboratory Analyses

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

Deviations

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 quantitation 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) were indicated in the protocol for this study:

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.98
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **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-110 (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, Analytical Methods).

- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve defined as a standard within ±30% of the theoretical value. (See Appendix D, Table 12.)
- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest.
- **Precision:** 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 54–109% of expected values were achieved for 12 of 14 matrix spikes prepared in urine. The remaining two spikes were recovered at 46% and 39%. Low recoveries were confirmed, but were not re-extracted. Associated results, which may be underestimated by more than 50%, are specifically identified in the report tables as not meeting the data quality objectives of this study.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards.

  External calibration was used in quantitation of data in this study due to the presence of an unidentified compound that interfered with the analysis of the surrogate.

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 contract lab reports in Appendix G for summary of quality control analysis results from contract labs.

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 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 purity factor, see Appendix E.

Centre Analytical Laboratories—

- Refer to the Centre Analytical Report for information regarding feces analysis (Appendix G).

- **Samples from Control Animals:** Low levels of PFOS were often detected in the sera, urine, feces and liver of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, PFOS levels found in the sera, urine, feces and liver of the test animals increased with each dose group within a particular time point. 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-110.

Statement of Conclusion

Under the conditions of the present studies, PFOS was observed in the livers, urine, feces and sera of all female rats dosed with the T-6295.12 during the in-life phase of the study. Additionally, PFOS was observed in fetal liver and serum taken during gestation from the same group of female rats.

Appendix A: Control Matrices and Dose Confirmation Analyses

Table 5. Description of the Control Matrices Used for Liver, Urine, Sera and Feces Analyses in Study FACT TOX-110

Location of Analyses	3M Environmental Laboratory	Battelle Memorial Institute	ABS	Centre Analytical
Control Matrix	**Human urine**	**Rat liver**	**Rat serum**	**Rat feces**
Source	Lampire Biological	Harlan Bioproducts for Science, Inc.	Harlan Bioproducts for Science, Inc.	Lampire Biological
Expiration Date	3 months	210 days	N/R	N/R
Storage Conditions	Frozen at -20°C±10°C	Frozen at ~ -20°C	Polypropylene vials frozen at -20°C	Frozen at <-10°C
Lot #	66-11066	Harlan Product #409	R80422 R80128	N/R
Physical Description	Human urine; no identity analyses performed	Rat liver; no identity analyses performed	Rat serum; no identity analyses performed	Rat feces; no identity analyses performed

N/R—not recorded

Table 6. Characterization of the Analytical Reference Substances used for Liver, Urine, Sera and Feces Analyses in Study FACT TOX-110

	3M Environmental Laboratory	Battelle Memorial Institute	Advanced Bioanalytical Services	Centre Analytical Laboratory
Chemical Name	**KPFOS** Potassium Perfluorooctanesulfonate*	**KPFOS** Potassium Perfluorooctanesulfonate*	**KPFOS** Potassium Perfluorooctanesulfonate*	**KPFOS** Potassium Perfluorooctanesulfonate*
Source	3M ICP/PCP Division	3M ICP/PCP Division	3M ICP/PCP Division	3M Toxicology Services, Medical Department
Expiration Date	8/31/01	8/31/01	8/31/01	8/31/01
Storage Conditions	Frozen ≤ -10°C	Frozen ≤ -10°C	Frozen ≤ -10°C	Frozen ≤ -10°C
Chemical Lot #	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%

*The reference standard used for analysis is KPFOS, but the target analyte is PFOS.

Table 7. Dose Confirmation Analyses for Perfluorooctanesulfonate for Test Samples From Study In-life #418-013—15 November 1998

Group Dose	Target Conc. PFOS (ng/mL)	Expected Conc. PFOS (ng/mL)[a]	Measured PFOS Conc. (ng/mL)[b]	%of Expected
Group 1 Control 0.0 mg/kg/day Diluted 1/1	0.00	0.00	0.00	NA
Group 2 0.1 mg/kg/day 0.02 mg/mL Diluted 1/40	20000	16100	14000	87%
Group 3 0.4 mg/kg/day 0.08 mg/mL Diluted 1/1000	80000	64500	61100	95%
Group 4 1.6 mg/kg/day 0.32 mg/mL Diluted 1/1000	320000	258000	246000	96%
Group 5 3.2 mg/kg/day 0.64 mg/mL Diluted 1/1000	640000	516000	417000	81%

NA=not applicable
[a]Calculations used to obtain *Expected Concentration PFOS* are given below
[b]*Measured PFOS Concentration* values are corrected using the PFOS correction factor and for purity

Formula used to calculate expected concentration from target concentration:

Target Concentration x PFOS Purity x PFOS Correction Factor = Expected Concentration PFOS

PFOS Purity = 86.9%
PFOS Correction Factor = 0.9275

640000 ng/mL x 0.869 x 0.9275 = 516000 ng/mL Expected Concentration

Appendix B: Protocol, Protocol Amendments and Deviation Summary

Table 8. Deviation Summary for FACT TOX-110

Deviation	Date(s) of Occurrence	Impact on Study
The original protocol states that the source of the control matrix used in the present study would be either Sigma Chemicals or Argus Research Laboratories. The physical description of the control substance was rat and/or rabbit liver and/or serum. Additional control substances used in this study were human urine and rat feces obtained from Lampire Biological Laboratories; Piperville, PA 18947.	Entire study	Successful validation studies using human urine and rat feces were performed; therefore, there was no adverse impact on the quality of the data.
ETS-8-96.0 states 2.0 mL will be used as the initial volume. Samples 19516F, F0, G1, control-GD0 premating had only 1.0 mL available.	9/14/99	The LOQ was maintained for all samples; therefore, there was no adverse impact on the outcome of the study. Data quality will not be affected.
Samples 19572F, F0, G5-3.2 MKD-G7, 24 hour had no sample available. Samples 19543F, F0, G3-0.4 MKD-GD21, 24 hour did not have enough sample for extracting. Initial volumes were recorded in the Excel spreadsheet for calculating final concentrations.	9/14/99	No data were generated.
ETS-8-96.0 specifies to add 0.5 mL methanol final volume. For the following samples, 1.0 mL of methanol final volume was added. 19540F, F0, G3-0.4MKD-GD15-Terminal 19542F, F0, G3-0.4MKD-GD15-Terminal 19543F, F0, G3-0.4MKD-GD15-Predose 19544F, F0, G3-0.4MKD-GD15-Terminal 19546F, F0, G3-0.4MKD-GD15-Predose	9/15/99	The LOQ was maintained for all samples; therefore, there was no adverse impact on the outcome of the study.
ETS-8-97.0, section 10.1.1 states that the methanol (water and extraction) blanks will be analyzed with each analytical batch. The water and extraction blanks were not analyzed during the following runs: 092099A and 092199a.	9/20/99, 9/21/99	All blanks were analyzed at least once during the study. Methanol blanks are often analyzed more frequently to monitor possible contamination, but since the study data here were well above any analyte levels in the blanks, there was no adverse impact on the quality of the data.
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.	9/21/99	The analysis of Tween samples was not conducted under GLPs (as per discussions with study director). This deficiency is included in the final report.
ETS-8-97.0 specifies the method of analysis requires the use of an internal calibration. The actual analyses used an external calibration method.	9/20/99, 9/21/99, 9/24/99, 9/27/99, 9/30/99, 10/28/99, 11/5/99	Unknown contaminant interfered with use of surrogate for internal calibration. Corrective action is an improvement. Data quality is satisfactory to ±50% accuracy.
The control of bias document requires the calibration curve to have percent deviations ≤30%. The actual percent deviation for the low calibration point is greater than 30%.	9/27/99	The data points are at the mid-range portion of the curve, so deviation at the low end curve points will not adversely affect the data.

Table 8. Deviation Summary for FACT TOX-110

Deviation	Date(s) of Occurrence	Impact on Study
Protocol FACT-TOX-110 and the method ETS-8-97.0 state the matrix spike/matrix spike duplicate analyses will be ±30% of the spiked concentration. The actual acceptable result for the matrix spike/matrix spike duplicate analyses will be ±50% of the spiked concentration.	11/4/99	Data quality will be expressed as having as accuracy of ±50% rather than ±30%.

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Protocol #FACT-TOX-110

3M

Study Title

Oral (Gavage) Pharmacokinetic 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-110
U2849

Study Identification

Oral (Gavage) Pharmacokinetic 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-09 651-778-5018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

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

1. STUDY

Oral (gavage) pharmacokinetic 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 #418-013. 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 #418-013.

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 the Argus Research protocol #418-013. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation and continue through day 14 or 20 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	16	0	0	5
2	16	0.1	0.02	5
3	16	0.4	0.08	5
4	16	1.6	0.32	5
5	16	3.2	0.64	5

9. SPECIMEN AND SAMPLE RECEIPT

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

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Fetus animals	Dam-Predose, Days 7, 15, and 21 Fetus-At termination of the study	320 Dam and 320 Fetus (pooled)
Urine and feces – Dam animals	Predose, Days 7, 15, and 21	320 urine and 320 feces
Liver – Dam and Fetus animals	Dam and Fetus-At termination of the study	80 Dam and 80 Fetus (pooled)
Milk Secreting Glands – Dam animals	At the termination of the study	80
Amniotic Fluid – Dam animals	Day 15 and Day 21	80

Table 2 Cont. Specimen Information		
Body tissue/fluid	**Collected**	**Expected # of specimens**
Embryo's with Placentae – Dam animals	Day 15 and Day 21	80
Carcass – Fetus animal	At the termination of the study	160
Placentae – Fetus animal	Day 15 and Day 21	80
Liver/lung – Fetus animal	At the termination of the study	80

Total number of expected specimens: 2000
Total number of test animals: 64
Total number of control animals: 16

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

10. PREPARATORY METHODS

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

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

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

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

11. ANALYTICAL METHODS

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

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

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

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

12. Data Quality Objectives

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

12.1 Linearity $r^2 \geq 0.98$

12.2 Limits of detection / quantitation

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

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

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

13. Sub-Contracted Analysis

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

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

14. Statistical Analysis

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

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

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

15. REPORT

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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

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

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

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

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

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

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

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

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

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

17. SPECIMEN RETENTION

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

18. PROTOCOL AMENDMENTS AND DEVIATIONS

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

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T Case — 9 June 1999

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

Kristen Hz — 6/16/99

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

Study Title
Oral (Gavage) Pharmacokinetic 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-TOX110
LIRN U2849

Protocol FACT-TOX110
Amendment No. 1

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

1. ***PROTOCOL READS:*** Section 2.0 states this study is designed to determine potassium perfluorooctane sulfonic acid (PFOS) in specimens of liver and serum of rats.

AMEND TO READ: This study is designed to determine PFOS in specimens of rat liver, serum, and urine. All Day -1 and Gestation Day 21 rat urine specimens will be extracted and analyzed by the 3M Environmental Laboratory.

REASON: The urine extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:*** Section 6.0 lists rat or rabbit liver and serum.

AMEND TO READ: Rat or rabbit urine from 3M Toxicology with a physical description of rat or rabbit urine.

REASON: The rat urine matrix was added after the protocol was approved.

3. ***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 to follow at the 3M Environmental Laboratory are:

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-8-7.0 "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"

3M Environmental Laboratory

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.
Methods ETS-8-96.0 and ETS-8-97.0 were not validated and approved until after the protocol was approved.

4. ***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~~ 1.0"

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

5. ***PROTOCOL READS:*** Section 12.2.1 b) Liver method detection limit is 15 ppb.

AMEND TO READ: 12.2.1 b) Liver method detection limit is 8.50 ppb (ng/g).
12.2.1 c) Urine method detection limit is 1.5 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 in liver. A validation in urine was performed, after protocol approval, to support this method detection limit.

DREuc 9/27/99

kjh 9/27/99

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

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

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 28, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX110
LIRN U2849

Protocol FACT-TOX110
Amendment No. 2

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

1. ***PROTOCOL READS:***
Section 2 states that all liver samples will be extracted and analyzed at Battelle Memorial Institute.

AMEND TO READ:
All dam liver specimens (female mother) and 27 fetus (pooled) liver specimens will be analyzed at Battelle Memorial Institute.

REASON:
The fetal liver/lung is not a target matrix and will not be extracted or analyzed.

2. ***PROTOCOL READS:***
Section 9 states that 80 dam liver specimens (female mother) and 80 fetus (pooled) liver specimens will be sent to the Environmental Laboratory.

AMEND TO READ:
Seventy-three dam liver specimens (female mother), 27 fetus (pooled) liver specimens, and 81 fetal liver/lung specimens (27 liver/lung specimens sampled in triplicate) were sent to the Environmental Laboratory.

REASON:
The number of animals changed during the course of the study.

3. ***PROTOCOL READS:***
Section 12.2.1 a) serum method detection limit is 1.75 ppb b) Liver method detection limit is 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.

4. ***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.

5. ***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 documentation when shipping specimens for analyses performed by a sub-contract laboratory.

Protocol FACT-TOX110
Amendment No. 2

Amendment Approval

Marvin T Case — 4 Oct 1999

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

Kristen Hansen — 5 Oct 1999

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

3M Environmental Laboratory

Study Title

Analytical Laboratory Report on the Determination of Perfluorooctanesulfonate (PFOS) Presence and Concentration in Serum, Liver, and Urine from the Gavage Study of T-6295.12

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

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-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

3M Environmental Laboratory

Protocol LRN-U2849
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.

