STUDY TITLE

Extraction of Potassium Perfluorooctanesulfonate from Quail Serum and Quail Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

DATA REQUIREMENTS

Analytical Method Requirements

STUDY DIRECTOR

Sean Gallagher

STUDY COMPLETED ON

September 06, 2001

PERFORMING LABORATORY / TESTING FACILITY

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STUDY SPONSOR

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PROJECT

Centre Protocol Number: 00P-023-041
Centre Study Number: 023-041

Total Pages: 102

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-041, entitled "Extraction of Potassium Perfluorooctanesulfonate from Quail Serum and Quail Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry," conducted for 3M Environmental Technology and Safety Services, was performed in compliance with US EPA TSCA Good Laboratory Practice Standards (40 CFR Part 792) by Centre Analytical Laboratories, Inc.

Emily R Decker — 9/6/01

Emily R. Decker
Principal Investigator
Centre Analytical Laboratories, Inc.

Date

Sean P. Gallagher — 9/10/01

Sean Gallagher
Study Director
Wildlife International, Ltd.

Date

Rochelle Robideau — 9/7/01

Rochelle Robideau
Sponsor Representative
3M Environmental Technology and Safety Services

Date

QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-041, entitled, "Extraction of Potassium Perfluorooctanesulfonate from Quail Serum and Quail Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry". All phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Centre Management	Date Reported to Study Director and Sponsor Management
1. Protocol Review	12/19/00	12/21/00	12/28/00
2. Extraction	12/19/00	12/21/00	12/28/00
3. Raw Data & Summary Table Review	2/28,3/1/01	3/19/01	3/23/01
4. Raw Data and Draft Report Review	3/23-26/01	4/9/01	5/7/01
5. Final Report Review	9/6/01	9/6/01	9/6/01

Naomi Lovallo
Sr. Quality Assurance Auditor

9/6/01
Date

CERTIFICATION OF AUTHENTICITY

This report, for Centre Study Number 023-041, is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.
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3M Environmental Technology and Safety Services

STUDY IDENTIFICATION

Extraction of Potassium Perfluorooctanesulfonate from Quail Serum and Quail Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

CENTRE PROTOCOL NUMBER:	00P-023-041
CENTRE STUDY NUMBER:	023-041
TYPE OF STUDY:	Analytical
SAMPLE MATRIX:	Quail Serum and Quail Liver
TEST SUBSTANCE:	Perfluorooctanesulfonate (PFOS)
SPONSOR:	3M Environmental Technology and Safety Services Building 2-3E-09 PO Box 33331 St. Paul, MN 55133-3331
STUDY DIRECTOR:	Sean Gallagher Wildlife International, Ltd. 8598 Commerce Drive Easton, MD 21601
SPONSOR REPRESENTATIVE:	Rochelle Robideau 3M Environmental Technology and Safety Services Building 2-3E-09 PO Box 33331 St. Paul, MN 55133-3331
TESTING FACILITY:	Centre Analytical Laboratories, Inc. 3048 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 12/08/00 Experimental Start Date: 12/18/00 Experimental Termination Date: 03/08/01 Study Completion Date: 09/06/01

PROJECT PERSONNEL

The Study Director for this project was Sean Gallagher at Wildlife International, Ltd. The following personnel from Centre Analytical Laboratories, Inc., were associated with various phases of the study:

Name	Title
Emily Stauffer	Scientist
Dave Bell	Scientist
Melissa Kennedy Whitsel	Supervisor
John Flaherty	Operations Manager
Tiffany Proctor	Technician
Angela Morgan	Technician
Michelle Arjmand	Technician
Lawrence Ord	Sample Custodian
Rickey Keller	Sample Custodian
Shawn Robb	Sample Custodian
Karen Risha	Scientist

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1.0 SUMMARY

Centre Analytical Laboratories, Inc. (Centre) extracted quail liver samples and quail serum samples for the determination of perfluorooctanesulfonate (PFOS) according to protocol 00P-023-041 (Appendix A).

The limit of quantation for quail liver was 10 ppb and for quail serum 10 ng/mL. These were determined in the method verification study (Centre study 023-054). PFOS in the quail liver samples ranged from non-quantifiable levels (a peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL)) to 8.37 µg/g. These results are reported on a wet weight basis. Residues of PFOS in the quail serum samples ranged from 0.00212 µg/mL levels to 263 µg/mL.

The average percent recovery ± standard deviation for PFOS in serum samples was 108% ± 10%. The average percent recovery ± standard deviation for PFOS in liver samples was 95% ± 11%.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of quail liver and quail serum using the analytical methods described in protocol 00P-023-041.

3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFOS in quail serum and quail liver, using the analytical methods entitled, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" with the modifications listed in Section 6.

The study was initiated on December 8, 2000, when the study director signed Centre protocol number 00P-023-041. The experimental start date was December 18, 2000, and the experimental termination date was March 8, 2001.

4.0 TEST SYSTEM

The control quail serum and liver used for the matrix blanks and matrix fortifications was received on November 28, 2000 from Wildlife International Ltd., Easton, MD.

The control quail liver was assigned the Centre ID of 0011423 and the control quail serum was assigned the Centre ID of 0011424.
Fifty-six quail serum samples and sixty quail liver samples were received at Centre on August 10, 2000. The samples were stored frozen until they were logged in by Centre personnel on August 16, 2000 and stored frozen. An additional twenty quail serum samples and twenty quail liver samples were received on February 23, 2001 and stored frozen until they were logged in by Centre personnel on February 26, 2001.

Sample login and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc. and a true copy of the storage records can be found in the raw data package associated with this study.

5.0 REFERENCE MATERIAL

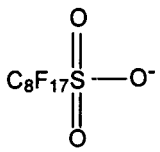
The analytical standard PFOS was received at Centre on June 3, 2000 from 3M Environmental Technology and Services.

The available information for the reference material is listed below. The reference material was stored frozen. Stability was not assessed in this study but the reference material was stored according to the certificate of analysis and was used prior to expiration. The solubility of the reference material was not relevant to this study.

Compound	Centre Control No.	TCR No.	Purity (%)	Expiration Date
PFOS	00-023-042	TCR 00017-46	97.9	08/31/01

The molecular structure of PFOS is given below.

PFOS
Chemical Name = Perfluorooctanesulfonate
Molecular weight = 499 ($C_8F_{17}SO_3^-$)



Note: The neutral molecule and standard form from which PFOS (anion) is derived, is potassium perfluorooctanesulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical methods entitled "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" with the modifications listed below were used for this study.

6.1 Extraction Procedure

A 100 µL aliquot of the serum and ~ 0.50 g sample of liver was used for the extraction procedure. After fortification of appropriate samples, the samples were vortexed for ~ 15 seconds. One mL of 0.5 M TBA was added to the liver samples and 0.5 mL was added to the serum samples. Two mL of 0.25 M sodium carbonate/sodium bicarbonate was added to the liver samples and 1 mL was added to the serum samples. Five mL of MTBE was added to both the serum and liver samples. Each sample was placed on wrist-action shaker for ~ 20 min. and then centrifuged for ~ 15 min. Four mL of the organic layer was taken and dried on an N-Evap and then reconstituted with 1 mL of methanol. Each sample was analyzed by LC/MS/MS electrospray.

6.2 Preparation of Standards and Fortification Solutions

Standard solutions were prepared on September 18, 2000 as specified in Centre Analytical Laboratories' protocol 00P-023-041. An individual stock standard solution of PFOS was prepared at a concentration of 100 µg/mL by dissolving 10 mg of the standard (corrected for purity and salt content) in methanol. From this solution, a 1.0 µg/mL fortification standard solution was prepared by taking 1 mL of the stock and bringing the volume up to 100 mL with methanol.

The 0.1 µg/mL fortification standard was prepared by taking 10 mL of the 1.0 µg/mL fortification standard and bringing the volume up to 100 mL with methanol. A 0.01 µg/mL standard was prepared by taking 10 mL of the 0.1 µg/mL standard and bringing to 100 mL with methanol.

A set of standards containing PFOS was prepared by dilution of the 0.1 µg/mL and 0.01 µg/mL solutions in the following manner.

Initial Conc. (µg/mL)[1]	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)
0.1	5	100	0.005
0.1	2	100	0.002
0.1	1	100	0.001
0.01	5	100	0.0005
0.01	2	100	0.0002
0.01	1	100	0.0001

[1] of PFOS

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

6.3 Chromatography

Quantification of PFOS was accomplished by LC/MS/MS analysis using electrospray LC/MS/MS. The retention time of PFOS was ~ 4.4 min. Peaks were detected in the control matrices corresponding to the analyte retention time and some of the amounts detected were significant enough to alter the results reported in this study.

6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run was equivalent to 0.0001 µg/mL of PFOS.

6.5 Description of Instrument and Operating Conditions

Mass Spec: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX TurboIon Spray Liquid Introduction Interface
Harvard infusion pump

Computer: Dell UltraScan P1110

Software: PE SciexAnalyst 1.1
Windows NT

HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column:Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in ASTM type I water
Mobile Phase (B): Methanol
Flow Rate: 0.3 mL/min.

Time	% A	% B
0	60	40
1.0	0	100
7.0	0	100
7.5	60	40
11.0	60	40

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFOS	negative	499 → 99	4.40

Tune File Parameters

Controls	Set
IS-Iospray	-4200.0
DP-Declustering Potential	-51.0
FP-Focusing Potential	-230.0
EP-Entrance Potential	10.0
CE-Collision Energy	-70
CXP-Collision Cell Exit Potential	-5
DF-Deflector	300.0
CEM-Channel Electron Multiplier	2800.0

Gas Flows	Set
Nebulizer Gas	12
Curtain Gas	13
Collision Gas	4
TIS Temperature	350°C

6.6 Quantitation and Example Calculation

Ten microliters of sample or calibration standard was injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x fit weighted linear regression) by Analyst software using six concentrations of standards. The concentration for quail serum and quail liver was determined from the following equations:

Use Equation 1 to calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program. Then use Equation 2 to calculate the amount of analyte found in µg/g (µg/mL for serum samples).

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

Equation 2:

$$\text{Analyte found (µg/g)} = \frac{\text{(anal. found (ng/mL)} \times \text{FV (mL)} \times \text{DF} \times \text{EV (mL))}}{\text{(AV (mL)} \times \text{sample wt. (g))}} \times \frac{1\ \mu g}{1000\ ng}$$

Where:
FV = Final Volume
DF = Dilution Factor
EV = Extraction Volume
AV = Aliquot Volume

For samples fortified with known amounts of PFOS prior to extraction, use Equation 3 to calculate the percent recovery.

Equation 3:

$$\text{Recovery (\%)} = \frac{\text{(anal. found (µg/g)} - \text{avg. analyte in ctrl (µg/g))} \times 1000\ ng \times 100\%}{\text{amount added (ng/g)} \times 1\ \mu g}$$

An example of a calculation using an actual sample follows:
Quail liver sample Centre ID 0007870 Spk A (Set: 121900A), fortified at 1000 ng/g with PFOS.
Where:

peak area	=	11483
intercept	=	463
slope	=	2790
dilution factor	=	100
ng/g added (fort level)	=	1000 ng/g
avg. amt in controls	=	0 (Not quantifiable)
final volume	=	1 mL
extraction volume	=	5 mL
aliquot volume	=	4 mL
sample weight	=	0.5 g

From equation 1:

$$\text{Analyte found (ng/mL)} = \frac{[11483 - 463]}{2790}$$

$$= 3.9\ \text{ng/mL}$$

From equation 2:

$$\text{Analyte found (µg/g)} = \frac{\text{(3.9 ng/mL} \times 1\ \text{mL} \times 100 \times 5\ \text{mL)}}{\text{(4 mL} \times 0.5\ \text{g)}} \times \frac{1\ \mu g}{1000\ ng}$$

$$= 0.975\ \mu g/mL$$

From equation 3:

$$\% \text{ Recovery} = \frac{(0.975\ \mu g/mL - 0\ \mu g/mL) \times (1000\ ng/1\mu g)}{1000\ ng/g} \times 100\%$$

$$= 98\%$$

7.0 EXPERIMENTAL DESIGN

Each set of samples (liver or serum) consisted of one reagent blank, one matrix blank, two matrix blanks fortified at known concentrations, and ~ 20 samples. Each sample was extracted using the appropriate method and then analyzed in duplicate.

8.0 RESULTS

The PFOS found in the reagent blanks are listed in **Table I**. The PFOS in the quail liver blanks and quail serum blanks are given in **Tables II & III**.

Individual recoveries for PFOS in the liver samples are detailed in **Table IV**. The average percent recovery ± standard deviation for PFOS in liver samples was 95% ± 11%. Individual recoveries for PFOS in the serum samples are detailed in **Table V**. The average percent recovery ± standard deviation for PFOS in serum samples was 108% ± 10%.

PFOS in the quail liver samples ranged from non-quantifiable levels (a peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL)) to 8.37 µg/g. Individual results are listed in **Tables VI-XIII**. The results are reported on a wet weight basis.

