STUDY TITLE

Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard 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-023-042
Centre Study Number: 023-042

Total Pages: 104

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-042, entitled "Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard 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

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

9/6/01
Date

Sean P. Gallagher

Sean Gallagher
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Date

Rochelle Robideau
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Date

QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-042, entitled, "Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry". All reviewed phases were inspected 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	11/20/00	11/30/00	12/28/00
2. Extraction, Fortification	11/20/00	11/30/00	12/28/00
3. Raw Data Review	12/12/00	12/21/00	12/28/00
4. Raw Data and Draft Report Review	3/18-21/00	3/29/01	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 023-042, is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.
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STUDY IDENTIFICATION

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

CENTRE PROTOCOL NUMBER:	00P-023-042
CENTRE STUDY NUMBER:	023-042
TYPE OF STUDY:	Analytical
SAMPLE MATRIX:	Mallard Serum and Mallard 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: 11/10/00 Experimental Start Date: 11/17/00 Experimental Termination Date: 03/13/01 Study Completion Date: 09/06/01

PROJECT PERSONNEL

The Study Director for this project was Sean Gallagher at Wildlife International, Ltd. The folowing 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	Group/Team Leader
Tiffany Proctor	Technician
Angela Morgan	Technician
Michelle Arjmand	Technician
Lawrence Ord	Sample Custodian
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1.0 SUMMARY

Centre Analytical Laboratories, Inc. (Centre) extracted mallard liver samples and mallard serum samples for the determination of perfluorooctanesulfonate (PFOS) in mallard serum and mallard liver according to protocol 00P-023-042 (Appendix A).

The limit of quantitation for mallard liver was 10 ppb and 10 ng/mL for mallard serum. The LOQ for each matrix was determined in a method verification study performed at Centre (Centre Study: 023-044). Residues of PFOS in the mallard 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 22.8 µg/g. These results are reported on a wet weight basis. PFOS in the mallard serum samples ranged from non-quantifiable levels to 218 µg/g.

The average percent recovery ± standard deviation for PFOS in liver samples was 95% ± 12%. The average percent recovery ± standard deviation for PFOS in serum samples was 93% ± 10%.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of mallard liver and mallard serum using the analytical methods described in protocol 00P-023-042.

3.0 INTRODUCTION

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

The study was initiated on November 10, 2000, when the study director signed Centre protocol number 00P-023-042. The experimental start date was November 17, 2000, and the experimental termination date was March 13, 2001.

4.0 TEST SYSTEM

The control mallard liver and serum used for the matrix blanks and matrix fortifications was received on May 18, 2000 from Wildlife International Ltd., Easton, MD. The control mallard liver was assigned the Centre ID of 0003804 and the control mallard serum was assigned the Centre ID of 0003803.

Sixty mallard liver samples and sixty mallard serum 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 mallard liver samples and forty mallard serum samples were received on February 24, 2001 and stored frozen until they were logged in by Centre personnel on March 1, 2001.

Sample log-in 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.

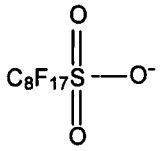
Compound	Centre Control No..	TCR No.	Purity (%)	Expiration Date
PFOS	00-023-042	TCR 00017-46	97.9	08/31/01

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 Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry" were used for this study.

6.1 Extraction Procedure

A ~ 0.50 g sample of liver and a 100 µL aliquot of the serum was used for the extraction procedure. After fortification of appropriate samples, the samples were vortexed for ~ 15 seconds. One milliliter of 0.5 M TBA was added to the liver samples and 0.5 milliliter was added to the serum samples. Two milliliters of 0.25 M sodium carbonate/sodium bicarbonate was added to the liver samples and 1 milliliter was added to the serum samples. Five milliliters of MTBE was added to both the liver and serum samples. Each sample was placed on wrist-action shaker for ~ 20 min. and then centrifuged for ~ 15 min. Four milliliters of the organic layer was taken and dried on a nitrogen evaporator and then reconstituted with 1 milliliter 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-023-042. 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.

A 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 serial 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 electrospray. The retention time of PFOS was ~ 4.4 min. Peaks were detected in the control matrices corresponding to the analyte retention time, but the amounts detected were only significant enough to alter several fortification recoveries and the rest were less than the lowest calibration standard (0.0001 µg/mL).

6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.0001 µg/mL of PFOS.

6.5 Description of Instrument and Operating Conditions

Instrument: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX TurboIon Spray Liquid Introduction Interface

Computer: Dell UltraScan P1110

Software: PE SciexAnalyst version 1.1
Windows NT, version 4

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

Ions monitored:

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

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 were 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 mallard liver (µg/g) and mallard serum (µg/mL) was determined from the equations below.

Equation 1 calculated 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 Equation 2 calculated the amount of analyte found in µg/mL (the equations for serum are shown).