Protocol LRN-U2849
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

Study Title

Oral (Gavage) Pharmacokinetic 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-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

Protocol LRN-U2849
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 Case 19 April 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

REPORT AMENDMENT NO. 5

Amendment Date:
26 July 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-110
ET&SS LRN-U2849

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

1. ***PROTOCOL READS:***
The amended analytical protocol (Amendment No. 1, section 4.0) states the extraction and analytical method to follow at Battelle Memorial Institute is "Method for Analysis of Perfluorooctance 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 listed in Amendment No. 1 had 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.

2. ***PROTOCOL READS:***
In the Argus in-life protocol #418-013, the testing facility is stated on page 1 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.

3. ***PROTOCOL READS:***
On the cover page, the type of document for FACT-TOX-110 is listed as a "Protocol." On page 3, under Proposed Study Timetable, the "Study Initiation Date" and "Study Completion Date" are listed.

AMEND TO READ:
Change the document type for FACT-TOX-110 to "Analytical Phase Protocol."
Change "Study Initiation Date" to "Phase Initiation Date." Change "Study Completion Date" to "Phase Completion Date."

REASON:
FACT-TOX-110 is the protocol for the analytical phase, while the Argus protocol #418-013

is the protocol for the in-life phase.

4. *PROTOCOL READS:*
The amended analytical protocol (Amendment No. 3, section 1) 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. protocol #418-013 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. This change is retroactive to February 10, 2000.

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

5. *PROTOCOL READS:*
The amended protocol (Amendment No. 4) states that rat feces samples will be analyzed for PFOS, as well as rat liver, urine 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.

Amendment Approval

John L Butenhoff by mrcase 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

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

PROTOCOL AMENDMENT NO. 6

Amendment Date:
October 3, 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-110
3M Laboratory Request No. U2849

3M Environmental Laboratory

Protocol FACT TOX-110
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

Protocol FACT TOX-110
Amendment #6

Amendment Approval

John L. Butenhoff — October 9, 2000

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

Marvin T Case — 9 October 2000

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

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

ETS-8-96.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLS-Electrospray/Mass Spectrometry/Mass Spectrometry," (14 pages)

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

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:

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

Final Concentration (µg/ml) of PFOS in matrix

14.0 Method Performance

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

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					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

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): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Blank fluid/identifier:
Method/revision: **Box Number:**
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:

D J B — 7/28/99
Laboratory Manager — Date

Kitter H — 7/28/99
Group Leader — Date

Lisa A. Clemen — 7/27/99
Technical Reviewer — 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

Appendix D: Data Summary Tables

Table 10. Average Results for the Analyses of Serum 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-6295.12 via Gavage

	0 mg/kg/day	0.1 mg/kg/day	0.4 mg/kg/day	1.6 mg/kg/day	3.2 mg/kg/day
DAY 0	0.0723	8.89	40.7	160	318
DAY 7	0.126	7.82	40.9	154	105
DAY 15	0.0926	8.80	41.4	156	275
DAY 21	0.0714	4.24	26.2	136	155
DAY 21 FETAL	0.125	9.07	34.3	101	165

NOTE: Serum concentrations are expressed as µg/mL and 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 (0.043 µg/mL). Individual animal data are located in Attachment E, Data Spreadsheets.

NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 11. 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-6295.12 via Gavage

Dose Group Units	PFOS Concentration (µg/g) Female Adult G0	PFOS Concentration (µg/g) Fetal Liver
0	0.288	0.169
0.1	29.2	7.93
0.4	107	30.6
1.6	347[a]	86.7
3.2	610	230

[a]Result from animal 19559 is an outlier and was not included in the average.

NOTE: Liver concentrations are expressed as the average of samples within a dose group from all animals tested, not including samples that tested at below the limit of quantitation (LOQ=0.112 µg/g). Individual animal data are located in Attachment E, Data Spreadsheets.

NOTE: Liver analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 12. Average Results for the Analysis 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-6295.12 via Gavage

	0 mg/kg/day	0.1 mg/kg/day	0.4 mg/kg/day	1.6 mg/kg/day	3.2 mg/kg/day
GESTATION DAY 0	<LOQ[a]	0.0497	0.302	0.959	1.53
GESTATION DAY 7	<LOQ—1 anomaly	0.0620	0.308	1.10	1.60
GESTATION DAY 15	0.00905	0.0685	0.526	0.622	0.563
GESTATION DAY 21	0.0194	0.0574	0.555	2.71	1.61

[a]LOQ=Limit of quantitation (≤ 0.004 µg/mL)

NOTE: Urine concentrations are expressed as µg/mL and are the average of samples from all animals tested, not including samples that tested at or below the limit of quantitation (0.004 µg/mL). Individual animal data are located in the study binder archived for this report and also in Appendix E, Data Spreadsheets.

Table 13. Average Results for the Analysis 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-6295.12 via Gavage

	0 mg/kg/day	0.1 mg/kg/day	0.4 mg/kg/day	1.6 mg/kg/day	3.2 mg/kg/day
GESTATION DAY 0	0.0380	0.499	2.42	10.3	23.9
GESTATION DAY 7	0.0155	0.490	2.16	9.19	33.0
GESTATION DAY 15	0.0322	0.662	2.93	11.1	29.5
GESTATION DAY 21	0.0342	0.416	2.39	9.94	20.1

*LOQ=Limit of quantitation (≤0.010 µg/g)

NOTE: Feces concentrations are expressed as µg/g and are the average of samples from all animals tested, not including samples that tested at or below the limit of quantitation (≤0.010 µg/g), those reported as None Detected, or those reported as Not Quantifiable.

Individual animal data are located in Appendix E, Data Spreadsheets and in Appendix G, Contract Lab Reports.

Table 14. LOQ Values Used in FACT TOX-110 Analyses

Method	Effective Date	LOQ
Urine		**µg/mL**
ETS-8-97.0	7/28/99	0.004 µg/mL
Liver		**µg/g**
Battelle	8/23/99	0.112 µg/g
Serum		**µg/mL**
Advanced Bioanalytical	8/26/99	0.043 µg/mL
Feces		**µg/g**
Centre	4/19/00	0.010 ng/g

Appendix E: Data Spreadsheets

Table 15. 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-6295.12 via Gavage

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 1 0.0 mg/kg/day	19501F	<LOQ	0.00891	<LOQ	NS
	19502F	<LOQ	<LOQ	0.0101	NS
	19503F	<LOQ	NS	NS	NS
	19504F	<LOQ	<LOQ	0.00406	<LOQ
	19505F	<LOQ	<LOQ	0.0141	<LOQ
	19506F	<LOQ	<LOQ	<LOQ	0.0334
	19507F	<LOQ	<LOQ	<LOQ	<LOQ
	19508F	<LOQ	<LOQ	<LOQ	NS
	19509F	<LOQ	<LOQ	<LOQ	0.0161
	19510F	<LOQ	<LOQ	<LOQ	NS
	19511F	<LOQ	<LOQ	<LOQ	NS
	19512F	<LOQ	<LOQ	0.00756	0.00887
	19513F	<LOQ	<LOQ	<LOQ	NS
	19514F	<LOQ	<LOQ	<LOQ	NS
	19515F	<LOQ	E	<LOQ	NS
	19516F	<LOQ	<LOQ	0.00951	<LOQ
Group 2 0.1 mg/kg/day	19517F	0.0396	0.0635	0.0456	NS
	19518F	0.0231	0.0648	0.00894	0.0496
	19519F	0.0487	0.0759	0.0324	0.0772
	19520F	0.0305	NS	NS	NS
	19521F	0.0677	0.121	0.0419	0.0420
	19522F	0.0475	0.0844	0.0990	NS
	19523F	0.0562	0.136	0.180	0.0726
	19524F	0.0374	0.0806	0.0981	0.0475
	19525F	0.0379	0.0508	0.0541	NS
	19526F	0.0268	0.0389	0.0493	NS
	19527F	0.0472	0.0319	0.0278	NS
	19528F	0.115	0.0357	0.0773	0.0608
	19529F	0.0444	0.0296	0.0641	0.0519
	19530F	0.0576	0.0266	0.132	NS
	19531F	0.0652	0.0541	0.0580	NS
	19532F	0.0504	0.0363	0.0601	NS

LOQ=Limit of quantitation (≤0.004 µg/mL)
NS=Not sampled
E=Not enough sample to complete the dilution/analysis
L=Sample lost during extraction
NOTE: PFOS concentrations in urine are expressed as micrograms of PFOS/mL of urine matrix (µg/mL)

Table 15. 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 3 0.4 mg/kg/day	19533F	0.219	0.343	0.673	0.383
	19534F	0.187	0.400	0.431	E
	19535F	0.494	0.191	0.436	NS
	19536F	0.212	0.533	0.600	E
	19537F	0.445	0.485	0.293	NS
	19538F	0.464	0.536	0.384	NS
	19539F	0.480	0.712	0.502	NS
	19540F	0.399	0.207	1.04	NS
	19541F	0.239	NS	NS	NS
	19542F	0.144	0.243	0.427	NS
	19543F	0.080	0.198	0.488	L
	19544F	0.274	0.0334	0.946	NS
	19545F	0.688	NS	NS	NS
	19546F	0.125	0.0769	0.534	0.593
	19547F	<LOQ	0.168	0.248	NS
	19548F	0.0879	0.192	0.359	0.688
Group 4 1.6 mg/kg/day	19549F	0.694	NS	NS	NS
	19550F	0.545	0.771	0.729	NS
	19551F	0.992	NS	NS	NS
	19552F	0.455	NS	NS	NS
	19553F	0.631	0.838	0.378	NS
	19554F	1.08	1.52	<LOQ	NS
	19555F	0.757	2.58	0.765	NS
	19556F	E	1.08	<LOQ	3.33
	19557F	1.11	NS	NS	NS
	19558F	1.32	1.05	0.640	NS
	19559F	0.904	0.297	0.700	5.35
	19560F	1.13	1.11	<LOQ	0.718
	19561F	1.20	1.04	0.805	NS
	19562F	1.90	1.11	<LOQ	1.45
	19563F	0.461	0.546	<LOQ	NS
	19564F	1.21	1.28	0.336	NS

LOQ=Limit of Quantitation (≤0.004 µg/mL)
NS=Not sampled
E=Not enough sample to complete the dilution/analysis
L=Sample lost during extraction
NOTE: PFOS concentrations in urine are expressed as micrograms of PFOS/mL of urine matrix (µg/mL)

Table 15. 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 5 3.2 mg/kg/day	19565F	1.27	0.908	0.585	NS
	19566F	2.14	1.63	0.188	NS
	19567F	1.15	0.525	<LOQ	NS
	19568F	0.961	1.82	0.381	1.39
	19569F	3.32	3.83	0.647	1.51
	19570F	1.04	1.03	0.150	NS
	19571F	1.14	1.32	0.391	1.32
	19572F	2.32	NS	0.973	1.46
	19573F	0.775	0.702	0.970	NS
	19574F	0.685	1.29	0.693	NS
	19575F	1.44	0.865	0.551	NS
	19576F	2.98	NS	NS	NS
	19577F	2.71	3.06	0.559	2.69
	19578F	1.15	NS	NS	NS
	19579F	0.629	2.36	0.557	1.31
	19580F	0.844	1.49	0.677	NS

LOQ=Limit of Quantitation (≤0.004 µg/mL)
NS=Not sampled
E=Not enough sample to complete the dilution/analysis
L=Sample lost during extraction
NOTE: PFOS concentrations in urine are expressed as micrograms of PFOS/mL of urine matrix (µg/mL)

Table 16. Results of Analyses of Serum 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-6295.12 via Gavage

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 1 0.0 mg/kg/day	19501F	0.0968	0.343	0.0694	NS
	19502F	0.0606	0.0462	0.0596	NS
	19503F	0.0524	NS	NS	NS
	19504F	0.0444	0.0800	<LOQ	<LOQ
	19505F	0.0475	0.0444	0.0526	<LOQ
	19506F	0.267	0.189	0.172	0.0708
	19507F	0.0471	0.051	0.0560	<LOQ
	19508F	0.0473	<LOQ	<LOQ	NS
	19509F	<LOQ	<LOQ	<LOQ	<LOQ
	19510F	0.0574	0.0550	0.0530	NS
	19511F	0.0535	0.216	0.152	NS
	19512F	<LOQ	0.170	0.206	0.0720
	19513F	0.0455	<LOQ	<LOQ	NS
	19514F	<LOQ	0.0677	0.0560	NS
	19515F	<LOQ	<LOQ	0.0502	NS
	19516F	0.0483	<LOQ	<LOQ	<LOQ
	Fetal Serum				
	19504	NS	NS	NS	0.0730
	19505	NS	NS	NS	0.0760
	19506	NS	NS	NS	0.231
	19507	NS	NS	NS	0.0727
	19509	NS	NS	NS	0.0881
	19512	NS	NS	NS	0.189
	19516	NS	NS	NS	0.143