PFOS in the quail serum samples ranged from 0.00212 µg/mL levels to 263 µg/mL. Individual results are listed in **Tables XIV-XXI**.

The average percent moistures for the quail liver samples are given in **Table XXII**.

9.0 CONCLUSIONS

The quail liver and serum samples were successfully extracted and analyzed according to protocol 00P-023-041.

10.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 40 CFR Part 792. Retained samples of reference substances are archived by the sponsor.

TABLES

Table I. Summary of PFOS in Reagent Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
na	Reagent Blank A	121800A	12/18/00	12/19/00	NQ
na	Reagent Blank A*	121800A	12/18/00	12/19/00	NQ
na	Reagent Blank A	121800B	12/18/00	12/20/00	NQ
na	Reagent Blank A*	121800B	12/18/00	12/20/00	NQ
na	Reagent Blank A	121900A	12/19/00	12/21/00	NQ
na	Reagent Blank A*	121900A	12/19/00	12/21/00	NQ
na	Reagent Blank A	121900B	12/19/00	12/21-22/00	NQ
na	Reagent Blank A*	121900B	12/19/00	12/21-22/00	NQ
na	Reagent Blank A	122000A	12/20/00	12/22/00	0.000200
na	Reagent Blank A*	122000A	12/20/00	12/22/00	0.000200
na	Reagent Blank A	122000B	12/20/00	12/22-23/00	NQ
na	Reagent Blank A*	122000B	12/20/00	12/22-23/00	NQ
na	Reagent Blank A	030501A	3/5/01	3/6-7/01	ND
na	Reagent Blank A*	030501A	3/5/01	3/6-7/01	ND
na	Reagent Blank A	030601A	3/6/01	3/7/01	NQ
na	Reagent Blank A*	030601A	3/6/01	3/7/01	NQ
				AVERAGE:	**NQ**
			STANDARD DEVIATION:		**NQ**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))
ND = Not Detected

For values recorded as ND,zero was used to calculate the average and standard deviation. For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table II. Summary of PFOS in Quail Liver Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
na	0011423 Blank A	121800A	12/18/00	12/19/00	NQ
na	0011423 Blank A*	121800A	12/18/00	12/19/00	NQ
na	0011423 Blank A	121800B	12/18/00	12/20/00	0.00113
Na	0011423 Blank A*	121800B	12/18/00	12/20/00	0.00109
454	0007870 Blank A	121900A	12/19/00	12/21/00	NQ
454	0007870 Blank A*	121900A	12/19/00	12/21/00	NQ
456	0007872 Blank A	030601A	3/6/01	3/7/01	0.00120
456	0007872 Blank A*	030601A	3/6/01	3/7/01	0.00110
				AVERAGE:	**0.000590**
				STANDARD DEVIATION:	**0.000578**

Table III. Summary of PFOS in Quail Serum Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
na	0011424 Blank A	121900B	12/19/00	12/21-22/00	0.0136
na	0011424 Blank A*	121900B	12/19/00	12/21-22/00	0.0144
na	0011424 Blank A	122000A	12/20/00	12/22/00	0.0138
na	0011424 Blank A*	122000A	12/20/00	12/22/00	0.0151
na	0011424 Blank A	122000B	12/20/00	12/22-23/00	0.0127
na	0011424 Blank A*	122000B	12/20/00	12/22-23/00	0.0109
na	0011424 Blank A	030501A	3/5/01	3/6-7/01	0.00555
na	0011424 Blank A*	030501A	3/5/01	3/6-7/01	0.00559
				AVERAGE:	**0.0115**
				STANDARD DEVIATION:	**0.00384**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/g (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table IV. Summary of PFOS Recoveries in Quail Liver

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/g)	% Recovery
na	0011423 Spk A	121800A	12/18/00	12/19/00	10	102
na	0011423 Spk A*	121800A	12/18/00	12/19/00	10	104
na	0011423 Spk B	121800A	12/18/00	12/19/00	2000	101
na	0011423 Spk B*	121800A	12/18/00	12/19/00	2000	103
na	0011423 Spk A	121800B	12/18/00	12/20/00	1000	113
na	0011423 Spk A*	121800B	12/18/00	12/20/00	1000	110
na	0011423 Spk B	121900A	12/18/00	12/20-21/00	20000	80
na	0011423 Spk B*	121900A	12/18/00	12/20-21/00	20000	80
454	0007870 Spk A	121900A	12/19/00	12/21/00	1000	99
454	0007870 Spk A*	121900A	12/19/00	12/21/00	1000	103
454	0007870 Spk B	121900A	12/19/00	12/21/00	20000	85
454	0007870 Spk B*	121900A	12/19/00	12/21/00	20000	84
456	0007872 Spk A	030601A	3/6/01	3/7/01	20	93
456	0007872 Spk A*	030601A	3/6/01	3/7/01	20	86
456	0007872 Spk B	030601A	3/6/01	3/7/01	200	82
456	0007872 Spk B*	030601A	3/6/01	3/7/01	200	84
					AVERAGE:	**94**
					STANDARD DEVIATION:	**11**
					RELATIVE STANDARD DEVIATION:	**12**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table V. Summary of PFOS Recoveries in Quail Serum

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
na	0011424 Spk A	121900BD	12/19/00	12/23-24/00	500	103
na	0011424 Spk A*	121900BD	12/19/00	12/23-24/00	500	106
na	0011424 Spk B	121900BD	12/19/00	12/23-24/00	100000	90
na	0011424 Spk B*	121900BD	12/19/00	12/23-24/00	100000	93
na	0011424 Spk A	122000A	12/20/00	12/22/00	500	106
na	0011424 Spk A*	122000A	12/20/00	12/22/00	500	103
na	0011424 Spk B	122000A	12/20/00	12/22/00	100000	97
na	0011424 Spk B*	122000A	12/20/00	12/22/00	100000	103
na	0011424 Spk A	122000B	12/20/00	12/22-23/00	500	108
na	0011424 Spk A*	122000B	12/20/00	12/22-23/00	500	110
na	0011424 Spk B	122000B	12/20/00	12/22-23/00	100000	113
na	0011424 Spk B*	122000B	12/20/00	12/22-23/00	100000	98
na	0011424 Spk A	030501A	3/5/01	3/6-7/01	100	122
na	0011424 Spk A*	030501A	3/5/01	3/6-7/01	100	122
na	0011424 Spk B	030501A	3/5/01	3/6-7/01	1000	107
na	0011424 Spk B*	030501A	3/5/01	3/6-7/01	1000	115
					AVERAGE:	**106**
					STANDARD DEVIATION:	**9**
					RELATIVE STANDARD DEVIATION:	**9**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table VI. Summary of PFOS in Adult Liver Samples at 0 ppm a.i. (Test Termination 19 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
451	0007867	121800AD	12/18/00	12/20/00	0.215
451*	0007867	121800AD	12/18/00	12/20/00	0.215
452	0007868	121800A	12/18/00	12/19/00	NQ
452*	0007868	121800A	12/18/00	12/19/00	NQ
453	0007869	121800AD	12/18/00	12/20/00	0.303
453*	0007869	121800AD	12/18/00	12/20/00	0.304
454	0007870	121800A	12/18/00	12/19/00	NQ
454*	0007870	121800A	12/18/00	12/19/00	NQ
455	0007871	121800AD	12/18/00	12/20/00	0.114
455*	0007871	121800AD	12/18/00	12/20/00	0.111
456	0007872	121800A	12/18/00	12/19/00	NQ
456*	0007872	121800A	12/18/00	12/20/00	NQ
457	0007873	121800AD	12/18/00	12/20/00	0.0762
457*	0007873	121800AD	12/18/00	12/20/00	0.0739
458	0007874	121800A	12/18/00	12/19/00	0.0103
458*	0007874	121800A	12/18/00	12/19/00	0.0104
459	0007875	121800AD	12/18/00	12/20/00	0.236
459*	0007875	121800AD	12/18/00	12/20/00	0.235
460	0007876	121800A	12/18/00	12/19/00	0.00117
460*	0007876	121800A	12/18/00	12/19/00	0.00117
				AVERAGE:	**0.0953**
				STANDARD DEVIATION:	**0.113**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/g (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Termination was actually on wk 20. This entire report incorrectly advances wk 19.

Table VII. Summary of PFOS in Adult Liver Samples at 1.8 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
E01-0259-20780	0102997	030601AD	3/6/01	3/8/01	0.800
E01-0259-20780*	0102997	030601AD	3/6/01	3/8/01	0.722
E01-0259-20781	0102998	030601AD	3/6/01	3/8/01	0.149
E01-0259-20781*	0102998	030601AD	3/6/01	3/8/01	0.134
E01-0259-20782	0102999	030601AD	3/6/01	3/8/01	0.341
E01-0259-20782*	0102999	030601AD	3/6/01	3/8/01	0.311
E01-0259-20783	0103000	030601AD	3/6/01	3/8/01	0.455
E01-0259-20783*	0103000	030601AD	3/6/01	3/8/01	0.422
E01-0259-20784	0103001	030601AD	3/6/01	3/8/01	0.0988
E01-0259-20784*	0103001	030601AD	3/6/01	3/8/01	0.0843
E01-0259-20790	0103007	030601AD	3/6/01	3/8/01	0.210
E01-0259-20790*	0103007	030601AD	3/6/01	3/8/01	0.210
E01-0259-20791	0103008	030601AD	3/6/01	3/8/01	0.177
E01-0259-20791*	0103008	030601AD	3/6/01	3/8/01	0.191
E01-0259-20792	0103009	030601A	3/6/01	3/7/01	0.0124
E01-0259-20792*	0103009	030601A	3/6/01	3/7/01	0.0121
E01-0259-20793	0103010	030601AD	3/6/01	3/8/01	0.0381
E01-0259-20793*	0103010	030601AD	3/6/01	3/8/01	0.0394
E01-0259-20794	0103011	030601AD	3/6/01	3/8/01	0.0629
E01-0259-20794*	0103011	030601AD	3/6/01	3/8/01	0.0720
				AVERAGE:	**0.227**
				STANDARD DEVIATION:	**0.224**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table VIII. Summary of PFOS in Adult Liver Samples at 6.2 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
E01-0259-20785	0103002	030601AD	3/6/01	3/8/01	1.15
E01-0259-20785*	0103002	030601AD	3/6/01	3/8/01	1.20
E01-0259-20786	0103003	030601AD	3/6/01	3/8/01	1.18
E01-0259-20786*	0103003	030601AD	3/6/01	3/8/01	1.19
E01-0259-20787	0103004	030601AD	3/6/01	3/8/01	1.43
E01-0259-20787*	0103004	030601AD	3/6/01	3/8/01	1.42
E01-0259-20788	0103005	030601AD	3/6/01	3/8/01	1.60
E01-0259-20788*	0103005	030601AD	3/6/01	3/8/01	1.51
E01-0259-20789	0103006	030601AD	3/6/01	3/8/01	1.06
E01-0259-20789*	0103006	030601AD	3/6/01	3/8/01	1.17
E01-0259-20795	0103012	030601AD	3/6/01	3/8/01	0.216
E01-0259-20795*	0103012	030601AD	3/6/01	3/8/01	0.208
E01-0259-20796	0103013	030601AD	3/6/01	3/8/01	0.115
E01-0259-20796*	0103013	030601AD	3/6/01	3/8/01	0.0934
E01-0259-20797	0103014	030601AD	3/6/01	3/8/01	0.0801
E01-0259-20797*	0103014	030601AD	3/6/01	3/8/01	0.0797
E01-0259-20798	0103015	030601AD	3/6/01	3/8/01	0.187
E01-0259-20798*	0103015	030601AD	3/6/01	3/8/01	0.218
E01-0259-20799	0103016	030601AD	3/6/01	3/8/01	0.0901
E01-0259-20799*	0103016	030601AD	3/6/01	3/8/01	0.0954
				AVERAGE:	**0.715**
				STANDARD DEVIATION:	**0.606**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table IX. Summary of PFOS in Adult Liver Samples at 17.6 ppm a.i. (Test Termination 19 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
481	0007877	121800AD	12/18/00	12/20/00	1.05
481*	0007877	121800AD	12/18/00	12/20/00	1.05
482	0007878	121800AD	12/18/00	12/20/00	4.10
482*	0007878	121800AD	12/18/00	12/20/00	4.17
483	0007879	121800AD	12/18/00	12/20/00	8.37
483*	0007879	121800AD	12/18/00	12/20/00	8.30
484	0007880	121800AD	12/18/00	12/20/00	1.41
484*	0007880	121800AD	12/18/00	12/20/00	1.43
485	0007881	121800AD	12/18/00	12/20/00	1.54
485*	0007881	121800AD	12/18/00	12/20/00	1.51
486	0007882	121800AD	12/18/00	12/20/00	0.415
486*	0007882	121800AD	12/18/00	12/20/00	0.392
487	0007883	121800AD	12/18/00	12/20/00	1.17
487*	0007883	121800AD	12/18/00	12/20/00	1.15
488	0007884	121800AD	12/18/00	12/20/00	0.511
488*	0007884	121800AD	12/18/00	12/20/00	0.509
489	0007885	121800AD	12/18/00	12/20/00	2.42
489*	0007885	121800AD	12/18/00	12/20/00	2.48
490	0007886	121800AD	12/18/00	12/20/00	0.351
490*	0007886	121800AD	12/18/00	12/20/00	0.360
				AVERAGE:	**2.13**
				STANDARD DEVIATION:	**2.39**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table X. Summary of PFOS in Juvenile Liver Samples at 0 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
9503	0007907	121800B	12/18/00	12/20/00	NQ
9503*	0007907	121800B	12/18/00	12/20/00	NQ
9506	0007908	121800B	12/18/00	12/20/00	NQ
9506*	0007908	121800B	12/18/00	12/20/00	NQ
9508	0007909	121800B	12/18/00	12/20/00	NQ
9508*	0007909	121800B	12/18/00	12/20/00	NQ
9512	0007910	121800B	12/18/00	12/20/00	NQ
9512*	0007910	121800B	12/18/00	12/20/00	NQ
9515	0007911	121800B	12/18/00	12/20/00	0.000785
9515*	0007911	121800B	12/18/00	12/20/00	0.000755
9518	0007912	121800B	12/18/00	12/20/00	NQ
9518*	0007912	121800B	12/18/00	12/20/00	NQ
9520	0007913	121800B	12/18/00	12/20/00	0.000734
9520*	0007913	121800B	12/18/00	12/20/00	0.000672
9522	0007914	121800B	12/18/00	12/20/00	0.000718
9522*	0007914	121800B	12/18/00	12/20/00	0.000656
9525	0007915	121800B	12/18/00	12/20/00	NQ
9525*	0007915	121800B	12/18/00	12/20/00	NQ
9526	0007916	121800B	12/18/00	12/20/00	NQ
9526*	0007916	121800B	12/18/00	12/20/00	NQ
				AVERAGE:	**0.000251**
				STANDARD DEVIATION:	**0.000316**

* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/g (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XI. Summary of PFOS in Juvenile Liver Samples at 1.8 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
9528	0007917	121800B	12/18/00	12/20/00	0.0130
9528*	0007917	121800B	12/18/00	12/20/00	0.0128
9529	0007918	121800B	12/18/00	12/20/00	0.00295
9529*	0007918	121800B	12/18/00	12/20/00	0.00295
9530	0007919	121800B	12/18/00	12/20/00	0.00904
9530*	0007919	121800B	12/18/00	12/20/00	0.00876
9531	0007920	121800B	12/18/00	12/20/00	0.00417
9531*	0007920	121800B	12/18/00	12/20/00	0.00435
9533	0007921	121800B	12/18/00	12/20/00	0.0119
9533*	0007921	121800B	12/18/00	12/20/00	0.0117
9535	0007922	121800B	12/18/00	12/20/00	0.0113
9535*	0007922	121800B	12/18/00	12/20/00	0.0114
9540	0007923	121800B	12/18/00	12/20/00	0.00635
9540*	0007923	121800B	12/18/00	12/20/00	0.00623
9541	0007924	121800B	12/18/00	12/20/00	0.00305
9541*	0007924	121800B	12/18/00	12/20/00	0.00297
9543	0007925	121800B	12/18/00	12/20/00	0.0126
9543*	0007925	121800B	12/18/00	12/20/00	0.0126
9544	0007926	121800B	12/18/00	12/20/00	0.00514
9544*	0007926	121800B	12/18/00	12/20/00	0.00521
				AVERAGE:	**0.00792**
				STANDARD DEVIATION:	**0.00393**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XII. Summary of PFOS in Juvenile Liver Samples at 6.2 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
9548	0007927	121900A	12/19/00	12/21/00	0.00303
9548*	0007927	121900A	12/19/00	12/21/00	0.00305
9549	0007928	121900A	12/19/00	12/21/00	0.0100
9549*	0007928	121900A	12/19/00	12/21/00	0.0105
9551	0007929	121900A	12/19/00	12/21/00	0.0123
9551*	0007929	121900A	12/19/00	12/21/00	0.0125
9552	0007930	121900A	12/19/00	12/21/00	0.00316
9552*	0007930	121900A	12/19/00	12/21/00	0.00302
9555	0007931	121900AD	12/19/00	12/23/00	0.0410
9555*	0007931	121900AD	12/19/00	12/23/00	0.0408
9559	0007932	121900AD	12/19/00	12/23/00	0.0340
9559*	0007932	121900AD	12/19/00	12/23/00	0.0341
9561	0007933	121900AD	12/19/00	12/23/00	0.0446
9561*	0007933	121900AD	12/19/00	12/23/00	0.0445
9562	0007934	121900A	12/19/00	12/21/00	0.0116
9562*	0007934	121900A	12/19/00	12/21/00	0.0121
9563	0007935	121900AD	12/19/00	12/23/00	0.0193
9563*	0007935	121900AD	12/19/00	12/23/00	0.0197
9564	0007936	121900A	12/19/00	12/21/00	0.00184
9564*	0007936	121900A	12/19/00	12/21/00	0.00201
				AVERAGE:	**0.0182**
				STANDARD DEVIATION:	**0.0156**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XIII. Summary of PFOS in Juvenile Liver Samples at 17.6 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
9567	0007937	121900A	12/19/00	12/21/00	0.00904
9567*	0007937	121900A	12/19/00	12/21/00	0.00912
9570	0007938	121900AD	12/19/00	12/23/00	0.0424
9570*	0007938	121900AD	12/19/00	12/23/00	0.0429
9575	0007939	121900A	12/19/00	12/21/00	0.00629
9575*	0007939	121900A	12/19/00	12/21/00	0.00623
9579	0007940	121900AD	12/19/00	12/23/00	0.0880
9579*	0007940	121900AD	12/19/00	12/23/00	0.0898
9580	0007941	121900AD	12/19/00	12/23/00	0.0461
9580*	0007941	121900AD	12/19/00	12/23/00	0.0441
9581	0007942	121900AD	12/19/00	12/23/00	0.0492
9581*	0007942	121900AD	12/19/00	12/23/00	0.0451
9582	0007943	121900AD	12/19/00	12/23/00	0.0747
9582*	0007943	121900AD	12/19/00	12/23/00	0.0733
9583	0007944	121900AD	12/19/00	12/23/00	0.156
9583*	0007944	121900AD	12/19/00	12/23/00	0.132
9584	0007945	121900AD	12/19/00	12/23/00	0.0983
9584*	0007945	121900AD	12/19/00	12/23/00	0.101
9588	0007946	121900AD	12/19/00	12/23/00	0.0350
9588*	0007946	121900AD	12/19/00	12/23/00	0.0370
				AVERAGE:	**0.0593**
				STANDARD DEVIATION:	**0.0417**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XIV. Summary of PFOS in Adult Serum Samples at 0 ppm a.i. (Test Termination 19 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
451	0007887	121900BD	12/19/00	12/23-24/00	11.6
451*	0007887	121900BD	12/19/00	12/23-24/00	11.8
452	0007888	121900B	12/19/00	12/21-22/00	0.0102
452*	0007888	121900B	12/19/00	12/21-22/00	0.00909
453	0007889	121900BD	12/19/00	12/23-24/00	9.04
453*	0007889	121900BD	12/19/00	12/23-24/00	10.4
454	0007890	121900B	12/19/00	12/21-22/00	0.00274
454*	0007890	121900B	12/19/00	12/21-22/00	0.00212
455	0007891	121900B	12/19/00	12/21-22/00	0.0125
455*	0007891	121900B	12/19/00	12/21-22/00	0.0123
456	0007892	121900BD	12/19/00	12/23-24/00	6.32
456*	0007892	121900BD	12/19/00	12/23-24/00	6.46
457	0007893	121900BD	12/19/00	12/23-24/00	6.58
457*	0007893	121900BD	12/19/00	12/23-24/00	6.35
458	0007894	121900BD	12/19/00	12/23-24/00	0.105
458*	0007894	121900BD	12/19/00	12/23-24/00	0.102
459	0007895	121900BD	12/19/00	12/23-24/00	8.75
459*	0007895	121900BD	12/19/00	12/23-24/00	8.50
				AVERAGE:	**4.78**
				STANDARD DEVIATION:	**4.64**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XV. Summary of PFOS in Adult Serum Samples at 1.8 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0259-20752	0102969	030501AD	3/5/01	3/7-8/01	10.9
E01-0259-20752*	0102969	030501AD	3/5/01	3/7-8/01	10.9
E01-0259-20753	0102970	030501AD	3/5/01	3/7-8/01	7.92
E01-0259-20753*	0102970	030501AD	3/5/01	3/7-8/01	8.01
E01-0259-20754	0102971	030501AD	3/5/01	3/7-8/01	16.4
E01-0259-20754*	0102971	030501AD	3/5/01	3/7-8/01	16.0
E01-0259-20755	0102972	030501AD	3/5/01	3/7-8/01	35.8
E01-0259-20755*	0102972	030501AD	3/5/01	3/7-8/01	33.1
E01-0259-20756	0102973	030501AD	3/5/01	3/7-8/01	24.3
E01-0259-20756*	0102973	030501AD	3/5/01	3/7-8/01	23.7
E01-0259-20762	0102979	030501AD	3/5/01	3/7-8/01	1.50
E01-0259-20762*	0102979	030501AD	3/5/01	3/7-8/01	1.89
E01-0259-20763	0102980	030501AD	3/5/01	3/7-8/01	5.01
E01-0259-20763*	0102980	030501AD	3/5/01	3/7-8/01	4.98
E01-0259-20764	0102981	030501AD	3/5/01	3/7-8/01	1.57
E01-0259-20764*	0102981	030501AD	3/5/01	3/7-8/01	1.84
E01-0259-20765	0102982	030501AD	3/5/01	3/7-8/01	0.134
E01-0259-20765*	0102982	030501AD	3/5/01	3/7-8/01	0.117
E01-0259-20766	0102983	030501AD	3/5/01	3/7-8/01	10.5
E01-0259-20766*	0102983	030501AD	3/5/01	3/7-8/01	9.79
				AVERAGE:	**11.2**
				STANDARD DEVIATION:	**10.7**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XVI. Summary of PFOS in Adult Serum Samples at 6.2 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0259-20757	0102974	030501AD	3/5/01	3/7-8/01	52.2
E01-0259-20757*	0102974	030501AD	3/5/01	3/7-8/01	56.1
E01-0259-20758	0102975	030501AD	3/5/01	3/7-8/01	102
E01-0259-20758*	0102975	030501AD	3/5/01	3/7-8/01	94.7
E01-0259-20759	0102976	030501AD	3/5/01	3/7-8/01	80.4
E01-0259-20759*	0102976	030501AD	3/5/01	3/7-8/01	84.6
E01-0259-20760	0102977	030501AD	3/5/01	3/7-8/01	69.2
E01-0259-20760*	0102977	030501AD	3/5/01	3/7-8/01	62.9
E01-0259-20761	0102978	030501AD	3/5/01	3/7-8/01	84.6
E01-0259-20761*	0102978	030501AD	3/5/01	3/7-8/01	86.7
E01-0259-20767	0102984	030501AD	3/5/01	3/7-8/01	4.57
E01-0259-20767*	0102984	030501AD	3/5/01	3/7-8/01	4.07
E01-0259-20768	0102985	030501AD	3/5/01	3/7-8/01	2.72
E01-0259-20768*	0102985	030501AD	3/5/01	3/7-8/01	2.61
E01-0259-20769	0102986	030501AD	3/5/01	3/7-8/01	4.02
E01-0259-20769*	0102986	030501AD	3/5/01	3/7-8/01	3.87
E01-0259-20770	0102987	030501AD	3/5/01	3/7-8/01	3.24
E01-0259-20770*	0102987	030501AD	3/5/01	3/7-8/01	3.31
E01-0259-20771	0102988	030501AD	3/5/01	3/7-8/01	2.10
E01-0259-20771*	0102988	030501AD	3/5/01	3/7-8/01	1.70
				AVERAGE:	**40.3**
				STANDARD DEVIATION:	**39.7**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XVII. Summary of PFOS in Adult Serum Samples at 17.6 ppm a.i. (Test Termination 19 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
481	0007896	121900BD	12/19/00	12/23-24/00	260
481*	0007896	121900BD	12/19/00	12/23-24/00	263
482	0007897	121900BD	12/19/00	12/23-24/00	43.1
482*	0007897	121900BD	12/19/00	12/23-24/00	42.4
484	0007898	121900BD	12/19/00	12/23-24/00	118
484*	0007898	121900BD	12/19/00	12/23-24/00	106
485	0007899	121900BD	12/19/00	12/23-24/00	179
485*	0007899	121900BD	12/19/00	12/23-24/00	172
487	0007900	121900BD	12/19/00	12/23-24/00	133
487*	0007900	121900BD	12/19/00	12/23-24/00	127
488	0007901	121900BD	12/19/00	12/23-24/00	15.8
488*	0007901	121900BD	12/19/00	12/23-24/00	16.8
489	0007902	030501AD	3/5/01	3/7-8/01	232
489*	0007902	030501AD	3/5/01	3/7-8/01	228
490	0007903	121900BD	12/19/00	12/23-24/00	6.87
490*	0007903	121900BD	12/19/00	12/23-24/00	6.80
				AVERAGE:	**122**
				STANDARD DEVIATION:	**93.0**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XVIII. Summary of PFOS in Juvenile Serum Samples at 0 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
9503	0007947	122000A	12/20/00	12/22/00	0.00486
9503*	0007947	122000A	12/20/00	12/22/00	0.00385
9506	0007948	122000A	12/20/00	12/22/00	0.00718
9506*	0007948	122000A	12/20/00	12/22/00	0.00754
9508	0007949	122000A	12/20/00	12/22/00	0.00365
9508*	0007949	122000A	12/20/00	12/22/00	0.00319
9512	0007950	122000A	12/20/00	12/22/00	0.00431
9512*	0007950	122000A	12/20/00	12/22/00	0.00457
9515	0007951	122000A	12/20/00	12/22/00	0.00789
9515*	0007951	122000A	12/20/00	12/22/00	0.00528
9518	0007952	122000A	12/20/00	12/22/00	0.00378
9518*	0007952	122000A	12/20/00	12/22/00	0.00481
9520	0007953	122000A	12/20/00	12/22/00	0.00367
9520*	0007953	122000A	12/20/00	12/22/00	0.00395
9525	0007954	122000A	12/20/00	12/22/00	0.00552
9525*	0007954	122000A	12/20/00	12/22/00	0.00438
9526	0007955	122000A	12/20/00	12/22/00	0.00354
9526*	0007955	122000A	12/20/00	12/22/00	0.00388
				AVERAGE:	**0.00477**
				STANDARD DEVIATION:	**0.00142**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XIX. Summary of PFOS in Juvenile Serum Samples at 1.8 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
9528	0007956	122000D	12/20/00	12/24/00	0.0762
9528*	0007956	122000D	12/20/00	12/24/00	0.0670
9529	0007957	122000A	12/20/00	12/22/00	0.0273
9529*	0007957	122000A	12/20/00	12/22/00	0.0284
9530	0007958	122000A	12/20/00	12/22/00	0.00785
9530*	0007958	122000A	12/20/00	12/22/00	0.00824
9531	0007959	122000A	12/20/00	12/22/00	0.0480
9531*	0007959	122000A	12/20/00	12/22/00	0.0476
9533	0007960	122000A	12/20/00	12/22/00	0.0488
9533*	0007960	122000A	12/20/00	12/22/00	0.0494
9535	0007961	122000A	12/20/00	12/22/00	0.0416
9535*	0007961	122000A	12/20/00	12/22/00	0.0413
9540	0007962	122000A	12/20/00	12/22/00	0.0192
9540*	0007962	122000A	12/20/00	12/22/00	0.0189
9541	0007963	122000A	12/20/00	12/22/00	0.0321
9541*	0007963	122000A	12/20/00	12/22/00	0.0334
9543	0007964	122000A	12/20/00	12/22/00	0.00969
9543*	0007964	122000A	12/20/00	12/22/00	0.00983
9544	0007965	122000A	12/20/00	12/22/00	0.0158
9544*	0007965	122000A	12/20/00	12/22/00	0.0166
				AVERAGE:	**0.0324**
				STANDARD DEVIATION:	**0.0198**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XX. Summary of PFOS in Juvenile Serum Samples at 6.2 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
9548	0007966	122000D	12/20/00	12/24/00	0.137
9548*	0007966	122000D	12/20/00	12/24/00	0.160
9549	0007967	122000D	12/20/00	12/24/00	0.382
9549*	0007967	122000D	12/20/00	12/24/00	0.390
9551	0007968	122000D	12/20/00	12/24/00	0.222
9551*	0007968	122000D	12/20/00	12/24/00	0.208
9552	0007969	122000B	12/20/00	12/22-23/00	0.0542
9552*	0007969	122000B	12/20/00	12/22-23/00	0.0535
9555	0007970	122000D	12/20/00	12/24/00	0.243
9555*	0007970	122000D	12/20/00	12/24/00	0.218
9559	0007971	122000D	12/20/00	12/24/00	0.161
9559*	0007971	122000D	12/20/00	12/24/00	0.168
9561	0007972	122000D	12/20/00	12/24/00	0.185
9561*	0007972	122000D	12/20/00	12/24/00	0.171
9562	0007973	122000D	12/20/00	12/24/00	0.231
9562*	0007973	122000D	12/20/00	12/24/00	0.229
9563	0007974	122000D	12/20/00	12/24/00	0.259
9563*	0007974	122000D	12/20/00	12/24/00	0.254
9564	0007975	122000D	12/20/00	12/24/00	0.171
9564*	0007975	122000D	12/20/00	12/24/00	0.173
				AVERAGE:	**0.203**
				STANDARD DEVIATION:	**0.0838**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XXI. Summary of PFOS in Juvenile Serum Samples at 17.6 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
9567	0007976	122000D	12/20/00	12/24/00	0.329
9567*	0007976	122000D	12/20/00	12/24/00	0.355
9570	0007977	122000D	12/20/00	12/24/00	0.483
9570*	0007977	122000D	12/20/00	12/24/00	0.512
9575	0007978	122000D	12/20/00	12/24/00	0.241
9575*	0007978	122000D	12/20/00	12/24/00	0.252
9579	0007979	122000D	12/20/00	12/24/00	0.406
9579*	0007979	122000D	12/20/00	12/24/00	0.424
9580	0007980	122000D	12/20/00	12/24/00	0.287
9580*	0007980	122000D	12/20/00	12/24/00	0.254
9581	0007981	122000D	12/20/00	12/24/00	0.295
9581*	0007981	122000D	12/20/00	12/24/00	0.285
9582	0007982	122000D	12/20/00	12/24/00	0.459
9582*	0007982	122000D	12/20/00	12/24/00	0.473
9583	0007983	122000D	12/20/00	12/24/00	0.521
9583*	0007983	122000D	12/20/00	12/24/00	0.524
9584	0007984	122000D	12/20/00	12/24/00	0.553
9584*	0007984	122000D	12/20/00	12/24/00	0.564
9588	0007985	122000D	12/20/00	12/24/00	0.532
9588*	0007985	122000D	12/20/00	12/24/00	0.509
				AVERAGE:	**0.413**
				STANDARD DEVIATION:	**0.114**

* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

Table XXII. Summary of Percent Moistures for Quail Liver Samples

Sponsor ID	Centre ID	% Moisture	Sponsor ID	Centre ID	% Moisture
451	0007867	66.7	9559	0007932	78.3
452	0007868	64.4	9561	0007933	80.6
453	0007869	68.4	9562	0007934	57.8
454	0007870	65.6	9563	0007935	63.4
455	0007871	64.1	9564	0007936	34.6
456	0007872	71.8	9570	0007938	85.2
457	0007873	59.5	9579	0007940	88.7
458	0007874	71.2	9584	0007945	59.6
460	0007876	69.0	9588	0007946	65.2
482	0007878	55.0	E01-0259-20780	0102997	70.3
484	0007880	60.6	E01-0259-20781	0102998	57.8
486	0007882	72.5	E01-0259-20782	0102999	70.6
488	0007884	61.5	E01-0259-20783	0103000	67.0
490	0007886	65.1	E01-0259-20784	0103001	67.7
9506	0007908	70.7	E01-0259-20785	0103002	64.5
9508	0007909	72.1	E01-0259-20786	0103003	72.3
9512	0007910	67.4	E01-0259-20787	0103004	67.6
9518	0007912	73.2	E01-0259-20788	0103005	68.0
9520	0007913	73.5	E01-0259-20789	0103006	68.0
9522	0007914	68.3	E01-0259-20790	0103007	69.3
9526	0007916	67.9	E01-0259-20791	0103008	70.9
9530	0007919	70.0	E01-0259-20792	0103009	67.0
9540	0007923	66.7	E01-0259-20793	0103010	65.3
9543	0007925	57.1	E01-0259-20794	0103011	74.5
9544	0007926	55.8	E01-0259-20795	0103012	65.7
9548	0007927	59.4	E01-0259-20796	0103013	71.7
9551	0007929	81.3	E01-0259-20797	0103014	72.8
9552	0007930	53.5	E01-0259-20798	0103015	71.3
9555	0007931	65.0	E01-0259-20799	0103016	72.1