LOQ=Limit of quantitation (≤0.043 µg/mL)
NS=Not sampled
NOTE: PFOS concentrations in serum are expressed as micrograms of PFOS/mL of serum matrix (µg/mL)
NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 16. Results of Analyses of Serum 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 2 0.1 mg/kg/day	19517F	7.84	7.46	9.94	NS
	19518F	8.53	8.90	9.24	5.91
	19519F	8.10	5.90	7.93	3.40
	19520F	8.41	NS	NS	NS
	19521F	9.59	7.62	12.2	4.26
	19522F	8.05	7.08	8.27	NS
	19523F	11.1	8.34	9.42	4.62
	19524F	8.81	8.99	7.95	3.55
	19525F	10.4	8.31	8.64	NS
	19526F	8.19	9.68	10.1	NS
	19527F	10.4	7.59	7.59	NS
	19528F	8.64	6.74	6.95	5.35
	19529F	10.0	8.90	6.32	2.63
	19530F	7.33	6.61	10.2	NS
	19531F	7.86	6.54	8.99	NS
	19532F	9.16	8.73	8.35	NS
	Fetal Serum				
	19518	NS	NS	NS	10.5
	19519	NS	NS	NS	7.36
	19521	NS	NS	NS	9.68
	19523	NS	NS	NS	8.81
	19524	NS	NS	NS	8.90
	19528	NS	NS	NS	9.33
	19529	NS	NS	NS	8.99

LOQ=Limit of quantitation (≤0.043 µg/mL)
NS=Not sampled
NOTE: PFOS concentrations in serum are expressed as micrograms of PFOS/mL of serum matrix (µg/mL)
NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 16. Results of Analyses of Serum 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 3 0.4 mg/kg/day	19533F	46.6	38.3	40.9	17.6
	19534F	40.5	43.2	48.5	17.7
	19535F	43.3	48.9	41.6	NS
	19536F	33.4	31.6	37.4	14.0
	19537F	48.9	50.5	43.8	NS
	19538F	40.2	41.6	46.2	NS
	19539F	39.1	38.5	32.1	NS
	19540F	37.1	39.6	42.0	NS
	19541F	38.4	NS	NS	NS
	19542F	39.1	35.1	38.0	NS
	19543F	40.0	39.8	37.9	19.9
	19544F	39.6	37.0	42.4	NS
	19545F	41.4	NS	NS	NS
	19546F	37.6	40.2	40.9	30.9
	19547F	36.5	36.5	37.4	NS
	19548F	49.8	51.8	50.1	56.8
	Fetal Serum				
	19533	NS	NS	NS	36.7
	19534	NS	NS	NS	35.8
	19536	NS	NS	NS	27.8
	19543	NS	NS	NS	29.5
	19546	NS	NS	NS	41.8

LOQ=Limit of quantitation (≤0.043 µg/mL)
NS=Not sampled
NOTE: PFOS concentrations in serum are expressed as micrograms of PFOS/mL of serum matrix (µg/mL)
NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 16. Results of Analyses of Serum 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 4 1.6 mg/kg/day	19549F	143	NS	NS	NS
	19550F	173	187	138	NS
	19551F	159	NS	NS	NS
	19552F	143	NS	NS	NS
	19553F	157	138	117	NS
	19554F	175	163	152	NS
	19555F	143	168	194	NS
	19556F	172	161	175	217
	19557F	171	NS	NS	NS
	19558F	169	148	142	NS
	19559F	148	138	166	205
	19560F	173	148	186	68.6
	19561F	167	156	189	NS
	19562F	160	155	123	55.0
	19563F	141	145	158	NS
	19564F	160	146	137	NS
	Fetal Serum				
	19560	NS	NS	NS	113
	19562	NS	NS	NS	88.1

LOQ=Limit of quantitation (≤0.043 µg/mL)
NS=Not sampled
NOTE: PFOS concentrations in serum are expressed as micrograms of PFOS/mL of serum matrix (µg/mL)
NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 16. Results of Analyses of Serum 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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/mL)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 5 3.2 mg/kg/day	19565F	320	339	298	NS
	19566F	291	242	269	NS
	19567F	316	283	254	NS
	19568F	293	294	290	208
	19569F	289	279	271	123
	19570F	303	291	232	NS
	19571F	301	321	266	143
	19572F	308	264	274	106
	19573F	314	296	274	NS
	19574F	315	340	258	NS
	19575F	357	334	245	NS
	19576F	331	NS	NS	NS
	19577F	360	341	312	191
	19578F	322	NS	NS	NS
	19579F	334	345	334	161
	19580F	327	308	279	NS
	Fetal Serum				
	19568	NS	NS	NS	197
	19569	NS	NS	NS	152
	19571	NS	NS	NS	143
	19572	NS	NS	NS	143
	19577	NS	NS	NS	195
	19579	NS	NS	NS	156

LOQ=Limit of quantitation (≤0.043 µg/mL)
NS=Not sampled
NOTE: PFOS concentrations in serum are expressed as micrograms of PFOS/mL of serum matrix (µg/mL)
NOTE: Serum analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

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-6295.12 via Gavage

Group/Dose	Sample # (all values in µg/g)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 1 0.0 mg/kg/day	19501F	ND	ND	ND	NS
	19502F	ND	NQ	ND	NS
	19503F	<LOQ	NS	NS	NS
	19504F	ND	NQ	ND	ND
	19505F	ND	ND	ND	ND
	19506F	0.0262	0.0155	0.0387	0.0107
	19507F	ND	ND	ND	ND
	19508F	<LOQ	<LOQ	ND	NS
	19509F	0.0348	ND	0.0210	0.0733
	19510F	ND	ND	<LOQ	NS
	19511F	ND	ND	ND	NS
	19512F	ND	ND	0.0503	0.0187
	19513F	ND	ND	ND	NS
	19514F	ND	ND	ND	NS
	19515F	ND	ND	ND	NS
	19516F	0.0529	NQ	0.0188	<LOQ
Group 2 0.1 mg/kg/day	19517F	0.609	0.356	0.659	NS
	19518F	0.396	0.488	0.584	0.639
	19519F	0.339	0.299	0.680	0.411
	19520F	0.587	NS	NS	NS
	19521F	0.430	0.404	0.549	0.351
	19522F	0.331	0.676	0.710	NS
	19523F	0.398	0.543	0.558	0.349
	19524F	0.724	0.500	0.725	0.429
	19525F	0.411	0.517	0.939	NS
	19526F	0.388	0.408	0.676	NS
	19527F	0.432	0.645	0.694	NS
	19528F	0.609	0.462	0.734	0.396
	19529F	0.693	0.647	0.629	0.340
	19530F	0.342	0.369	0.630	NS
	19531F	0.638	0.542	0.675	NS
	19532F	0.650	0.499	0.494	NS

LOQ=Limit of quantitation (≤0.010 µg/g)
NS=Not sampled
ND=None detected
NQ=Not quantifiable
NOTE: PFOS concentrations in feces are expressed as micrograms of PFOS/g of feces matrix (µg/g)

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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/g)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 3 0.4 mg/kg/day	19533F	2.26	2.21	2.40	1.46
	19534F	3.26	2.34	4.19	2.70
	19535F	2.43	1.59	3.15	NS
	19536F	2.76	2.83	2.77	1.61
	19537F	2.41	2.27	2.95	NS
	19538F	2.68	2.55	2.73	NS
	19539F	3.20	1.82	2.69	NS
	19540F	1.78	1.97	3.61	NS
	19541F	1.83	NS	NS	NS
	19542F	2.86	2.61	1.64	NS
	19543F	1.84	2.43	2.97	1.94
	19544F	2.98	1.98	2.93	NS
	19545F	2.39	NS	NS	NS
	19546F	2.03	1.32	2.28	1.94
	19547F	1.93	1.79	3.36	NS
	19548F	2.03	2.47	3.40	4.71
Group 4 1.6 mg/kg/day	19549F	12.3	NS	NS	NS
	19550F	14.7	13.0	12.1	NS
	19551F	9.03	NS	NS	NS
	19552F	11.4	NS	NS	NS
	19553F	11.9	10.7	12.9	NS
	19554F	13.5	10.2	12.3	NS
	19555F	8.98	8.35	14.1	NS
	19556F	8.91	8.88	7.88	15.9
	19557F	7.79	NS	NS	NS
	19558F	5.95	5.12	6.23	NS
	19559F	6.67	5.99	6.50	9.19
	19560F	14.4	6.10	12.9	9.72
	19561F	10.5	7.71	15.2	NS
	19562F	8.33	9.42	9.92	4.94
	19563F	6.49	13.0	7.78	NS
	19564F	14.7	11.9	15.1	NS

LOQ=Limit of quantitation (≤0.010 µg/g)
NS=Not sampled
ND=None detected
NQ=Not quantifiable
NOTE: PFOS concentrations in feces are expressed as micrograms of PFOS/g of feces matrix (µg/g)

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-6295.12 via Gavage (Continued)

Group/Dose	Sample # (all values in µg/g)	GD0 external std.	GD7 external std.	GD15 external std.	GD21 external std.
Group 5 3.2 mg/kg/day	19565F	20.4	44.9	30.6	NS
	19566F	25.7	23.0	13.2	NS
	19567F	26.3	31.2	17.7	NS
	19568F	21.0	20.0	40.8	21.8
	19569F	23.5	37.3	31.6	18.8
	19570F	17.1	32.5	27.0	NS
	19571F	26.0	37.4	24.7	18.7
	19572F	22.1	33.7	17.9	13.0
	19573F	20.0	23.2	34.6	NS
	19574F	25.5	31.6	41.1	NS
	19575F	20.0	30.8	35.4	NS
	19576F	31.6	NS	NS	NS
	19577F	29.8	59.4	41.1	23.5
	19578F	30.2	NS	NS	NS
	19579F	22.0	31.2	29.1	24.6
	19580F	21.6	26.1	28.4	NS

LOQ=Limit of quantitation (≤0.010 —µg/g)
NS=Not sampled
ND=None detected
NQ=Not quantifiable
NOTE: PFOS concentrations in feces are expressed as micrograms of PFOS/g of feces matrix (µg/g)

Table 18. Results of 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-6295.12 via Gavage

Group/Dose	Sample #	Concentration of PFOS (all values in µg/g)	Group/Dose	Sample #	Concentration of PFOS (all values in µg/g)
Group 1 0.0 mg/kg/day	19501F	0.346	Group 2 0.1 mg/kg/day	19517F	42.6
	19502F	0.305		19518F	21.8
	19503F	NS		19519F	18.9
	19504F	0.125		19520F	15.4
	19505F	0.174		19521F	20.1
	19506F	0.403		19522F	32.0
	19507F	0.168		19523F	17.7
	19508F	0.230		19524F	19.6
	19509F	0.175		19525F	36.5
	19510F	0.203		19526F	34.0
	19511F	0.828		19527F	46.4
	19512F	0.353		19528F	26.4
	19513F	0.258		19529F	21.7
	19514F	0.325		19530F	47.8
	19515F	0.286		19531F	32.1
	19516F	0.138		19532F	34.6
	Fetal Liver			Fetal Liver	
	19504	0.153		19518	7.32
	19505	<LOQ		19519	8.64
	19506	0.149		19521	7.23
	19507	<LOQ		19523	7.68
	19509	0.204		19524	8.54
	19512	<LOQ		19528	9.33
	19516	<LOQ		19529	6.73

LOQ=Limit of quantitation (≤ 0.112 µg/g)
NS=Not sampled
NOTE: PFOS concentrations in liver are expressed as micrograms of PFOS/g of liver matrix (µg/g)
NOTE: Liver analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 18. Results of 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-6295.12 via Gavage (Continued)

Group/Dose	Sample #	Concentration of PFOS (all values in µg/g)	Group/Dose	Sample #	Concentration of PFOS (all values in µg/g)
Group 3 0.4 mg/kg/day	19533F	67.0	Group 4 1.6 mg/kg/day	19549F	NS
	19534F	88.1		19550F	342
	19535F	111		19551F	NS
	19536F	88.1		19552F	NS
	19537F	143		19553F	351
	19538F	144		19554F	358
	19539F	105		19555F	497
	19540F	110		19556F	304
	19541F	96.8		19557F	268
	19542F	108		19558F	334
	19543F	85.9		19559F	881
	19544F	113		19560F	250
	19545F	NS		19561F	397
	19546F	83.5		19562F	215
	19547F	119		19563F	426
	19548F	137		19564F	416
	Fetal Liver			Fetal Liver	
	19533	27.5		19560	105
	19534	34.1		19562	68.0
	19536	19.0			
	19543	30.7			
	19546	41.6			

LOQ=Limit of quantitation (≤ 0.112 µg/g)
NS=Not sampled
NOTE: PFOS concentrations in liver are expressed as micrograms of PFOS/g of liver matrix (µg/g)
NOTE: Liver analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Table 18. Results of 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-6295.12 via Gavage (Continued)

Group/Dose	Sample #	Concentration of PFOS (all values in µg/g)
Group 5 3.2 mg/kg/day	19565F	381
	19566F	594
	19567F	623
	19568F	596
	19569F	468
	19570F	740
	19571F	448
	19572F	455
	19573F	787
	19574F	735
	19575F	802
	19576F	NS
	19577F	521
	19578F	NS
	19579F	612
	19580F	782
	Fetal Liver	
	19568	260
	19569	183
	19571	199
	19572	184
	19577	339
	19579	213

LOQ=Limit of quantitation (≤ 0.112 µg/g)
NS=Not sampled
NOTE: PFOS concentrations in liver are expressed as micrograms of PFOS/g of liver matrix (µg/g)
NOTE: Liver analysis were conducted at a contract laboratory but were corrected by 3M to reflect the official purity values from the COA. Revised reports from the final contract laboratories will be added as a report amendment at a later date.

Appendix F: Example Calculations

Formula Used for Urine Analyses in Study FACT TOX-110

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

Calculation Used for Group 2, GD15, Animal ID 19522F

$$123.57\ \text{ng/mL} \times 1 \times 0.9275 \times \frac{1.0\ \mu g}{1000\ ng} \times 0.864 = 0.0990\ \mu g\ \text{PFOS/mL urine}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
SC—PFOS salt correction constant (0.9275)
PC—PFOS purity correction factor (86.4%)

Appendix G: Contract Lab Reports

This Appendix includes the following contract laboratory reports:

Battelle Memorial Institute, "Oral (Gavage) Pharmacokinetic Study of PFOS in Rats," 63 pages

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

Advanced Bioanalytical Services, Inc., "Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FAC T-TOX-110 Using Turbo Ion Spray LC/MS," 24 pages

Advanced Bioanalytical Services, Inc., "Addendum—Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-110 Using Turbo Ion Spray LC/MS," 3 pages

Centre Analytical Laboratories, Inc., "Oral (Gavage) Pharmacokinetic Study of PFOS in Rats," 103 pages

BIOLOGICAL SAMPLE ANALYSIS

Battelle . . . Putting Technology To Work

Battelle Study Number: N003296-F

3M Toxicology Services Protocol Number: FACT-TOX-110

FINAL REPORT

ORAL (GAVAGE) PHARMACOKINETIC 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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

FINAL REPORT

ORAL (GAVAGE) PHARMACOKINETIC STUDY OF PFOS IN RATS

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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

ORAL (GAVAGE) PHARMACOKINETIC 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 investigated.

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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

QUALITY ASSURANCE STATEMENT

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

Phase Inspected	Inspection Date	Date Reported to Battelle Task Leader/ Battelle Management	Date Reported to Offsite Study Director/ Management
Sample homogenization	8/27/1999	9/20/1999	9/29/1999
Sample weights	8/27/1999	9/20/1999	9/29/1999
Sample analysis	8/30/1999	9/20/1999	9/29/1999
Sample processing	8/30/1999	9/20/1999	9/29/1999
Audit final report	9/20/1999	9/20/1999	9/29/1999
Audit study file	9/20/1999	9/20/1999	9/29/1999
Audit final report	2/16/2001	2/16/2001	2/20/2001

Quality Assurance Unit — Date
Battelle Memorial Institute

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

Study Title: ORAL (GAVAGE) PHARMACOKINETIC 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 — 4-24-01.

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

Date

Marvin T Case — 30 April 2001

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

Date

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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-110 and the results from this analysis. See Appendix E for a copy of the study protocol, amendments, and deviation reports.

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 418-013. The samples were stored at approximately –20°C.

4.0 Analysis of Samples

4.1 Summary of Method

Samples were analyzed by a previously validated method (Battelle study number N003604-A). The current version of the method is attached to this report in Appendix 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, although present in the stock solutions, were not monitored. Quadratic regressions weighted 1/x were used to construct the calibration curves.

Battelle Study Number: N003296-F
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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 25 µL/min into the MS		
Column temp	Ambient		
HPLC pressure	Approximately 1000 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: 4.3 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 each sample set are provided in Appendix B. Representative chromatograms are given in Appendix D.

4.2.2 Sample Results

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

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

5.0 Conclusions

All analyses met acceptance criteria unless otherwise noted.

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

7.0 Specimen Storage and Record Archives

Refer to Appendix E, protocol amendment 2 for record 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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

APPENDIX A -RESULTS

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

RAT LIVER SAMPLE RESULTS
STUDY: N003296-F
ANALYSIS DATE AND INSTRUMENT ID: 25Aug99; 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 19501	0	0.400		
Animal 19502	0	0.353		
Animal 19504	0	0.145		
Animal 19504P	0	0.177		
Animal 19505	0	0.201		
Animal 19505P	0	BLOQ		
Animal 19506	0	0.466		
Animal 19506P	0	0.173		
Animal 19507	0	0.194		
Animal 19507P	0	BLOQ		
Animal 19508	0	0.266		
Animal 19509	0	0.203		
Animal 19509P	0	0.236		
Animal 19510	0	0.235		
Animal 19511	0	0.958		
Animal 19512	0	0.408		
Animal 19512P	0	BLOQ		
Animal 19513	0	0.299		
Animal 19514	0	0.376		
Animal 19515	0	0.331		
Animal 19516	0	0.160		
Animal 19516P	0	BLOQ		
Animal 19520	0 (SEE NOTE 2)	ALOQ		YES (1)
Animal 19517	0.1	ALOQ		YES (1)
Animal 19518P	0.1	8.47		
Animal 19533	0.4	ALOQ		YES (1)
Animal 19533P	0.4	ALOQ		YES (1)
Animal 19550	1.6	ALOQ		YES (1)
Animal 19560P	1.6	ALOQ		YES (1)
Animal 19565	3.2	ALOQ		YES (1)
Animal 19568P	3.2	ALOQ		YES (1)

BLOQ = BELOW CONC OF LOWEST STANDARD IN CALIBRATION CURVE
ALOQ = ABOVE CONC OF HIGHEST STANDARD IN CALIBRATION CURVE
(1) = SAMPLE TO BE DILUTED BEFORE REPEAT EXTRACTION
(2) = SAMPLE INITIALLY MARKED AS 0.1 mg/kg/day; LABEL HAND-CORRECTED TO INDICATE VEHICLE

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

RAT LIVER SAMPLE RESULTS
STUDY: N003296-F
ANALYSIS DATE AND INSTRUMENT ID: 28Aug99; 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 19517	0.1	49.3	YES (1)	
Animal 19518	0.1	25.2		
Animal 19519	0.1	21.9		
Animal 19519P	0.1	10.0		
Animal 19520	0 (SEE NOTE 2)	17.8	YES (1)	
Animal 19521	0.1	23.3		
Animal 19521P	0.1	8.37		
Animal 19522	0.1	37.0		
Animal 19523	0.1	20.5		
Animal 19523P	0.1	8.89		
Animal 19524	0.1	22.7		
Animal 19524P	0.1	9.89		
Animal 19525	0.1	42.2		
Animal 19526	0.1	39.4		
Animal 19527	0.1	53.7		
Animal 19528	0.1	30.5		
Animal 19528P	0.1	10.8		
Animal 19529	0.1	25.1		
Animal 19529P	0.1	7.79		
Animal 19530	0.1	55.3		
Animal 19531	0.1	37.2		
Animal 19532	0.1	40.1		
Animal 19533	0.4	77.6	YES (1)	
Animal 19533P	0.4	31.8	YES (1)	
Animal 19550	1.6	396	YES (1)	
Animal 19560P	1.6	122	YES (1)	
Animal 19565	3.2	441	YES (1)	
Animal 19568P	3.2	301	YES (1)	

BLOQ = BELOW CONC OF LOWEST STANDARD IN CALIBRATION CURVE
ALOQ = ABOVE CONC OF HIGHEST STANDARD IN CALIBRATION CURVE
(1) = SAMPLE REPEATED FROM 25AUG99 ANALYSIS
(2) = SAMPLE INITIALLY MARKED AS 0.1 mg/kg/day; BAG LABEL HAND-CORRECTED TO INDICATE VEHICLE

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

RAT LIVER SAMPLE RESULTS
STUDY: N003296-F
ANALYSIS DATE AND INSTRUMENT ID: 30Aug99; 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 19534	0.4	102		
Animal 19534P	0.4	39.5		
Animal 19535	0.4	128		
Animal 19536	0.4	102		
Animal 19536P	0.4	22.0		
Animal 19537	0.4	165		
Animal 19538	0.4	167		
Animal 19539	0.4	121		
Animal 19540	0.4	127		
Animal 19541	0.4	112		
Animal 19542	0.4	125		
Animal 19543	0.4	99.4		
Animal 19543P	0.4	35.5		
Animal 19544	0.4	131		
Animal 19546	0.4	96.6		
Animal 19546P	0.4	48.2		
Animal 19547	0.4	138		
Animal 19548	0.4	159		
Animal 19562P	1.6	78.7		
Animal 19569P	3.2	212		
Animal 19571P	3.2	230		
Animal 19572P	3.2	213		
Animal 19577P	3.2	392		
Animal 19579P	3.2	246		

BLOQ = BELOW CONC OF LOWEST STANDARD IN CALIBRATION CURVE
ALOQ = ABOVE CONC OF HIGHEST STANDARD IN CALIBRATION CURVE

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

RAT LIVER SAMPLE RESULTS
STUDY: N003296-F
ANALYSIS DATE AND INSTRUMENT ID: 01Sep99; 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 19553	1.6	406		
Animal 19554	1.6	414		
Animal 19555	1.6	575		
Animal 19556	1.6	352		
Animal 19557	1.6	310		
Animal 19558	1.6	387		
Animal 19559	1.6	1.02E+03		
Animal 19560	1.6	289		
Animal 19561	1.6	460		
Animal 19562	1.6	249		
Animal 19563	1.6	493		
Animal 19564	1.6	482		
Animal 19566	3.2	687		
Animal 19567	3.2	721		
Animal 19568	3.2	690		
Animal 19569	3.2	542		
Animal 19570	3.2	856		
Animal 19571	3.2	518		
Animal 19572	3.2	527		
Animal 19573	3.2	911		
Animal 19574	3.2	851		
Animal 19575	3.2	928		
Animal 19577	3.2	603		
Animal 19579	3.2	708		
Animal 19580	3.2	905		

BLOQ = BELOW CONC OF LOWEST STANDARD IN CALIBRATION CURVE
ALOQ = ABOVE CONC OF HIGHEST STANDARD IN CALIBRATION CURVE

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

METHOD DETECTION LIMIT (MDL) RESULTS
STUDY: N003296-F
ANALYSIS DATE AND INSTRUMENT ID: 28Aug99; 9053
DATA ENTERED: Electronically
SPREADSHEET SOFTWARE: Excel 97

All concs in µg/g

	PFOS
Calculated Concentration of Replicate 1	0.1184
Calculated Concentration of Replicate 2	0.1192
Calculated Concentration of Replicate 3	0.1251
Calculated Concentration of Replicate 4	0.1239
Calculated Concentration of Replicate 5	0.1351
Calculated Concentration of Replicate 6	0.1248
Calculated Concentration of Replicate 7	0.1225
Mean Concentration	0.1241
Std. Dev.	0.0055
Spike Level	0.1340
MDL determined	0.01729
S/N	22.57
Valid	Yes
LOQ (det. from 10 x std.dev. "noise")	0.05501
LOQ (det. from cal curve low std.)	0.1340
Curve Coeff of Determination	0.9981
Date analyzed	08/28/99
Method	LC/MS/MS

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

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

APPENDIX B-DAILY ACCEPTANCE CRITERIA SUMMARY

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

DAILY ACCEPTANCE CRITERIA SUMMARY
PFOS IN RAT LIVER
STUDY: N003296-F
SPREADSHEET SOFTWARE: Excel 97
DATA ENTERED MANUALLY NA=Not Applicable

Analysis Date	Cal Curve Coeff of Det	Sys Suit %RSD	Homogenizer Fort Ave Recov	Diln Std Ave Recov	Ave QC 1 %RE (%RSD)	Ave QC 2 %RE (%RSD)	Ave QC 3 %RE (%RSD)	Ave QC 4 %RE (%RSD)
August 25, 1999	0.9899	4.0	111.1	NA	-7.1 (15.8)	0.0 (14.2)	-5.5 (13.7)	2.5 (16.8)
August 28, 1999	0.9981	3.1	112.4	102.4	2.7 (0.9)	2.3 (3.4)	-3.8 (4.1)	-6.9 (0.9)
August 30, 1999	0.9943	7.5	110.9	116.0	-1.8 (4.0)	-1.9 (4.2)	-6.5 (6.4)	1.4 (1.1)
September 1, 1999	0.9951	5.3	118.1	110.1	1.9 (4.6)	1.6 (5.6)	-10.7 (4.6)	18.0 (7.4)

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

APPENDIX C-METHOD

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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: 15 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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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.

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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	X 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		

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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.