FIGURES

Figure 1. Typical Calibration Curve for PFOS

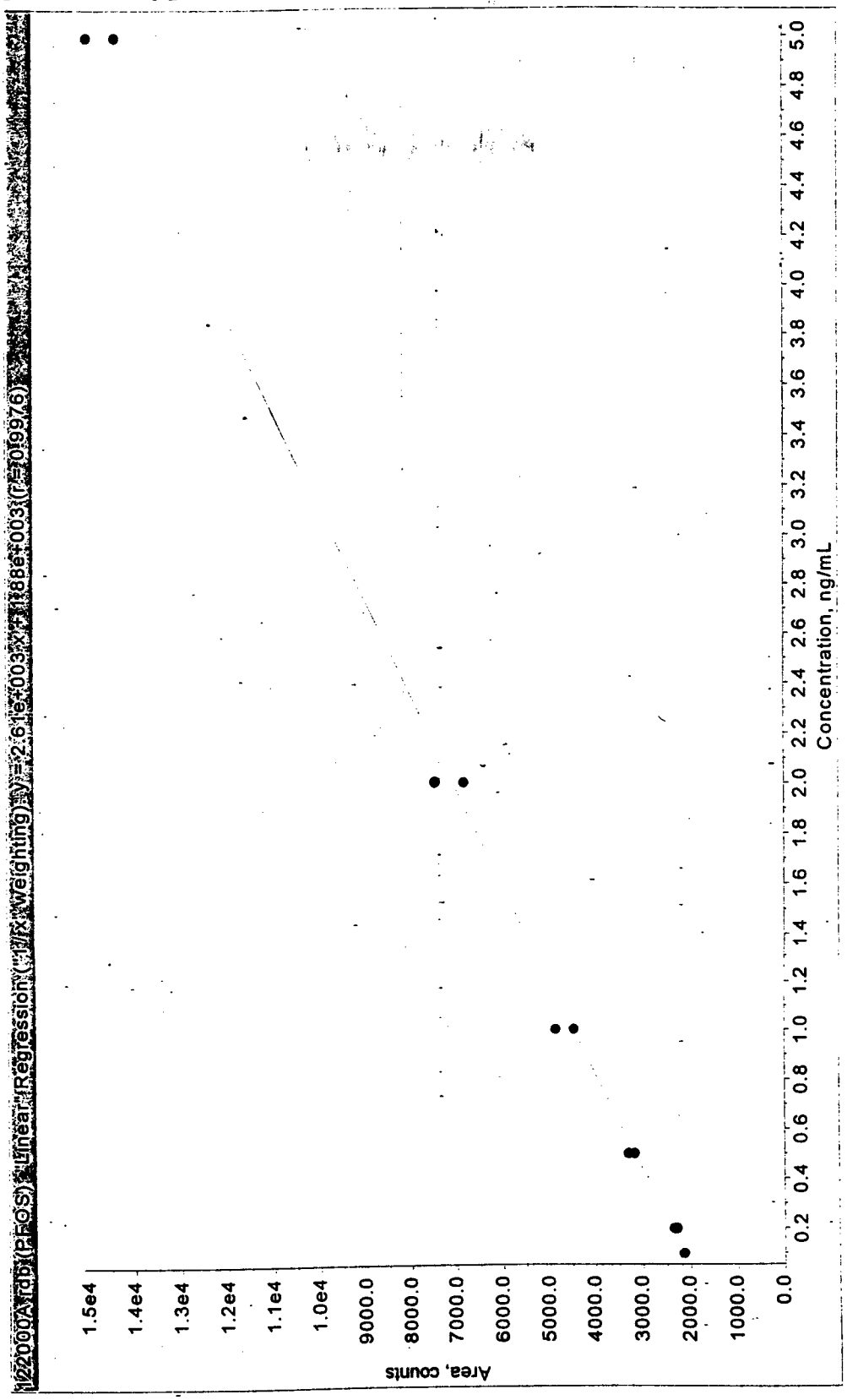


Figure 2. Chromatogram Representing a 0.1 ng/mL standard for PFOS

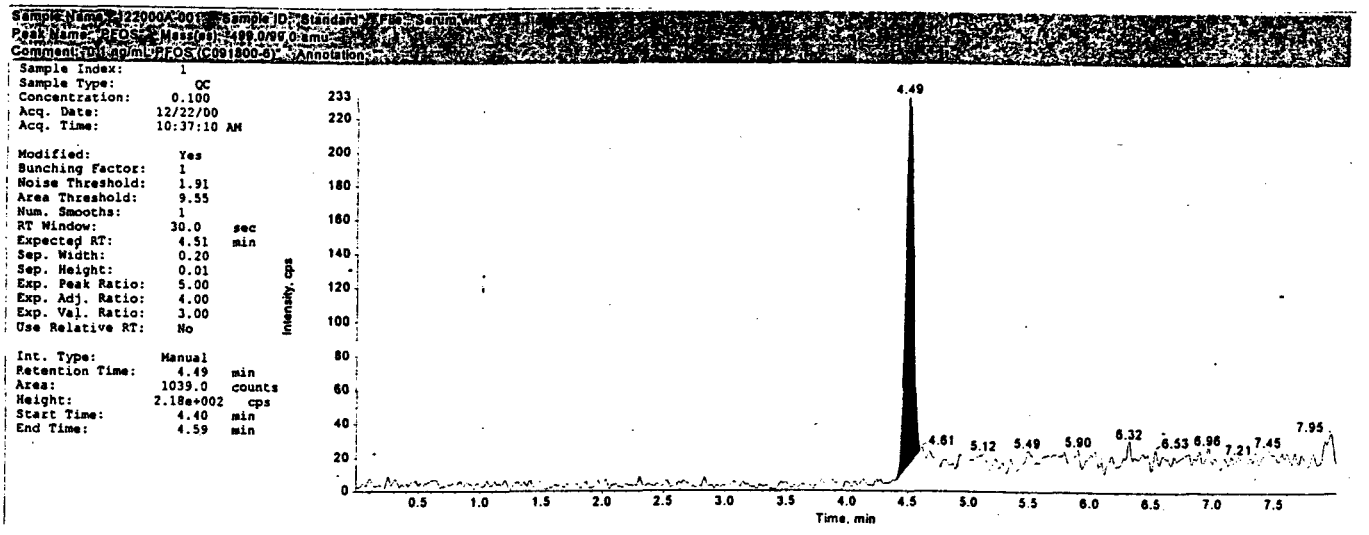


Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS

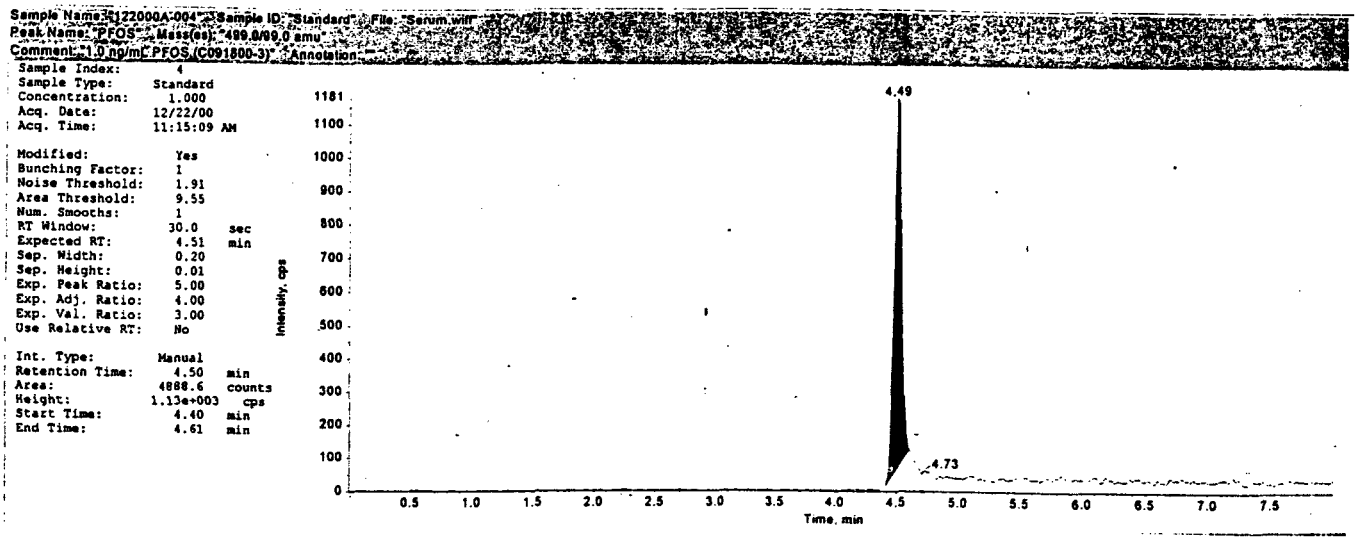


Figure 4. Chromatogram Representing a Reagent Blank for PFOS (Centre ID Reagent Blank A, Set: 122000A)

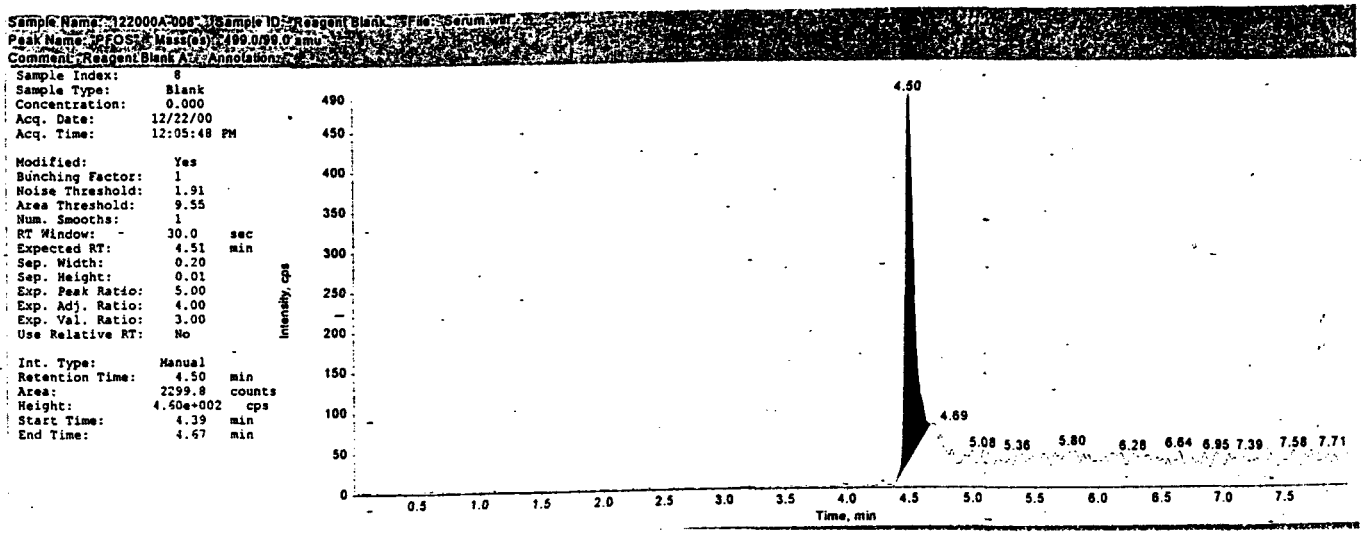


Figure 5. Chromatogram Representing Control Quail Serum for PFOS (Centre ID: 0011424 Blank A, Set: 122000A)

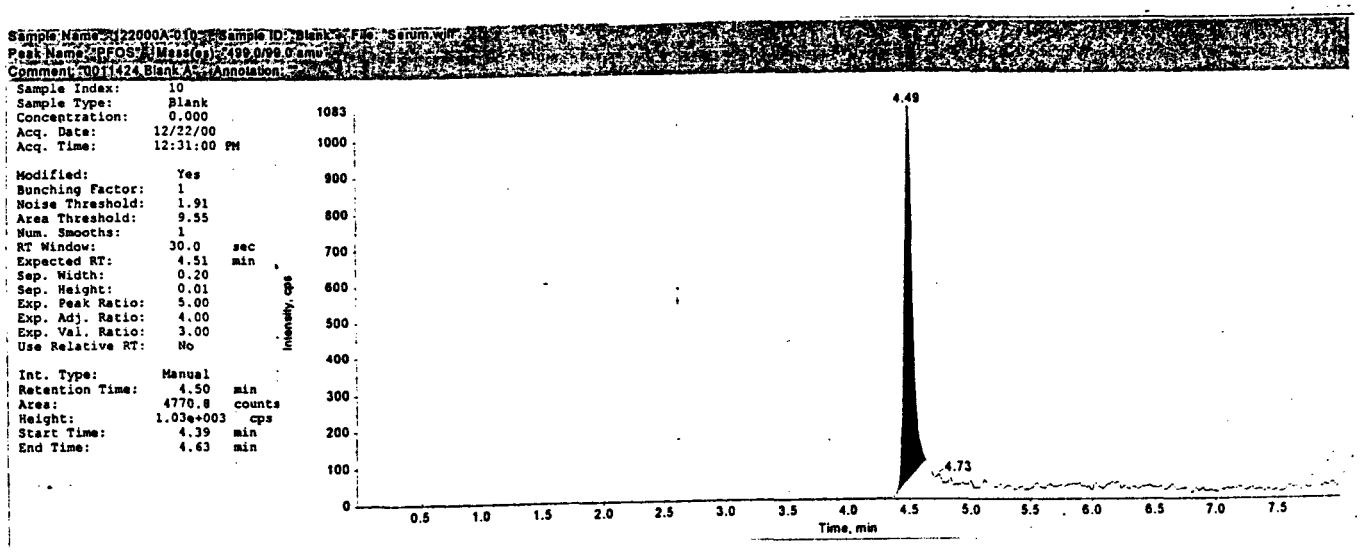


Figure 6. Chromatogram Representing Control Quail Liver for PFOS, (Centre ID: 0007870 Blank A, Set: 121900A)

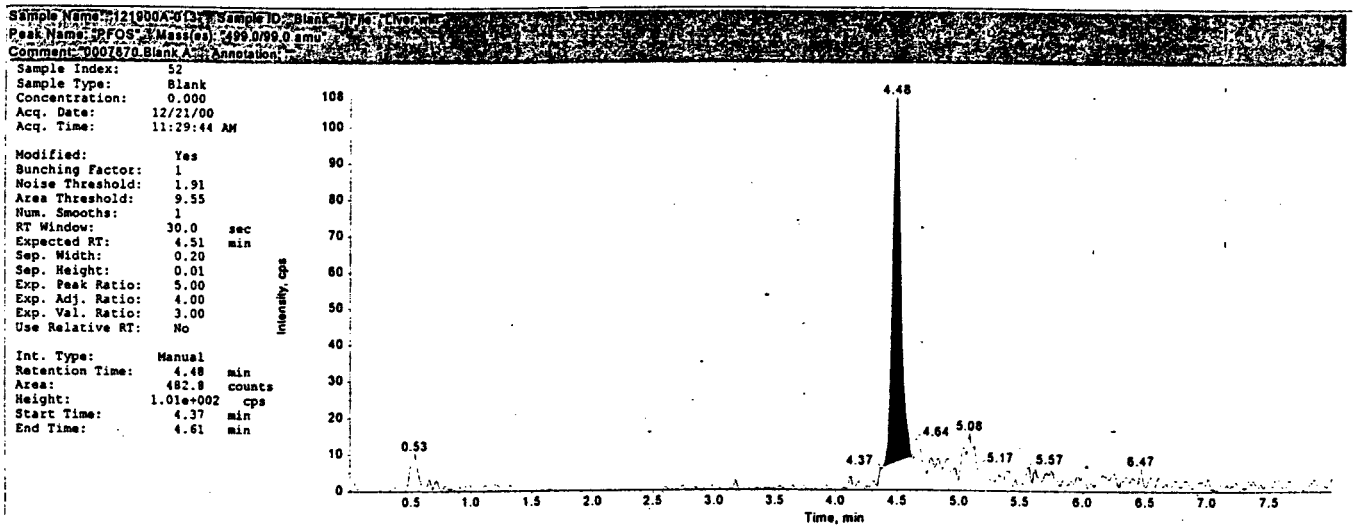


Figure 7. Chromatogram Representing Control Quail Serum Fortified with 500 ng/mL of PFOS (Centre ID: 0011424 Spk A, Set: 122000A)