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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: _____

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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

Version 1.0

Study No.: _____
Analyst/Date: _____

2. WSS (1000 ng/mL):

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

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

H. Preparation of Calibration Solvent Stocks and Working Standards

1. Solvent Stocks:

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

2. Mixed Solvent Stocks:

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

Date of preparation: _____
Study No: _____

3. Working Standards (WS):

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

Date of preparation: _____

Table 3. Calibration Solvent Stocks and Working Standards

Standard	Source	Target Amount	Actual Amount Analytical Std. Stock, or WS	Target Final Vol (mL)	Actual Final Vol. (mL)	Nominal Conc (ng/mL)
Stock 1A	PFOS	50 ± 1 mg	_____ 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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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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Table 5. Calibration Standards and Blanks

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

Date of preparation of cal stds/blank: _____

J. Preparation of Quality Control Liver Samples (QCs)

1. Quality Control Working Standards

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

Table 6. QC WS Preparation

Soln ID	Source	Vol Source, mL	Final Vol, mL	Conc, ng/mL
QC WS 1	Mixed Stock A	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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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

②WN PCP 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, ~~13~~ ① *20 V (V) PFOSA, PFOSAA, PFOSEA, M-556, M570		
Collision energy	*40 eV (eV) PFOS, ~~15~~ 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 ( )		

①SHOULD READ "55"; LE PCP 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:

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

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

8. Calculate the regression equation relating the peak response ratio (test article/SS) of each calibration standard (y-axis) to test article concentration in liver (x-axis) for PFOS, M-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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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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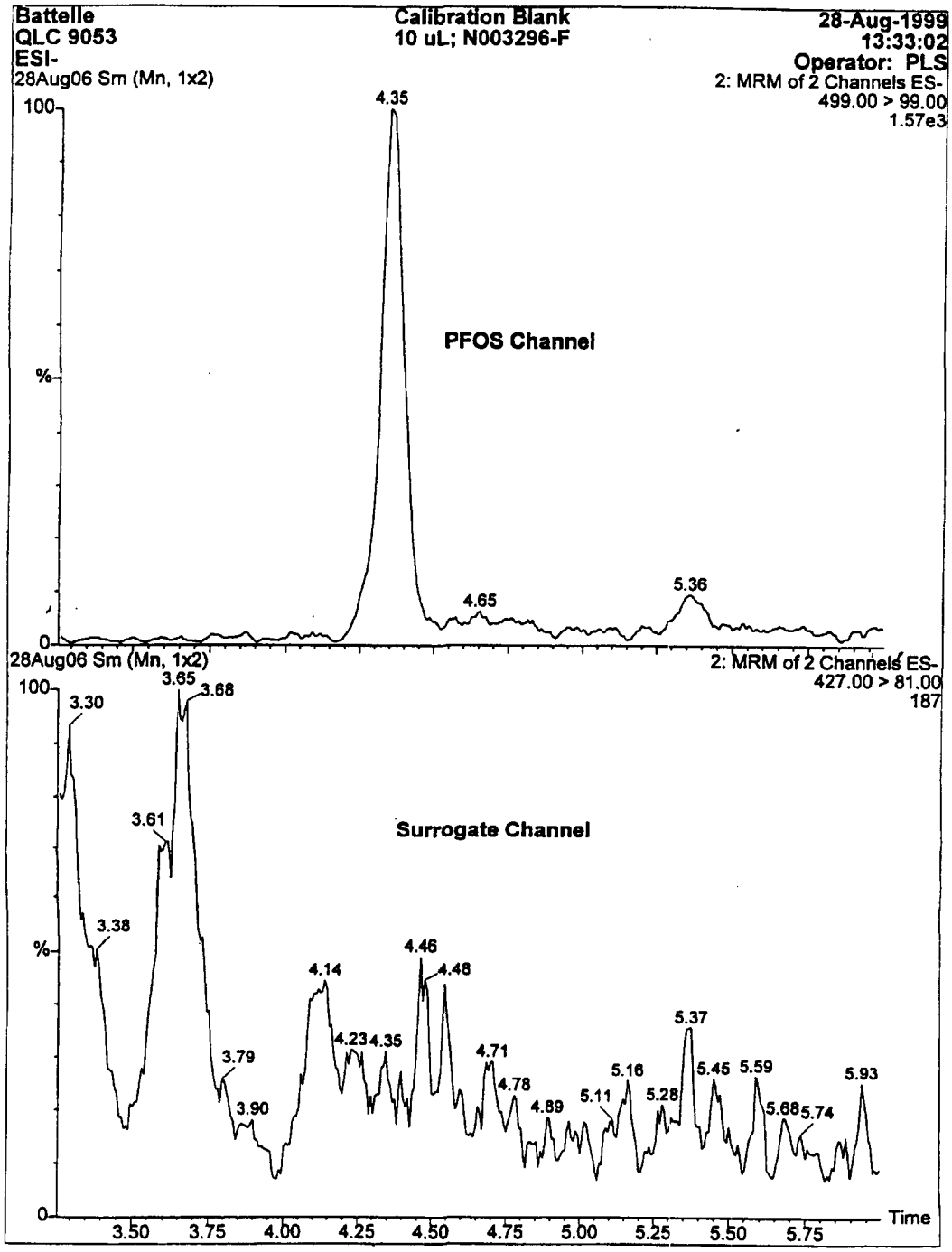

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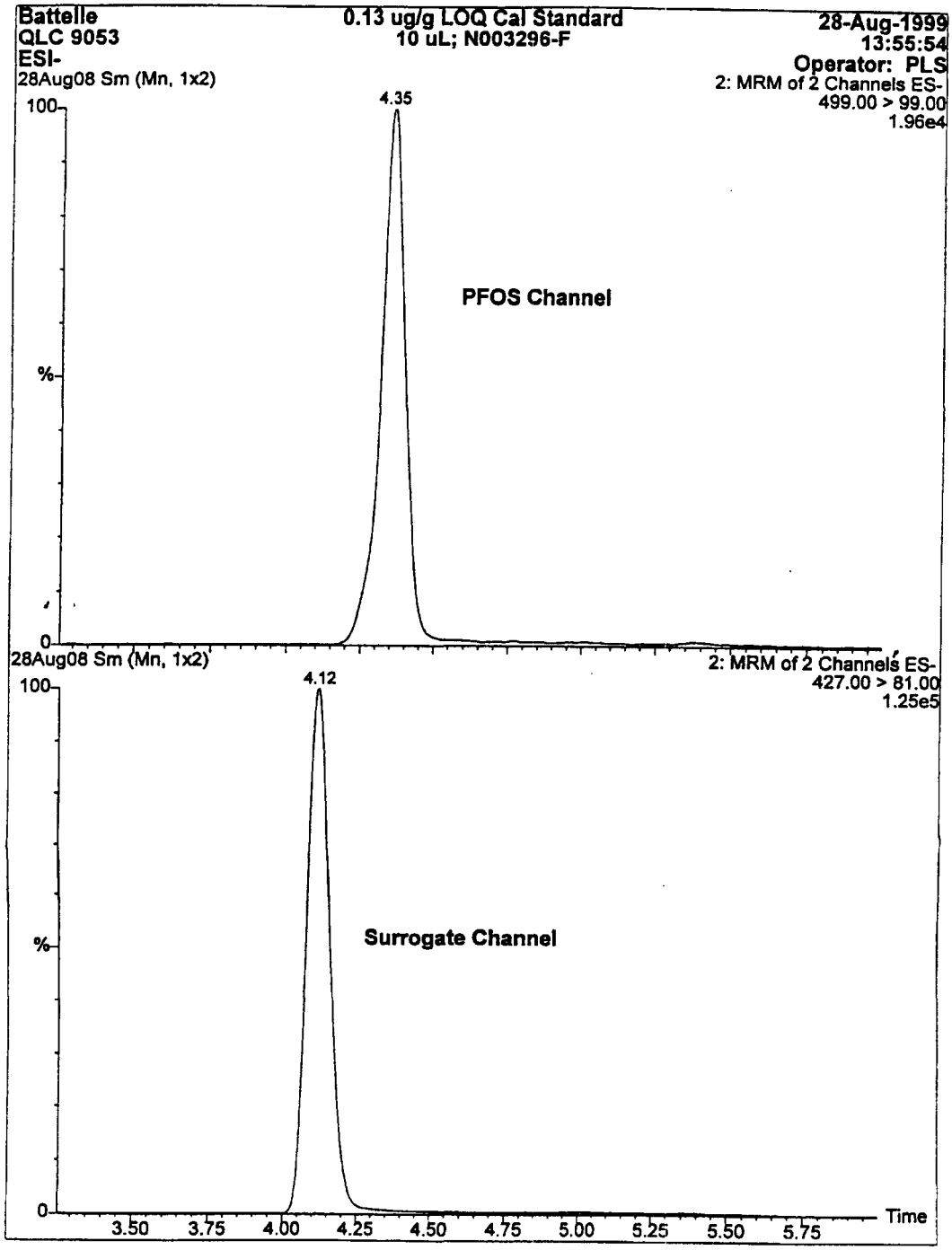

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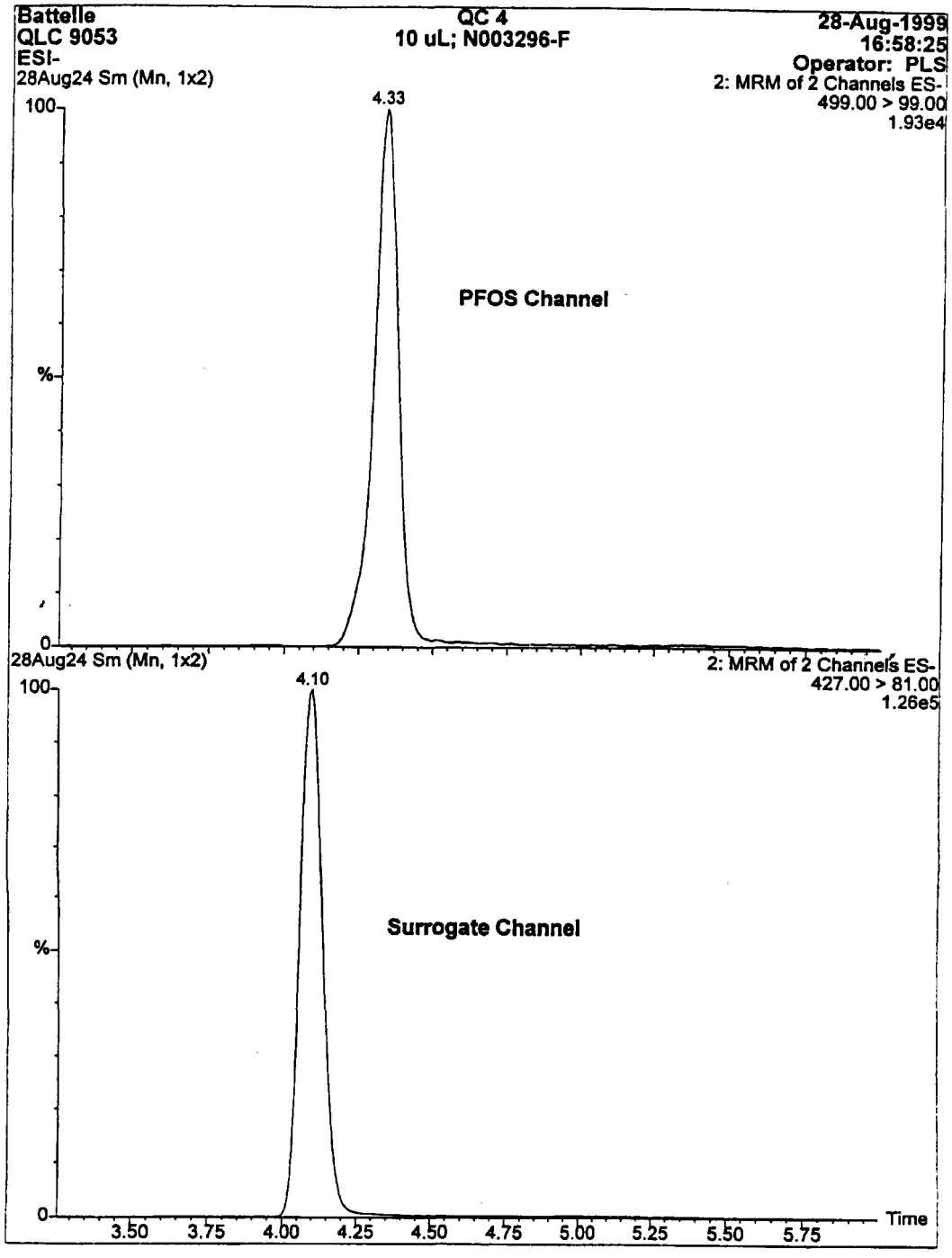

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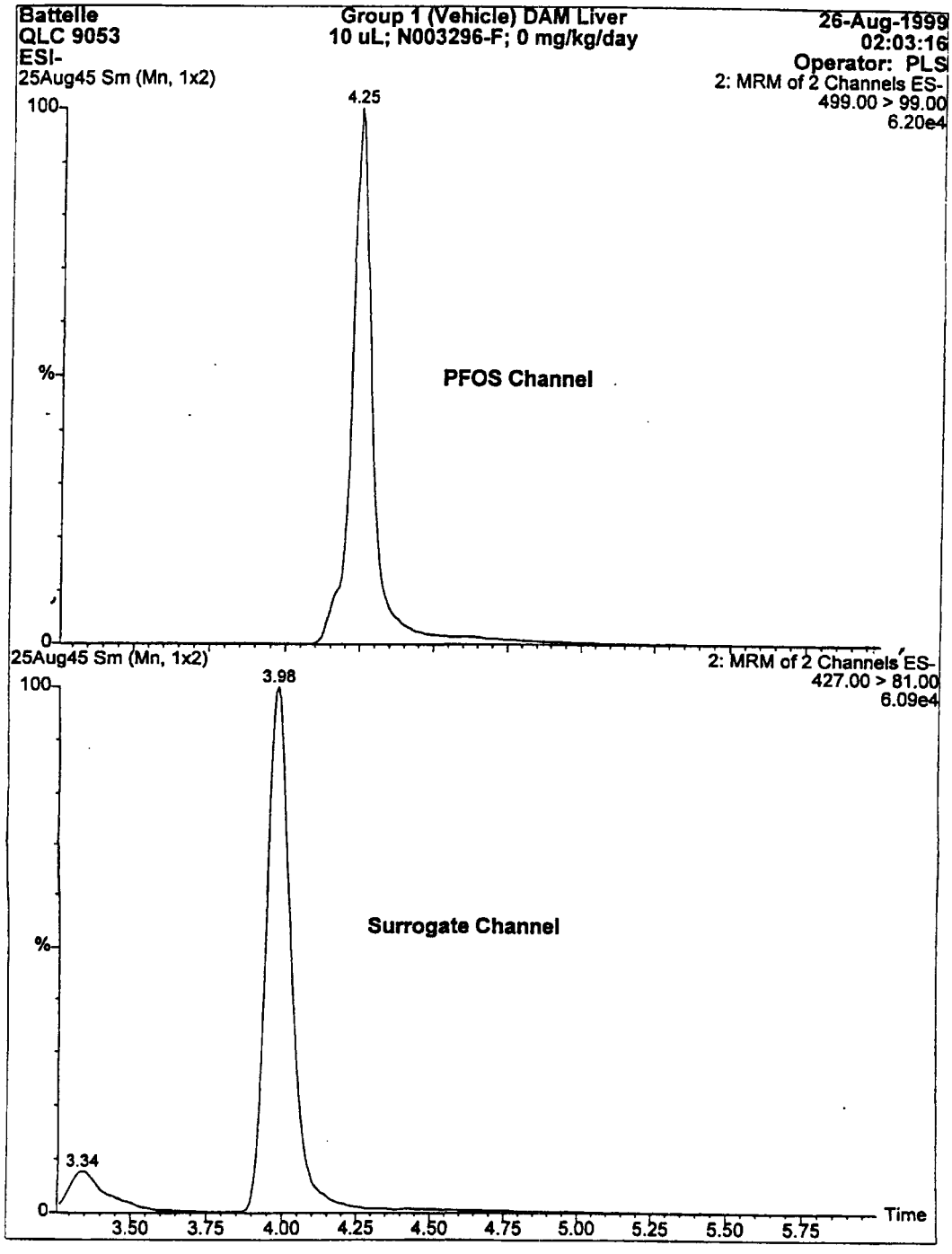

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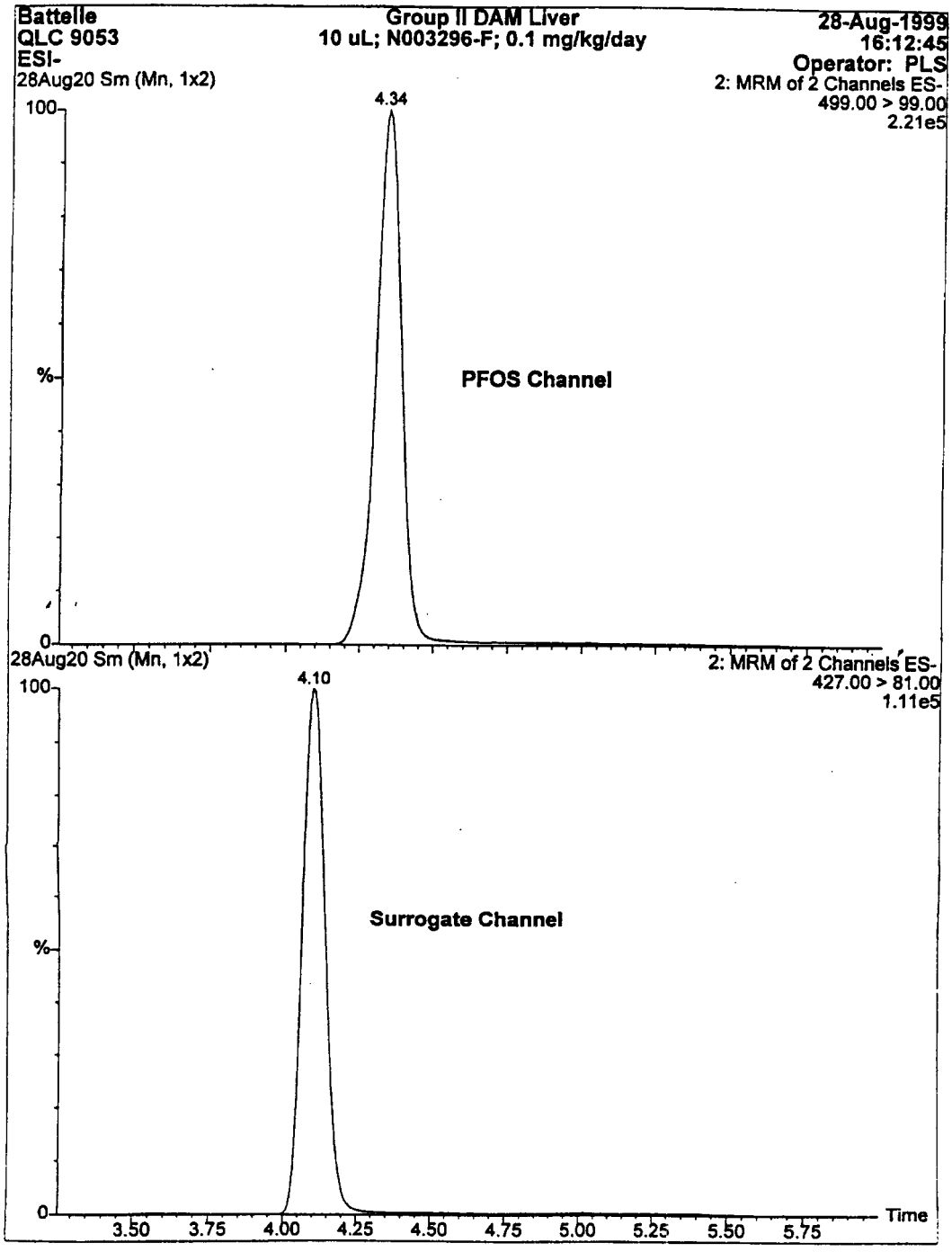

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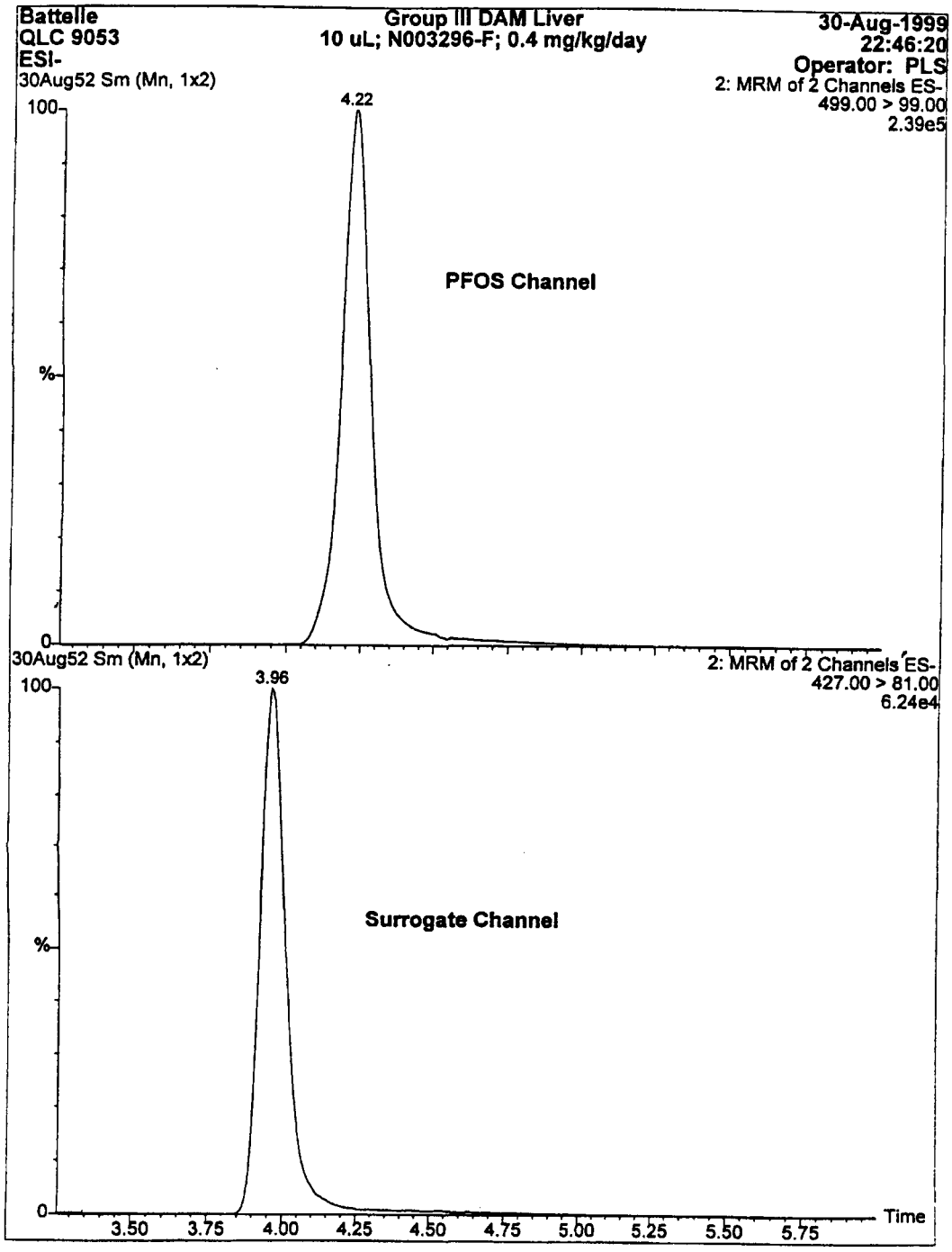

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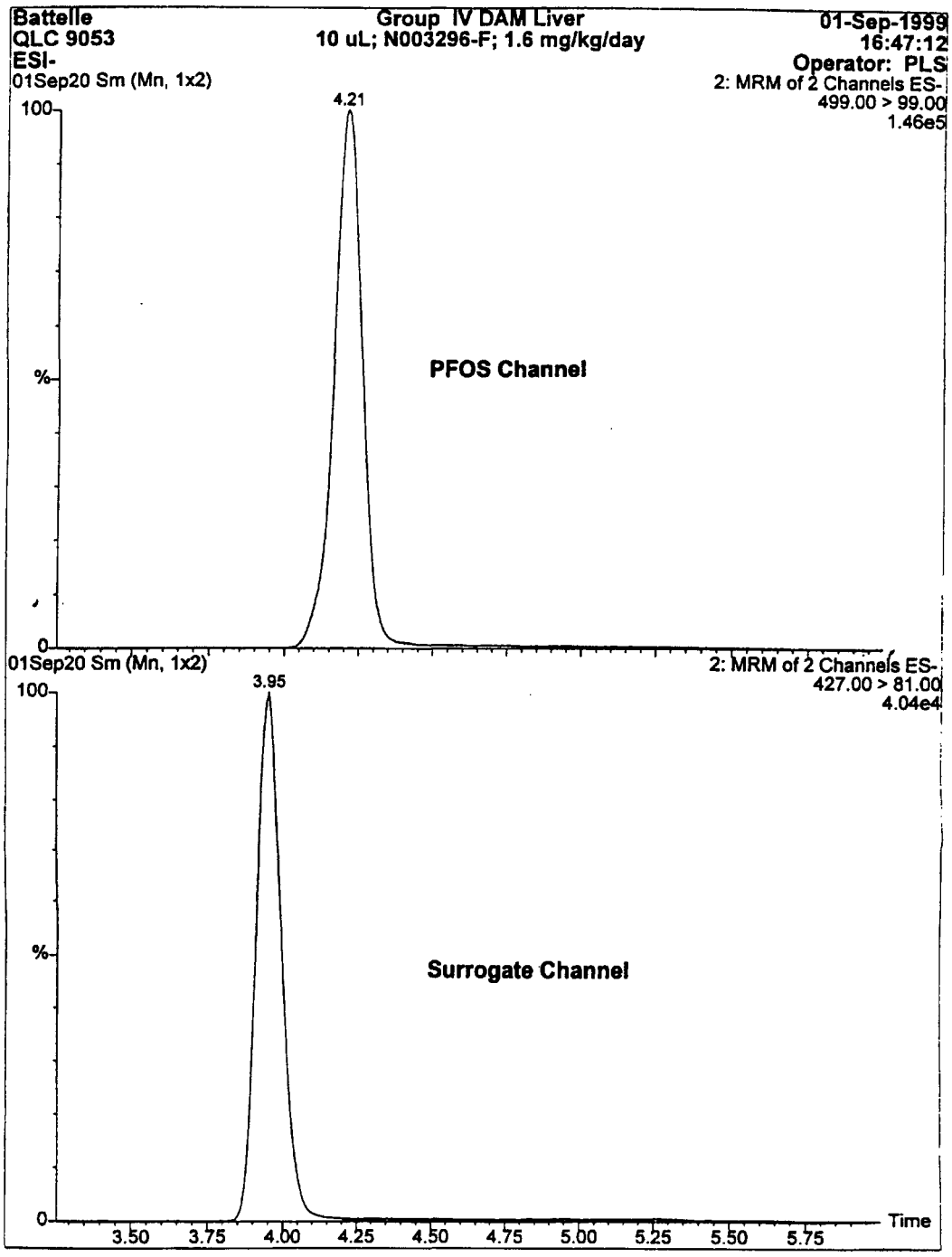