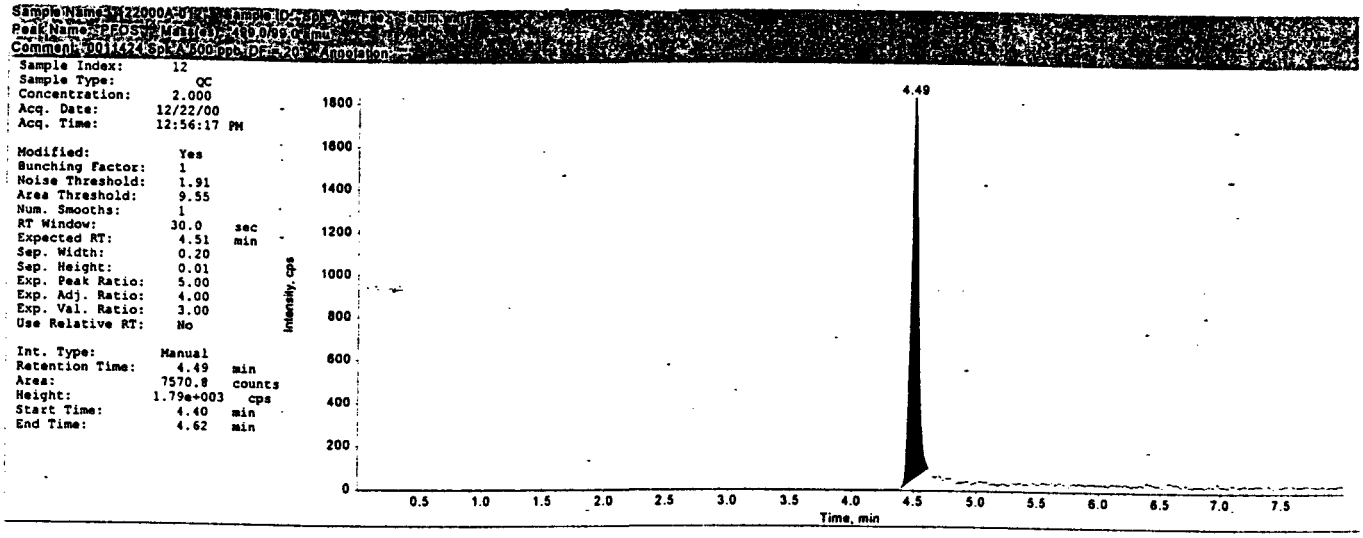


Figure 8. Chromatogram Representing Control Quail Liver Fortified at 10 ppb with PFOS (Centre ID: 0011423 Spk A, Set: 121800A)

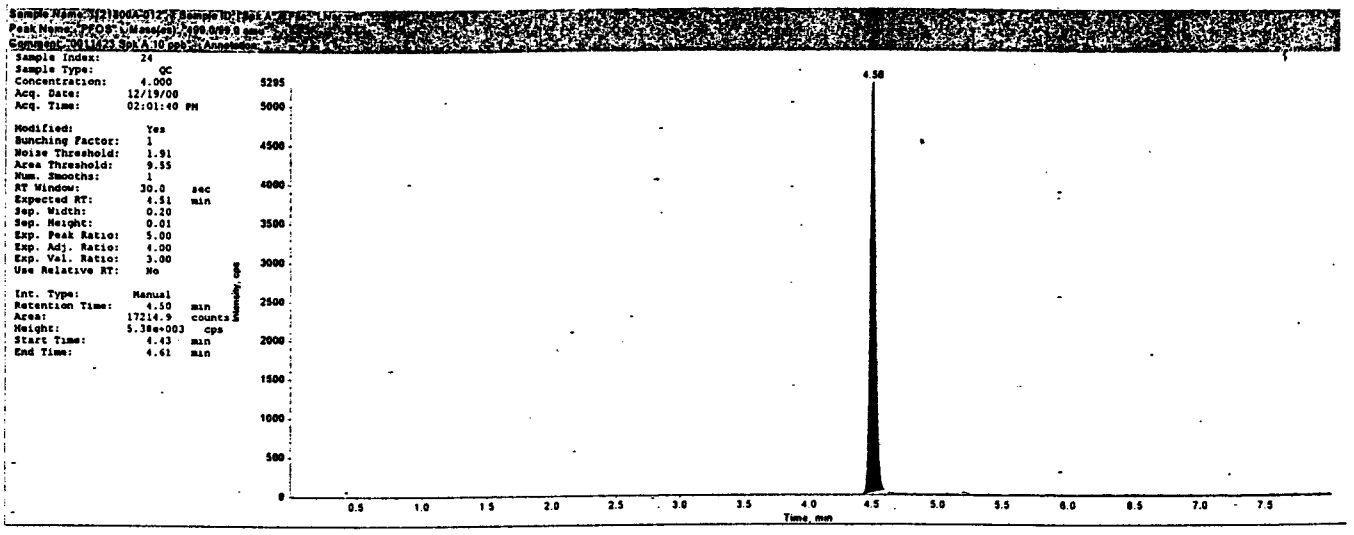


Figure 9. Chromatogram Representing Juvenile Quail Serum Sample (Centre ID: 0007965, Sponsor ID: 9544, Set: 122000A)

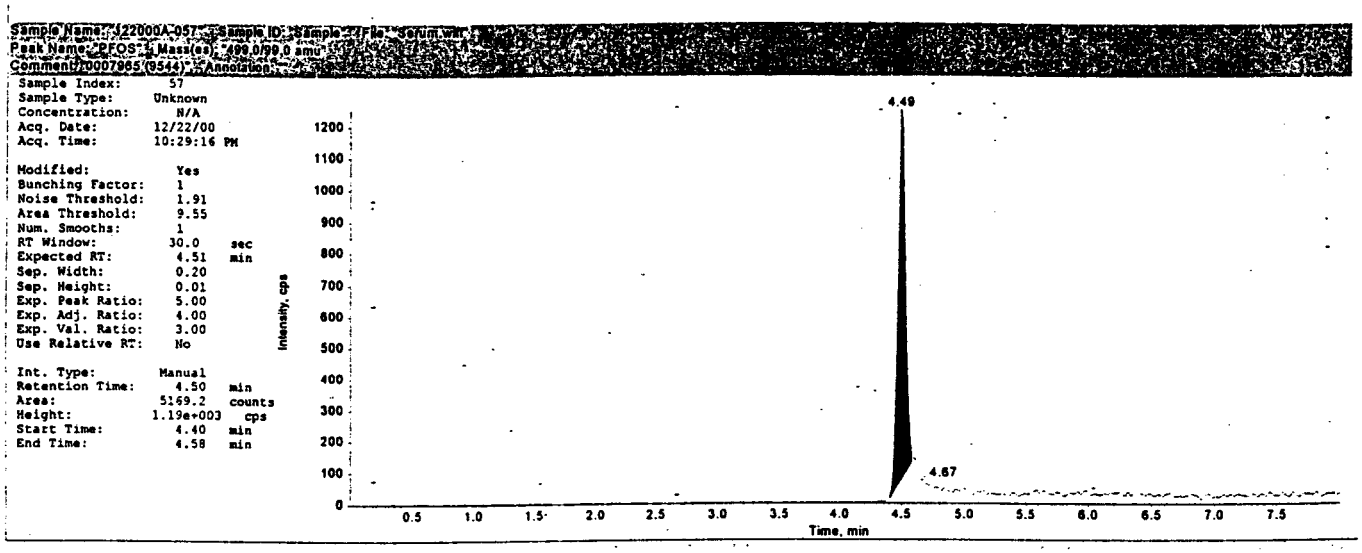
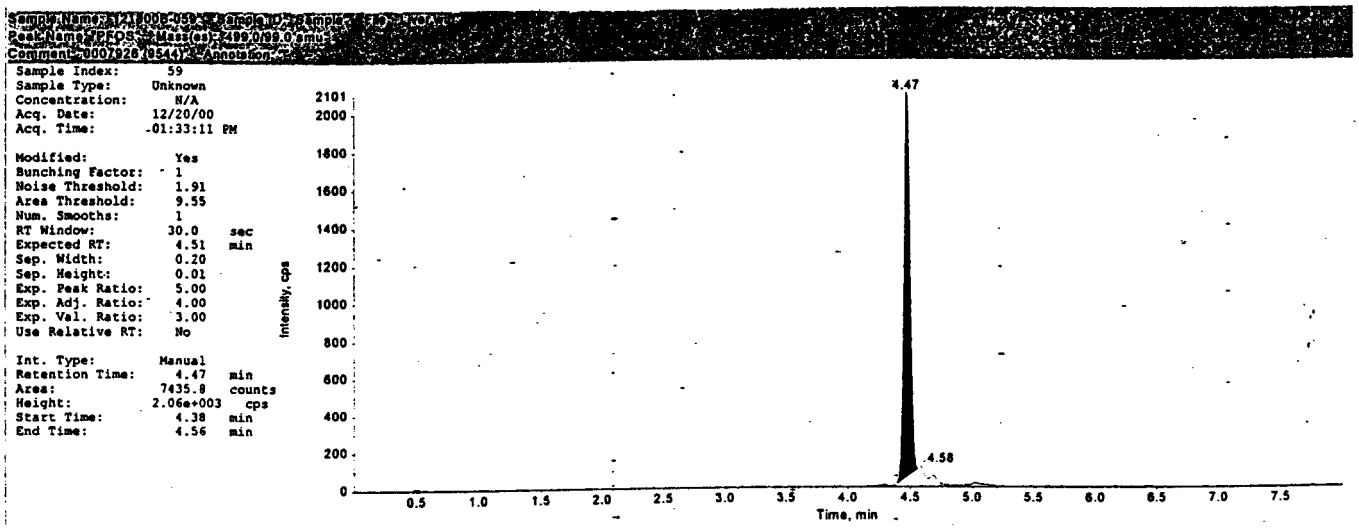


Figure 10. Chromatogram Representing Juvenile Quail Liver Sample (Centre ID: 0007926, Sponsor ID: 9544, Set: 121800B)



APPENDIX A

Study Protocol
00P-023-041
(Centre Study No. 023-041)
and
Amendments and Deviations

Centre Protocol No. 00P-023-041

STUDY PROTOCOL

STUDY TITLE

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM QUAIL SERUM AND QUAIL LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

SPONSOR

3M Environmental Technology and Safety Services
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

DATA REQUIREMENTS

Analytical Method Requirements

TESTING LABORATORY

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

PROTOCOL IDENTIFICATION NUMBER

00P-023-041

Centre Protocol No. 00P-023-041

Title: EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM QUAIL SERUM AND QUAIL LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

TABLE OF CONTENTS

Study Title:
EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM QUAIL SERUM AND QUAIL LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

1. PURPOSE

The purpose of this study is to analyze quail serum samples and quail liver samples for residues of perfluorooctanesulfonate (PFOS) using methods entitled, "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) and "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Liver For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-6.0) with the modifications listed in Section 8.

The Quality Assurance Unit of Centre Analytical Laboratories, Inc., will audit the study for compliance with EPA TSCA Good Laboratory Practice standards 40 CFR Part 792 (1).

2. TEST MATERIALS

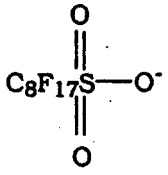
The following analytical standards will be used:

Test Material	TCR Number	Purity (%)	Expiration Date
PFOS	TCR00017-46	97.9	08/31/01

Chemical name and structure of the compound is presented below.

PFOS

Chemical Name: Perfluorooctanesulfonate
IUPAC Name: 1-Octanesulfonic acid,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt
CAS Number: 2795-39-3
Molecular Weight: 499 ($C_8F_{17}SO_3^-$)



Note: The neutral molecule and standard form that PFOS (anion) is derived from is perfluorooctanesulfonate potassium salt [$C_8F_{17}SO_3K$], molecular weight 538.

A record of test and reference substance receipt, storage conditions, and a record of use will be maintained at Centre Analytical Laboratories, Inc. Forms documenting chain-of-custody and shipping records for tracking of the test substances will be included as part of the raw data package.

All standards/test substances and any prepared solutions must be identified with a unique label or number on the container or cross-referenced to the container.

HAZARD INFORMATION

A current MSDS for the chemical(s) used in this study will be maintained at the testing facility.

3. SPONSOR

3M Environmental Technology and Safety Services
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

STUDY DIRECTOR:
Rochelle Robideau
TELEPHONE: 651-778-7065
FAX NUMBER: 651-778-6176

4. TESTING FACILITY AND PERFORMING LABORATORY

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

PRINCIPAL INVESTIGATOR:
Emily R. Stauffer, Centre
TELEPHONE: 814-231-8032
FAX NUMBER: 814-231-1580

5. PROPOSED EXPERIMENTAL TIME-FRAME

Experimental Start Date	Dec. 11, 2000
Experimental Termination Date	Dec. 15, 2000
Report Issued	Dec. 30, 2000

6. JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

Quail serum samples and liver samples collected by Wildlife International, Ltd. will be used as the test systems for this study. Quails represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. Quail habitats include bushy pastures, grassy roadsides,

farmlands and open woodlands, and they are considered land grazers. The use of these matrices for the study was to determine if PFOS was deposited in the liver and serum of mating adults that were fed PFOS.

A complete description of the test system along with all of the in-life parameters is documented in the sponsor's protocol no. 454/010600/QP/SUB454, Project No. 454-104 that is associated with this analytical protocol.

7. SAMPLE PROCESSING, STORAGE AND IDENTIFICATION

No processing will be required for the serum samples. Due to the small amount of sample available, the liver samples will not be homogenized prior to extraction; however, they will be homogenized by laying the samples on a flat surface and mashing them by hand as best as possible.

Each sample will be assigned a unique sample identification number at Centre, which will be used for tracking and identification of the samples. The samples will be stored in a temperature-monitored freezer, maintained at < -10° C, except when removed for extraction and analysis as described in the method. The samples will be kept isolated from the test substance during storage.

Sample receipt and storage location and conditions during the study will be documented. All samples and any resulting sample extracts will be identified with a unique label or sample number. Such identification will be either on the container or cross-referenced to the container.

8. ANALYTICAL METHOD

The serum samples will be analyzed according to the analytical method titled "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) and the control liver samples will be analyzed according to the method titled "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Liver For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-6.0). The following modifications will be applied to each method:

1. There will be no surrogate standard used in the extraction procedure. The stock, fortification, and calibration standards will be prepared as follows:

Stock Solution
Prepare a stock solution of PFOS at 100 µg/mL by weighing out 10.0 mg of analytical standard (corrected for percent salt and percent purity). Adjust final volume to 100 mL with methanol in a 100-mL volumetric flask. Store this stock solution (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

Fortification Solutions

a. 1.0 µg/mL Fortification Solution – Pipette 1.0 mL of the 100 µg/mL stock solution into a 100 mL volumetric flask. Bring up to volume with methanol.

b. 0.1 µg/mL Fortification Solution – Pipette 10.0 mL of the 1.0 µg/ml fortification solution into a 100-mL volumetric flask and bring up to volume with methanol.

c. 0.01 µg/mL Fortification Solution – Pipette 10.0 mL of the 0.1 µg/mL fortification solution into a 100-mL volumetric flask and bring up to volume with methanol

Store all fortification standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

Calibration Standards

Prepare six LC/MS/MS calibration standards in methanol via dilution of the 0.1 µg/mL and the 0.01 µg/mL fortification solutions.

This is a typical example; additional concentrations may be prepared as needed.

Initial Conc. (µg/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)
0.1	5	100	0.005
0.1	2	100	0.002
0.1	1	100	0.001
0.01	5	100	0.0005
0.01	2	100	0.0002
0.01	1	100	0.0001

Store all calibration standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

2. The liver samples will not be processed to make the homogenate described in the method Section 12.2 to 12.12. Due to limited sample available, samples will be mashed on a flat surface by hand prior to extraction.

3. The sample weight for liver will be 0.50 g (± 0.05) and the sample volume for serum will be 100 µL. The amount of 0.5 M TBA added to the serum sample will be 0.5 mL and the amount of 0.25 M sodium carbonate/sodium bicarbonate buffer added will be 1.0 mL. Samples will be placed on a wrist-action shaker for ~ 20 min. at level 10 and then centrifuged for ~ 15 min @ ~3000 rpm. The samples will only be filtered with 0.2 µm nylon mesh filter if

necessary. Samples will be reconstituted in a final volume of 1 mL of methanol for both matrices.

4. The samples will be analyzed under the following conditions:

LC/MS/MS System and Operating Conditions (TurboIonspray)

Mass Spec: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX TurboIon Spray Liquid Introduction Interface
Harvard infusion pump

Computer: Power Macintosh G3

Software: PE SciexAnalyst 1.1
Windows NT

HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column:Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in ASTM type I water
Mobile Phase (B): Methanol
Flow Rate: 0.3 mL/min.

Time	% A	% B
0	60	40
1.0	0	100
7.0	0	100
7.5	40	60
11.0	40	60

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g., 2.1 mm x 30 mm) and columns from different manufacturers (Keystone Betasil C_{18} etc.) can be used, provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFOS	negative	499 → 99	4.20

On a day-to-day basis, the retention times may vary slightly depending on the batch of mobile phase, etc.

Example Tune File Parameters

The following values are provided as an example. Actual values may vary from instrument to instrument. Also, these values may be changed from time to time in order to optimize for greatest sensitivity.

The mass spectrometer is tuned using a 0.5µg/mL PFOS solution, prepared via dilution of the stock solution in methanol. The solution is infused (using a "T" connector) at 10 µL/min into a 0.2 mL/min stream of mobile phase consisting of 40% methanol and 60% 2 mM ammonium acetate. The analytes are initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a "tune file". This tune file is then used during routine analysis. The tuning procedure may be repeated as necessary to ensure optimal sensitivity.

Controls	Set
IS-Iospray	-4000.0
OR-Orifice	-61.0
RNG-Focus Ring	-270.0
Q0-Quad 0 Rod Offset	10.0
IQ1- Inter quad 1 lens	9.3
ST-Stubbies	-15.0
RO1-Quad1 Rod Offset	9.3
IQ2-Inter quad 2 lens	20.0
RO2-Quad 2 rod offset	84.0
ST3-Stubbies	100.0
RO3- Quad 3 rod offset	86.0
DF-CEM Deflection Plate	300.0
CEM-Channel Electron Multiplier	2800.0

Gas Flows	Set
Nebulizer Gas	12
Curtain Gas	13
Collision Gas	4
TIS Temperature	350°C

Calibration Procedures

a. Inject the same volume (between 10 to 20 µL) of each calibration standard (prepared in MeOH) into the LC/MS/MS.

b. Use linear, 1/x weighted standard curves for quantification. Linear standard curves are generated for each set by linear regression using the appropriate software system. Any calibration standards falling outside ± 30%, based on its calculated concentration, must be excluded from the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 30% of the total number of standards injected.

c. The correlation coefficient (r) for calibration curves generated must be ≥0.9925 (r^2≥0.985). If calibration results fall outside these limits, then appropriate steps should be taken to adjust instrument operation, and the relevant set of samples must be reanalyzed.

Sample Analysis

Inject the same volume used for the calibration standards (between 10 to 20 µL) of each sample, fortification, control, etc. into the LC/MS/MS. Each sample must be analyzed in duplicate.

a. Standards corresponding to at least six concentrations must be included in an analytical set.

b. Inject an entire set of standards (six) at the beginning of the run and inject standards interspersed about every 5-10 samples. All sample injections must be bracketed by standard injections.

c. Each set of samples analyzed (not to exceed 25) must include at least one reagent control (extraction solvents only), at least one matrix control, and two matrix control samples fortified at known concentrations and carried through the procedure to verify recovery.

d. The concentration of each sample, fortification, control, etc. is determined from the standard curve based on the peak area of the analyte in all standards injected during a set. The standard responses must bracket responses of the residue found in the sample set. If necessary, dilute the samples and re-analyze to give a response within the standard curve range.

e. Fortification recoveries within 80 to 120% are acceptable for fortifications at the LOQ level. Recoveries between 80 to 120% are acceptable for fortifications at levels greater than the LOQ. Failure to meet these criteria requires an investigation of cause and a full reanalysis of the affected samples.

f. Samples in which no peaks are detected at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention times

but are less than 3:1 signal:noise will be reported as NQ (not quantifiable).

g. Background levels of analyte found in control/blank samples that correspond to values below the LOQ, but are still quantifiable, will be used to correct fortification recoveries.

h. If samples are not loaded on the instrument to be analyzed the day they are extracted, samples must be stored refrigerated at approximately 2°C to 6°C until analysis and analyzed preferably within a week.

9. EXPERIMENTAL DESIGN

Samples obtained by Wildlife International Ltd. have been shipped to Centre Analytical Laboratories, Inc. for analysis.

All samples will be analyzed according to methods, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" with the modifications detailed in Section 8.

Methods to control bias will include assay of untreated control samples, fortification of untreated control samples to obtain recovery data, and replicate analysis of fortified samples to provide an indication of reproducibility.

The average recovery and relative standard deviation of the fortified samples will be calculated. Also, the average residue found and standard deviation for each matrix will be calculated.

Moisture determination will be performed on all liver samples according to Centre SOP CAL-10B/11 with the following modifications:

1. The sample weight will be ~ 0.5 g.
2. The drying time will be ~ 12 hours.

10. PROTOCOL AMENDMENTS AND DEVIATIONS

Any deviations from the protocol or from the analytical method as provided will be documented and reported promptly to the Study Director. Planned changes to this protocol or to the analytical method will be made in writing as an amendment and approved by the Principal Investigator and the Study Director. Any amendments or deviations will be appended to this protocol and included in the final report.

1. Protocol amendments: Planned changes to the approved protocol shall be documented by amendments that clearly describe the change, justification for the change, and impact on the study. Amendments will be signed and dated by the Principal Investigator and Study Director. Copies of amendments will be sent to the quality assurance unit.

2. Protocol deviations: Protocol deviations, which are one time and unplanned deviations from the protocol, shall be documented in the study records, noting the nature of the deviation, potential effect or impact on the study, and corrective action if required. Protocol deviations are signed and dated by the Principal Investigator and Study Director and reviewed by QAU.

11. RECORDS

Records to be maintained include, but are not limited the following (as appropriate):

1. Sample tracking sheet(s)
2. Sample receipt records, storage history, and chains of custody
3. History and preparation of standards (stock, fortification, calibration)
4. Description of any modifications to the method
5. Instrument run sheets, bench-sheets or logs
6. Analytical data tables
7. All chromatographic and instrumental conditions
8. Sample extraction and analysis dates
9. A complete listing of study personnel, signatures and initials
10. Chronological presentation of all study correspondence
11. Any other data necessary for the reconstruction of the study

All chromatograms will contain the following:

a. Sample identification, date, arrow or other indication of the area of interest, and injection number corresponding to the run.
b. Additionally, fortifications will include the fortification level of the analyte.
c. Analytical standard chromatograms will additionally include the concentration (e.g., µg/ml, ng/mL, ppb, ppt, etc.).

Each data set will contain information on temperatures, flow rates, column parameters, gases, instrument parameters, and instrument type, etc. The Centre study number will also be recorded on the first chromatogram of each daily analytical run.

12. QUALITY ASSURANCE

Centre QA Unit will review the protocol, audit the study conduct and the study documentation (including raw data and final report) to ascertain that all QA/GLP procedures are adhered to. Centre QA Unit will inspect the study at intervals adequate to assure compliance to GLP's, and will report the findings of the audits to the Principal Investigator, Centre Management, and the Study Director.

13. DATA AND REPORT

1. All raw data and the original signed protocol will be maintained in the study file. This data includes the protocol amendments, protocol deviations, laboratory notebooks, analytical standard solution preparation, sample chain of custody sheets, sample work sheets, chromatograms, calibration curves, and any other appropriate data generated. Raw data not used will be stored in the study file. The reason for exclusion will be documented.

2. A final report will be issued by Centre, approved and signed by the Study Director and sufficient for submission to EPA. The report contents should include, but not limited to:

    1. Objectives and procedures stated in the protocol
    2. Analytical and statistical methods used
    3. Test materials identified by name, lot, purity, and other characteristics
    4. Name of testing facility and study initiation and completion dates
    5. Statement prepared and signed by the quality assurance unit
    6. Tables containing all applicable data
    7. All chromatographic and instrumental conditions
    8. A complete listing of Centre study personnel

Centre will send a copy of the draft report to the Study Director, who will return the report with comments. Centre Analytical will make revisions and finalize the report with the approval of the Study Director. The Centre QA unit will conduct an audit of the final draft report and data files to assure accuracy and GLP compliance. The final report must be approved by the Study Director prior to signature. A statement of QAU inspections and a statement of GLP compliance will be included in the final report.

Any corrections or additions to the final report shall be in the form of an amendment by the Principal Investigator. The amendment shall clearly identify the part of the final report that is being corrected and the justification for correction. The amendment shall be signed and dated by the Principal Investigator and the Study Director.

REPORT DISTRIBUTION:
Original: Study Director
Copy: Centre Archives

14. ARCHIVE STATEMENT

Study records to be maintained: Records to be maintained for the study include all raw data, observations recorded during the conduct of the study, documentation, chromatograms, specimen tracking information, and study related correspondence. This includes a description of equipment used during the conduct of the study. All characterization data and any shipping records shall be retained.

Document archives: Upon completion of the study, the study records, protocol and amendments, and the final report and amendments shall be retained in the 3M document archives. If it is necessary to substitute a copy for an original record, it will be certified as an exact copy. Centre will retain facility-related originals and a copy of the final report and raw data package.

15. REFERENCES

1 U.S. Environmental Protection Agency. Toxic Substances Control Act (TSCA). Good Laboratory Practice Standards. Final Rule, 40 CFR Part 792.

16. PROTOCOL APPROVAL

This protocol was audited by the Quality Assurance Unit of Centre Analytical Laboratories, Inc.

Quality Assurance Reviewer, Centre

Naomi Lovallo, Ph.D.
Quality Assurance Auditor — Date: 12/19/00

Principal Investigator, Centre

Emily Stauffer
Scientist — Date: 12/19/00

Facility Management, Centre

John Flaherty
Laboratory Manager — Date: 12/19/00

Richard A. Grazzini, Ph.D.
President — Date: 19-DEC-00

Study Director, 3M

Rochelle Robideau
Environmental Technologist — Date: 12/8/00

APPENDIX: ANALYTICAL METHODS

A. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

B. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

3M ENVIRONMENTAL LABORATORY

METHOD

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

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

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date 4/27/99

Group Leader — Date 4/26/99

Technical Reviewer — Date 04/26/99

1.0 SCOPE AND APPLICATION

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

1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

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

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

3.0 Definitions

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

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

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

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

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

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

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

4.0 Warnings and Cautions

4.1 Health and safety warnings

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes

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

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser, 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 Reagents and Standards

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 Fluorochemical standards

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

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

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

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

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

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

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{17}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

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

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

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

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

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

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

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{17}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

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

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Solvent Blanks, Method blanks and matrix blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 Calibration and Standardization

11.1 Prepare matrix calibration standards.

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

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

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

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

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

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

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

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

Table 1
Approximate spiking amounts for standards and spikes
Using 1.0 mL of matrix

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

12.0 PROCEDURE

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

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

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

12.4 Record the initial volume on the extraction worksheet.

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

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

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

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

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

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

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

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

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

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

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

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

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

12.18 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

Blank Std # amount mL

Serum Extraction Method — Date & Initials

Vortex 15 sec.