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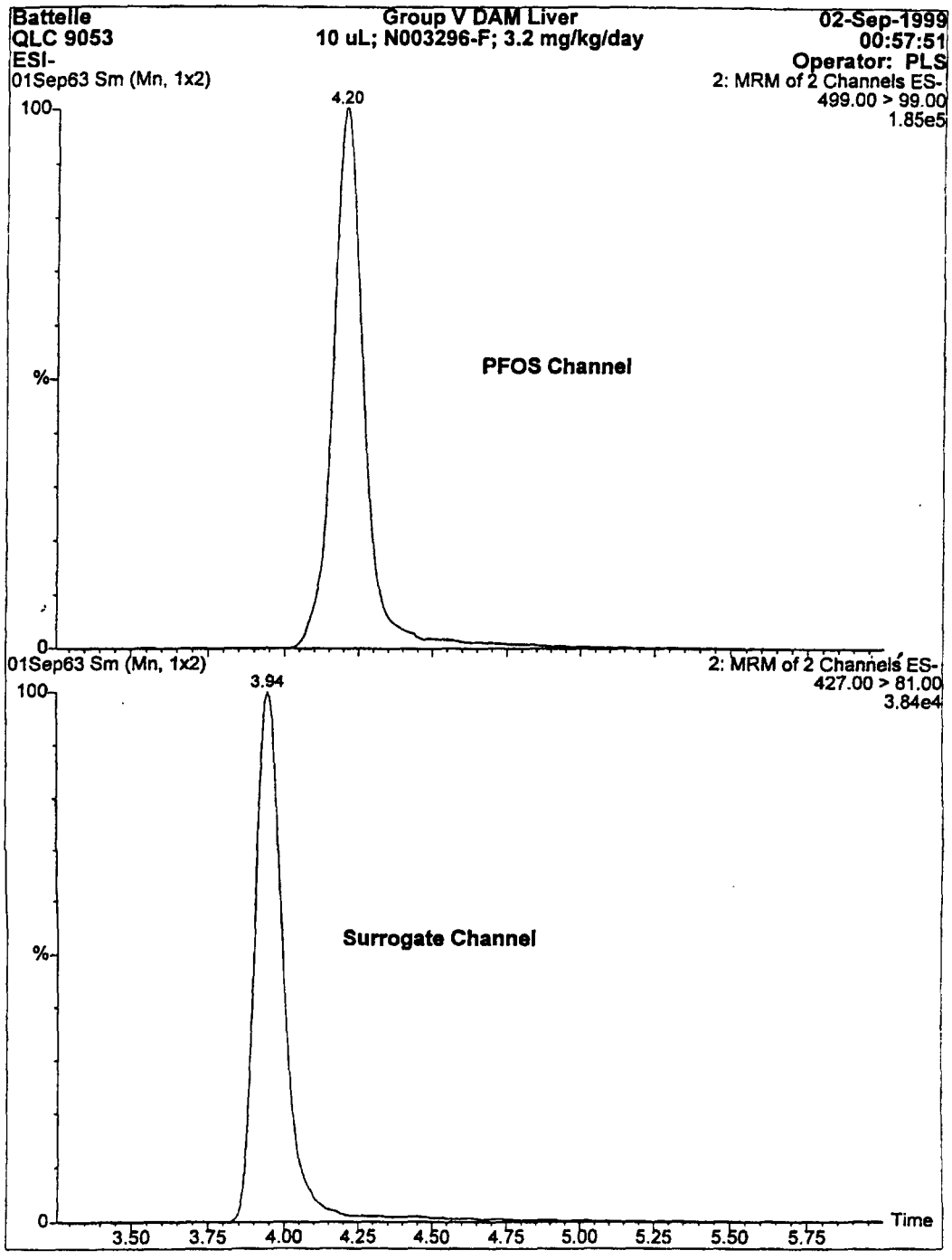

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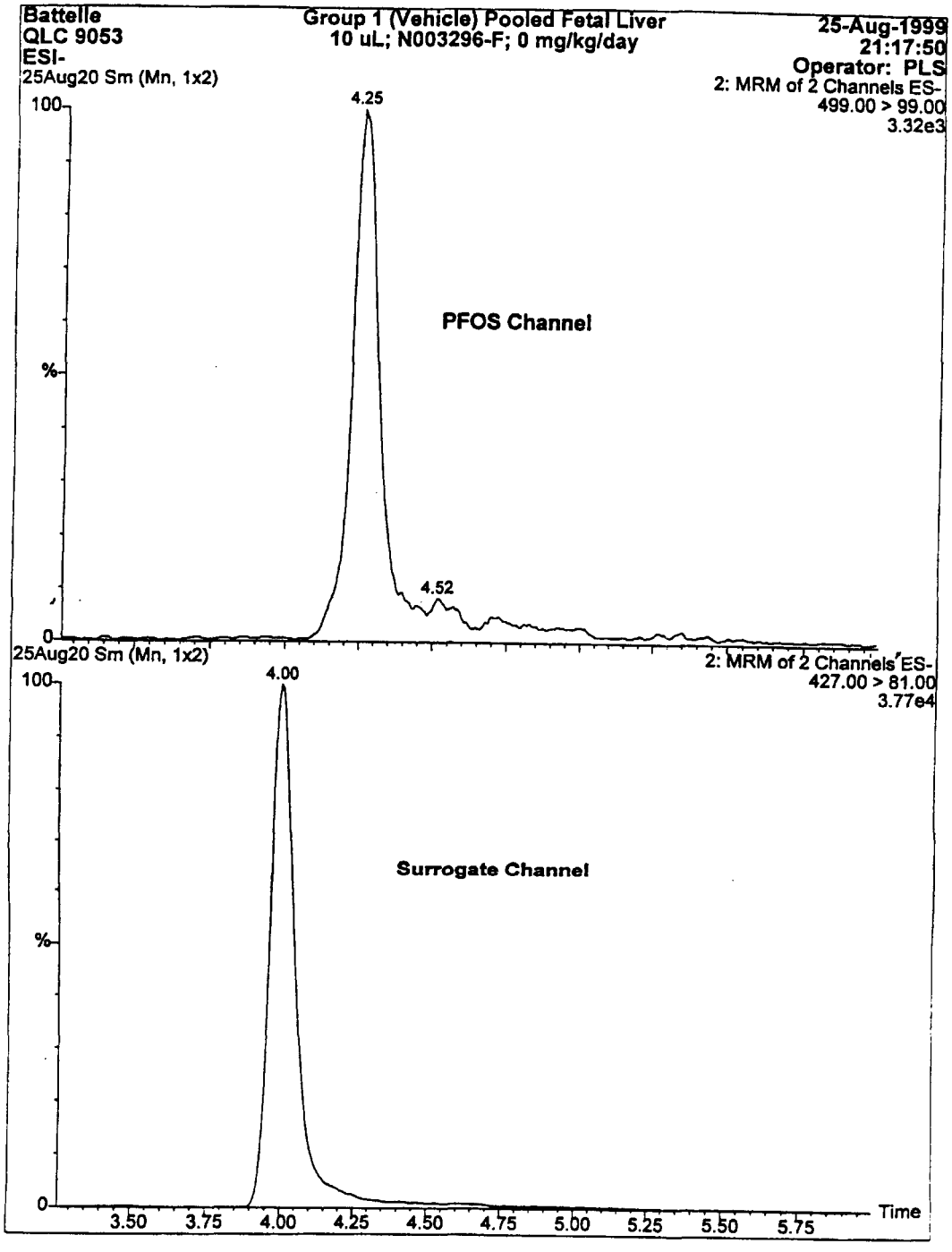

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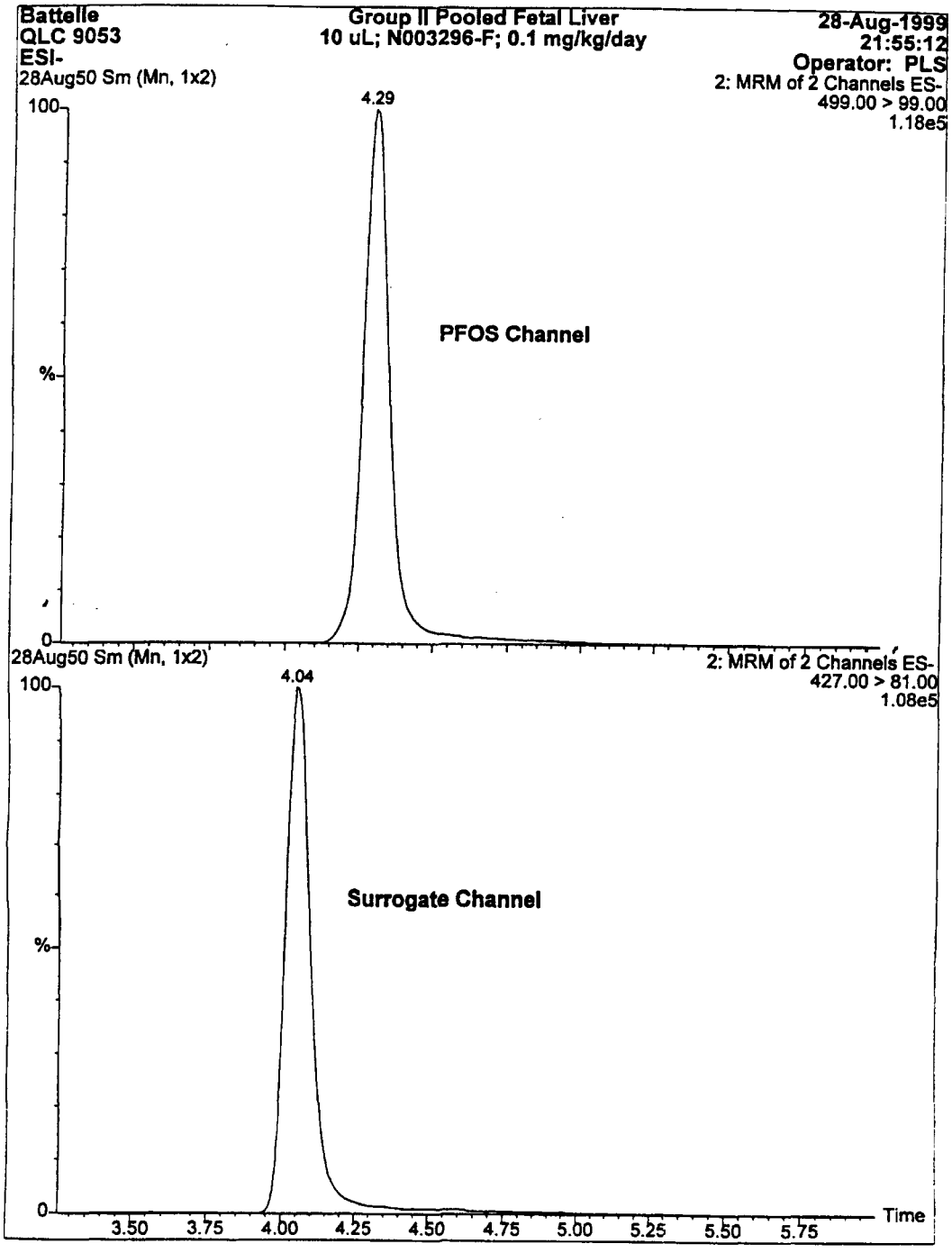