Pipette Matrix Volume mL

Pipette 1 mL of 0.5 M TBA, pH 10. pH = Std. #

Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer Std. #

Dispense 5 mL of methyl-t-butyl ether TN-A-

Shake 20 min. Shaker speed:

Centrifuge 20-25 min. Centrifuge speed:

Remove a 4 mL aliquot of organic layer

Put on Nitrogen Evaporator to dryness Temperature:

Add methanol Volume mL TN-A-

Vortex 30 sec.

Filter using a 3cc B-D syringe with a 0.2um filter into a 1.5 mL autosample vial

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

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	1.74	5.55	5 ppb - 1000 ppb
PFOSA	1.51	4.79	5 ppb - 1000 ppb
PFOSAA	3.46	20.5	5 ppb - 1000 ppb
EtFOSE-OH	11.4	36.2	5 ppb - 1000 ppb
M556	6.03	19.2	5 ppb - 1000 ppb
PFOSEA	5.71	18.2	5 ppb - 1000 ppb

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

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

Attachment B: MDL/LOQ Summary ETS-8-4.1 Extraction of PFOS from Serum Page 11 of 14

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Attachment B: MDL/LOQ Summary ETS-I-4.1 Extraction of PFOS from Serum Page 13 of 14

Ion Pair Standard Curves – Fluids

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

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

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Amt spiked mL	All Final vol mL
0.500	0.507	0.532	0.501	0.521	0.501	0.010	1.015
0.500	0.507	0.532	0.501	0.521	0.501	0.020	1.025
5.00	5.07	5.32	5.01	5.21	5.01	0.005	1.010
5.00	5.07	5.32	5.01	5.21	5.01	0.010	1.015
5.00	5.07	5.32	5.01	5.21	5.01	0.020	1.025
50.0	50.1	53.2	50.1	52.1	50.1	0.005	1.010
50.0	50.1	53.2	50.1	52.1	50.1	0.010	1.015
50.0	50.1	53.2	50.1	52.1	50.1	0.015	1.020
50.0	50.1	53.2	50.1	52.1	50.1	0.020	1.025

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Amt spiked mL
4.93	5.00	5.24	4.94	5.01	5.13	100	0.005
9.76	9.89	10.4	9.78	9.93	10.2		
24.8	25.1	26.3	24.8	25.2	25.8	Surrogate	
49.3	50.0	52.4	49.4	50.1	51.3	Final conc ng/mL	
97.6	98.9	104	97.8	99.3	102	500	
248	251	263	248	252	258		
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-6.0 Adoption Date: 07/22/99

Revision Date: NR

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager Date 7/22/99

Group Leader Date 7/14/99

Technical Reviewer Date 07/14/99

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1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

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

2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 Definitions

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

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

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

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

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

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

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

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

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

7.5 Volumetric flasks, glass, type A

7.6 I-CHEM vials, 40 mL glass

7.7 Plastic sampule vials; Wheaton, 6 mL (or appropriate size)

7.8 Centrifuge tubes, polypropylene, 15 mL

7.9 Labels

7.10 Oxford Dispensor – 3.0 to 10.0 ml

7.11 Syringes, capable of measuring 5 µL to 50 µL

7.12 Graduated pipettes

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

7.16 Crimp cap autovials and caps

7.17 Crimpers

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

8.0 Reagents and Standards

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Liver, frozen from supplier

8.9 Dry ice from supplier

8.10 Fluorochemical standards

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

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

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

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

8.10.5 PFOSSA (3M Specialty Chemical Division), molecular weight = 527

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

8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{17}SO_3H$) molecular weight = 428

8.10.8 Other fluorochemicals, as appropriate

Reagent preparation

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

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

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

8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.13 Surrogate stock standard preparation

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

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

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

9.0 Sample Handling

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

10.0 Quality Control

10.1 Matrix blanks and method blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration verifications

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.

11.1.3 Refer to 13.0 to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.

11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.

11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.

11.1.8 Refer to validation reports ETS-8-6.0 and ETS-8-7.0-V-1 or Attachment B, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.9 Use Attachment C as an aid in calculating the concentrations of the working standards. Refer to 13.0 to calculate actual concentrations of PFOS in calibration standards.

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

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

Table 1
Approximate Spiking Amounts for Calibration Standards

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

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

12.3 Weigh the sample directly into a tared plastic sampule vial.

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

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

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

* refer to 13.1.1 for details.

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

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

*refer to 13.1.2 for details.

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. Refer to attached worksheet for documenting the remaining steps.

12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.

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

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

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

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

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

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

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

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

12.21 Label a fresh 15 mL centrifuge tube with the same information as in 12.10.

12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.

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

12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.

12.25 Vortex mix for 30 seconds.

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

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

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

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

16.0 Records

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 References

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

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

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

19.0 Affected Documents

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

20.0 Revisions

Revision Number	Reason For Revision	Revision Date

Study # / Matrix / Box # / Wk/Day / Date Spiked/Analyst	Surrogate Std approx. ppm actual ppm #	FC Mix Std approx. 0.5 ppm actual ppm #	FC Mix Std approx. 5 ppm actual ppm #	FC Mix Std approx. 50 ppm actual ppm #	Comments
CCV					
MS					
MSD					

Blank Liver Homogenate: Std # Liver amount =	
Liver Extraction Method	Date & Initials
Spike surrogate and Standard mix, Vortex 15 sec.	
Pipette 1 mL of Liver Solution	
Pipette 1 mL of 0.5 M TBA, pH 10. pH = Std. #	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer Std. #	
Dispense 5ml of Methyl-t-Butyl Ether TN-A-	
Shake 20 min. Shaker Speed	
Centrifuge 20-25 min. Centrifuge Speed	
Remove a 4 mL aliquot of organic layer	
Put on Nitrogen Evaporator to dryness Evaporator Temperature	
Add 1.0 mL of Methanol TN-A-	
Vortex 30 sec.	
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial	

Cont. Cal. Verifications used the same matrix as for the standard curve.

MDL/LOQ values for rabbit liver

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

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

Attachment C: Standard Calculations ETS-8-6.0 Extraction of PFOS from Liver Page 14 of 14

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

PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 12/18/00

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF AMENDED SECTION

1. § 9: Experimental Design

AMENDED TO

1. Change the last paragraph to read as follows:
   Moisture determination will be performed on all liver samples according to AOAC Official Method 950.46 Section B. (a). with the following modifications:
   1. The sample weight will be ~ 0.5 g.
   2. The drying time will be ~ 12-14 hours.

RATIONALE

1. At the request of the sponsor, moisture determinations will be done for every liver sample. Due to limited sample size, the sample weight used will be ~0.5 g instead of ~2 g. The drying time required for such a small sample size does not have to be 16-18 hours, so the samples will be dried for ~ 12-14 hours

IMPACT ON THE STUDY

1. No negative impact on the study.

Emily R Stauffer
Principal Investigator Signature — Date 12/20/00

Sponsor Representative (Study Director) Signature — Date 1/8/01

Centre QAU Review NCL 12/20/00

February 12, 1998/1

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

PROTOCOL AMENDMENT
Amendment Number: 2
Effective Date: 03/28/01

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF AMENDED SECTION

1. § 8: Sample Analysis (f)
2. § 9: Experimental Design
3. § 8: LC/MS/MS System and Operating Conditions (TurboIonspray)

AMENDED TO

1. Change the last sentence to read the following:
   Samples in which peaks are detected at the corresponding analyte retention times but are less than the lowest concentration of the calibration standards (0.0001 µg/mL) will be reported as NQ (not quantifiable).
2. Add the following:
   The following equations will be used for quantitation of the samples:

   1.Analyte Found (ng/mL) = (peak area – intercept) / slope

   2.Analyte Found (µg/g) = $\frac{\text{(analyte found (ng/mL) x FV(mL) x DF x EV (mL) x 1 µg}}{\text{AV (mL) x sample wt. (g) x 1000 ng}}$

   Where FV = final volume, DF = dilution factor, EV = extraction volume, AV = aliquot volume

   3.Recovery (%) = $\frac{\text{(anal. found (µg/mL) – avg. analyte in ctrl (µg/mL)) x 1000 ng x 100}}{\text{amount added (ng/mL) x 1 µg}}$
3. Change the computer listed to Dell UltraScan P1110

RATIONALE

1-3. Clarify how the samples are quantitated and reported and the instruments used to acquire data.

IMPACT ON THE STUDY

1-3. No negative impact on the study.

Emily R Decker
Principal Investigator Signature 9/6/01 Date

Study Director Signature 9/6/01 Date

Centre QAU Review NOC 3/30/01

February 12, 1998/1

RECEIVED TIME SEP. 6. 1:32PM

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

PROTOCOL AMENDMENT
Amendment Number: 3
Date of Occurrence: 08/24/01

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF AMENDED SECTION

1. § 3: SPONSOR

AMENDED TO

1. Change the study director from Rochelle Robideau at 3M Environmental Technology and Safety Services to Sean Gallagher at Wildlife International, LTD, 8598 Commerce Drive, Easton, MD 21601, phone: 410-822-8600, fax: 410-822-0632.
2. Change Rochelle Robideau to the Sponsor Representative.

RATIONALE

1-2. By request of Rochelle Robideau (fax dated 08/24/01 included in correspondence section of the raw data associated with this study)

IMPACT ON THE STUDY

1-2. No negative impact on the study.

John M Hebert — Testing Facility Management Signature — 8/30/01 — Date

Outgoing Study Director Signature — 9/5/01 — Date

Sean P. Gallagher — Incoming Study Director Signature — 8/31/01 — Date

Centre QAU Review BBK 8/30/01

February 12, 1998/1

Centre Analytical Laboratories, Inc.
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PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 12/21/00

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF DEVIATION

1. § Amendment #1: Did not perform moisture determination on the following samples because there was no sample available after extraction:

Centre Sample ID	Client ID	Centre Sample ID	Client ID
0007875	459	0007920	9531
0007879	483	0007921	9533
0007881	485	0007922	9535
0007883	487	0007924	9541
0007885	489	0007928	9549
0007907	9503	0007937	9567
0007911	9515	0007941	9580
0007915	9525	0007942	9581
0007917	9528	0007943	9582
0007918	9529	0007944	9583

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc.,

1. Protocol deviation issued.

Recorded By/Date: Emily P Stauffer 3/15/01

IMPACT ON THE STUDY

1. No results for the moisture determination will be available for these samples.

Emily P. Stauffer 3/16/01
Principal Investigator Signature Date

Study Director/Sponsor Representative Signature 03/19/01
Date

CAL QAU Review AMC 3/15/01

February 12, 1998/2

RECEIVED TIME SEP. 6. 1:32PM

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

PROTOCOL DEVIATION

Deviation Number: 2

Date of Occurrence: (1) 12/21/00

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF DEVIATION

1. § Amendment #1: Weighed less than – 0.5 g for the moisture determination on the following samples because it was all of the sample available after extraction:

Centre Sample ID	Client ID	Weight (g)
0007913	9520	0.34
0007867	451	0.30
0007880	484	0.33
0007927	9548	0.32
0007936	9564	0.26
0007938	9570	0.27

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 3/15/01

IMPACT ON THE STUDY

1. No negative impact because moisture was still calculated and the results did not vary significantly from the moisture results for samples that weighed –0.5g.

Emily R Stauffer 3/14/01
Principal Investigator Signature Date

4/4/01
Study Director/Sponsor Representative Signature Date

CAL QAU Review NEC 3/15/01

February 12, 1998/2

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

PROTOCOL DEVIATION

Deviation Number: 3

Date of Occurrence: (1) 12/21/00, (2) 12/21/00 and 3/12/01

Centre Study Number: 023-041 Centre Protocol Number: 00P-023-041

DESCRIPTION OF DEVIATION

1. § Amendment #1: Did not perform moisture determination on the following samples because there was no sample available after extraction:

Centre Sample ID	Client ID
0007877	481
0007939	9575
0011423	na

2. § Amendment #1: The oven temperature for the moisture determinations was 110°C.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1-2. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 3/28/01

IMPACT ON THE STUDY

1. No results for the moisture determination will be available for these samples.
2. No negative impact.

Emily R Deeter
Principal Investigator Signature 9/6/01 Date

Study Director/Sponsor Representative Signature 9/6/01 Date

CAL QAU Review NLL 3/29/01

February 12, 1998/2

RECEIVED TIME SEP. 6. 1:32PM