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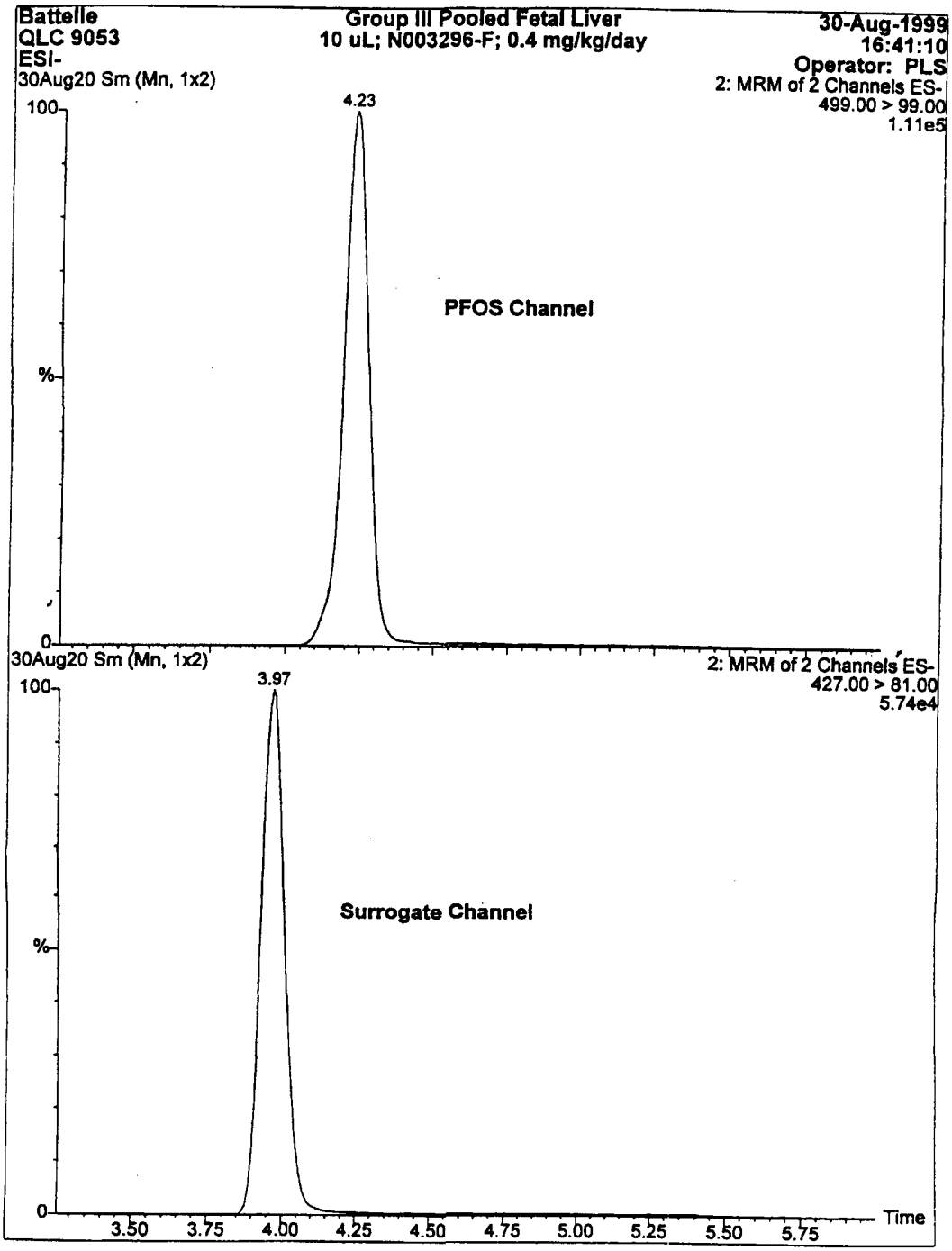
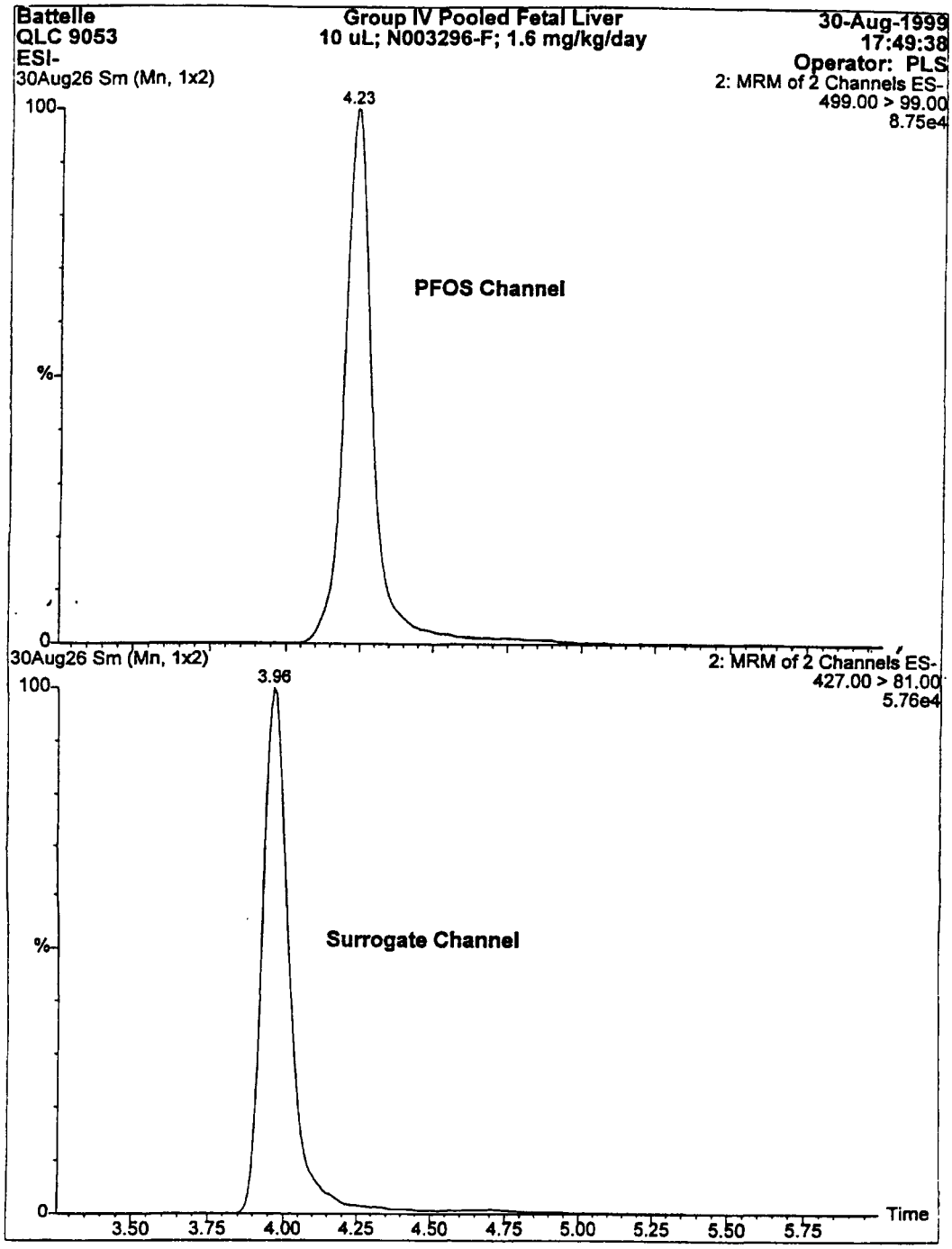
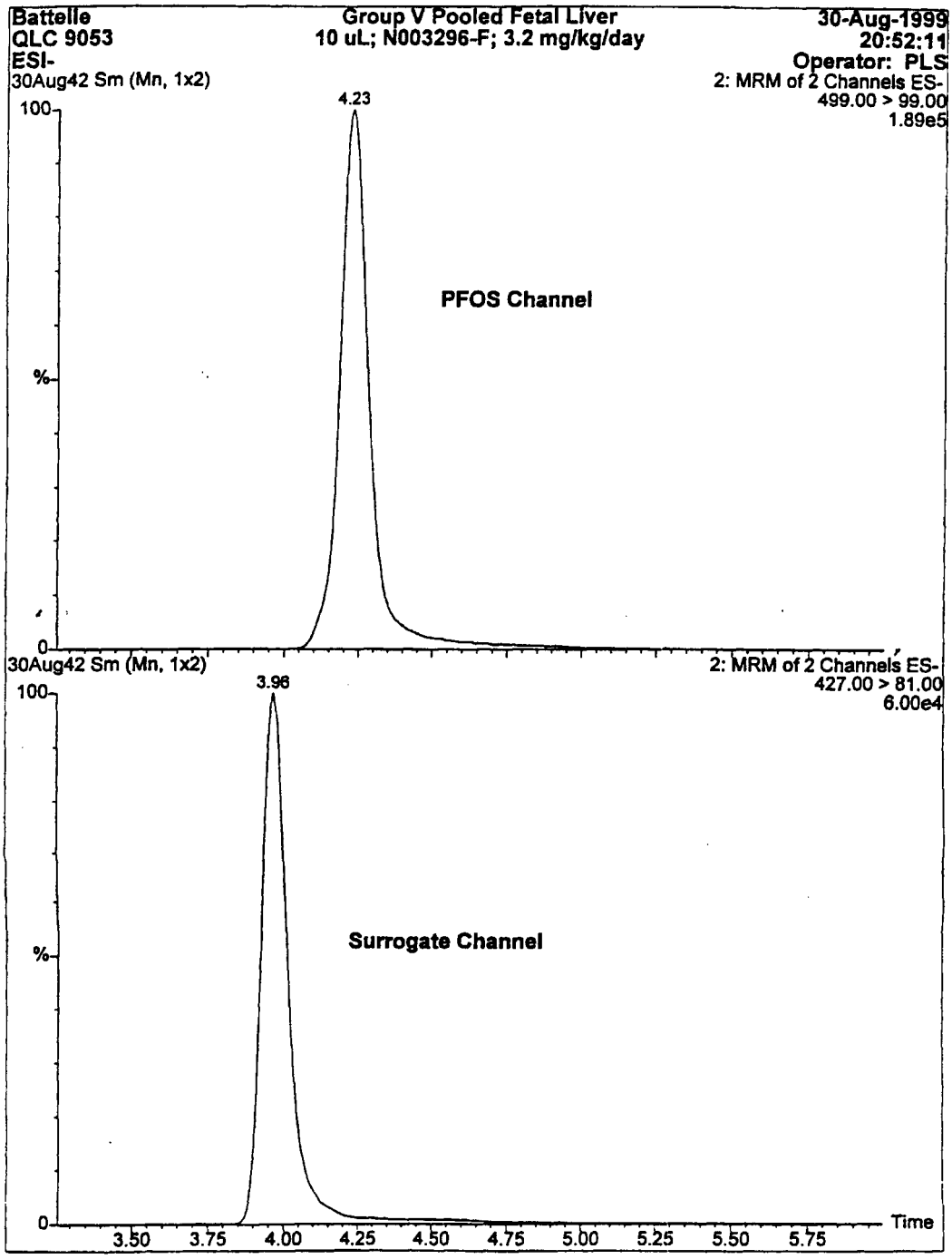


Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110



Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110



Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

APPENDIX E-PROTOCOL, AMENDMENTS, AND DEVIATION

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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

Protocol #FACT-TOX-110

3M

Study Title

Oral (Gavage) Pharmacokinetic 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-110
U2849

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

Study Identification

Oral (Gavage) Pharmacokinetic 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

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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

1. STUDY

Oral (gavage) pharmacokinetic 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 #418-013. 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 #418-013.

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 the Argus Research protocol #418-013. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation and continue through day 14 or 20 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	16	0	0	5
2	16	0.1	0.02	5
3	16	0.4	0.08	5
4	16	1.6	0.32	5
5	16	3.2	0.64	5

9. SPECIMEN AND SAMPLE RECEIPT

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

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Fetus animals	Dam-Predose, Days 7, 15, and 21 Fetus-At termination of the study	320 Dam and 320 Fetus (pooled)
Urine and feces – Dam animals	Predose, Days 7, 15, and 21	320 urine and 320 feces
Liver – Dam and Fetus animals	Dam and Fetus-At termination of the study	80 Dam and 80 Fetus (pooled)
Milk Secreting Glands – Dam animals	At the termination of the study	80
Amniotic Fluid – Dam animals	Day 15 and Day 21	80

Table 2 Cont.
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Embryo's with Placentae – Dam animals	Day 15 and Day 21	80
Carcass – Fetus animal	At the termination of the study	160
Placentae – Fetus animal	Day 15 and Day 21	80
Liver/lung – Fetus animal	At the termination of the study	80

Total number of expected specimens: 2000
Total number of test animals: 64
Total number of control animals: 16

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

10. Preparatory Methods

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

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

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

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

11. Analytical Methods

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

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

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

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

12. Data Quality Objectives

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

12.1 Linearity $r^2 \geq 0.98$

12.2 Limits of detection / quantitation

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

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

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

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

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

13. Sub-Contracted Analysis

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

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

14. Statistical Analysis

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

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

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

15. REPORT

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

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

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

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

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

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

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

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

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

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

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

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

17. SPECIMEN RETENTION

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

18. PROTOCOL AMENDMENTS AND DEVIATIONS

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

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

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

Kristen Hz 6/10/99
Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director Date

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

Study Title
Oral (Gavage) Pharmacokinetic 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-TOX110
LIRN U2849

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 1

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

1. ***PROTOCOL READS:*** Section 2.0 states this study is designed to determine potassium perfluorooctane sulfonic acid (PFOS) in specimens of liver and serum of rats.

AMEND TO READ: This study is designed to determine PFOS in specimens of rat liver, serum, and urine. All Day -1 and Gestation Day 21 rat urine specimens will be extracted and analyzed by the 3M Environmental Laboratory.

REASON: The urine extraction and analytical methods were not validated and approved prior to protocol approval.

2. ***PROTOCOL READS:*** Section 6.0 lists rat or rabbit liver and serum.

AMEND TO READ: Rat or rabbit urine from 3M Toxicology with a physical description of rat or rabbit urine.

REASON: The rat urine matrix was added after the protocol was approved.

3. ***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 to follow at the 3M Environmental Laboratory are:

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-8-7.0 "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"

3M Environmental Laboratory

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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Protocol FACT-TOX110
Amendment No. 1

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.
Methods ETS-8-96.0 and ETS-8-97.0 were not validated and approved until after the protocol was approved.

4. ***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.

5. ***PROTOCOL READS:*** Section 12.2.1 b) Liver method detection limit is 15 ppb.

AMEND TO READ: 12.2.1 b) Liver method detection limit is 8.50 ppb (ng/g).
12.2.1 c) Urine method detection limit is 1.5 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 in liver. A validation in urine was performed, after protocol approval, to support this method detection limit.

DREAC 9/27/99

KH 9/27/99

3M Environmental Laboratory

SEP 28 '99 12:21

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 1

Amendment Approval

Marvin Case

Marvin Case, D.V.M., Ph.D., Sponsor Representative — 17 August 1999 Date

Kris J. Hansen

Kris J. Hansen, Ph.D., Study Director — 8/18/99 Date

3M Environmental Laboratory

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

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 28, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX110
LIRN U2849

4. ***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.

5. ***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 documentation when shipping specimens for analyses performed by a sub-contract laboratory.

Protocol FACT-TOX110
Amendment No. 2

Amendment Approval

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

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

Study Title

Analytical Laboratory Report on the Determination of Perfluorooctanesulfonate (PFOS) Presence and Concentration in Serum, Liver, and Urine from the Gavage Study of T-6295.12

PROTOCOL AMENDMENT NO. 3

Amendment Date:

20 January 2000

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-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

Protocol LRN-U2849
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.

Protocol LRN-U2849
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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

DEVIATION REPORT

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

ORAL (GAVAGE) PHARMACOKINETIC STUDY
OF PFOS IN RATS

TYPE OF DEVIATION: PROTOCOL

DATE OF DEVIATION: August 19, 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 THE 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 — September 15, 1999
Jon C. Andre, Ph.D. — Date
Battelle Principal Investigator

Kristen Hansen — 10/1/99
Kristen J. Hansen, Ph.D. — Date
Study Director

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

APPENDIX F - PFOS PURITY REPORT

Battelle Study Number: N003296-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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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-F
3M Toxicology Services Protocol Number: FACT-TOX-110

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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%

ADVANCED BIOANALYTICAL SERVICES, INC.

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.
Holly D. Ross, M.S.

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 — 26Aug99

Kathleen Cormack, B.S. — Date

Quality Auditor

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 — 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