Equation 1:

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

Equation 2:

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

Where:

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

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

Equation 3:

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

An example of a calculation using an actual sample follows:

Mallard serum sample Centre ID 0003803 Spk A (Set: 112100B), fortified at 10 ng/mL with PFOS, where:

peak area	=	6558
intercept	=	515
slope	=	7740
dilution factor	=	1
ng added (fort level)	=	1 ng
avg. amt in controls	=	0 (Not quantifiable)
final volume	=	1 mL
extraction volume	=	5 mL
aliquot volume	=	4 mL
sample weight (volume)	=	0.1 mL

From equation 1:

$$\text{Analyte found (ng/mL)} = \frac{[6558 - 515]}{7740}$$

$$= 0.8\ \text{ng/mL}$$

From equation 2:

$$\text{Analyte found (µg/mL)} = \frac{(0.8\ \text{ng/mL} \times 1\ \text{mL} \times 1 \times 5\ \text{mL})}{(4\ \text{mL} \times 0.1\ \text{mL})} \times \frac{1\ \mu g}{1000\ ng}$$

$$= 0.00976\ \mu\text{g/mL}$$

From equation 3:

$$\% \text{ Recovery} = \frac{((0.00976\ \mu g/mL \times -0\ ng/mL) \times 1000)}{10\ ng/mL} \times 100$$

$$= 98\%$$

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 mallard liver blanks and mallard 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% ± 12%. Individual recoveries for PFOS in the serum samples are detailed in **Table V**. The average percent recovery ± relative standard deviation for PFOS in serum samples was 93% ± 10%.

PFOS in the mallard 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 22.8 µg/g. Individual results are listed in **Tables VI-XIII**. The results are reported on a wet weight basis.

PFOS in the mallard serum 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 218 µg/g. Individual results are listed in **Tables XIV-XXIII**.

The percent moistures for the mallard liver samples are given in **Table XXIV**.

9.0 CONCLUSIONS

The mallard liver and serum samples were successfully extracted and analyzed according to protocol 00P-023-042.

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/g)
na	Reagent Blank A	111700A	11/17/00	11/18-19/00	NQ
na	Reagent Blank A*	111700A	11/17/00	11/18-19/00	NQ
na	Reagent Blank A	112000A	11/20/00	11/20-21/00	NQ
na	Reagent Blank A*	112000A	11/20/00	11/20-21/00	NQ
na	Reagent Blank A	112100A	11/21/00	11/22-23/00	NQ
na	Reagent Blank A*	112100A	11/21/00	11/22-23/00	NQ
na	Reagent Blank A	112100B	11/21/00	11/23/00	NQ
na	Reagent Blank A*	112100B	11/21/00	11/23/00	NQ
na	Reagent Blank A	112700A	11/27/00	11/28-29/00	NQ
na	Reagent Blank A*	112700A	11/27/00	11/28-29/00	NQ
na	Reagent Blank A	112700B	11/27/00	11/29/00	NQ
na	Reagent Blank A*	112700B	11/27/00	11/29/00	NQ
na	Reagent Blank A	030701A	3/7/01	3/9/01	NQ
na	Reagent Blank A*	030701A	3/7/01	3/9/01	NQ
na	Reagent Blank A	030701B	3/7/01	3/9-10/01	NQ
na	Reagent Blank A*	030701B	3/7/01	3/9-10/01	NQ
na	Reagent Blank A	030801A	3/8/01	3/11/01	NQ
na	Reagent Blank A*	030801A	3/8/01	3/11/01	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))

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 II. Summary of PFOS in Mallard Liver Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/g)
na	0003804 Blank A	111700A	11/17/00	11/18-19/00	0.000294
na	0003804 Blank A*	111700A	11/17/00	11/18-19/00	0.000300
na	0003804 Blank A	112000A	11/20/00	11/20-21/00	NQ
na	0003804 Blank A*	112000A	11/20/00	11/20-21/00	NQ
na	0003804 Blank A	112100A	11/21/00	11/22-23/00	NQ
na	0003804 Blank A*	112100A	11/21/00	11/22-23/00	NQ
na	0003804 Blank A	030801A	3/8/01	3/11/01	NQ
na	0003804 Blank A*	030801A	3/8/01	3/11/01	NQ
				AVERAGE:	**NQ**
				STANDARD DEVIATION:	**NQ**

Table III. Summary of PFOS in Mallard Serum Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
na	0003803 Blank A	112100B	11/21/00	11/23/00	NQ
na	0003803 Blank A*	112100B	11/21/00	11/23/00	NQ
na	0003803 Blank A	112700A	11/27/00	11/28-29/00	NQ
na	0003803 Blank A*	112700A	11/27/00	11/28-29/00	NQ
na	0003803 Blank A	112700B	11/27/00	11/29/00	NQ
na	0003803 Blank A*	112700B	11/27/00	11/29/00	0.00159
na	0003803 Blank A	030701A	3/7/01	3/9/01	0.00826
na	0003803 Blank A*	030701A	3/7/01	3/9/01	0.00825
na	0003803 Blank A	030701B	3/7/01	3/9-10/01	0.00159
na	0003803 Blank A*	030701B	3/7/01	3/9-10/01	0.00397
				AVERAGE:	**0.00239**
				STANDARD DEVIATION:	**0.00333**

* 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))

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

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.

Table IV. Summary of PFOS Recoveries in Mallard Liver

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/g)	% Recovery
na	0003804 Spk A	111700AD	11/17/00	11/19/00	10	79
na	0003804 Spk A*	111700AD	11/17/00	11/19/00	10	77
na	0003804 Spk B	111700A	11/17/00	11/18-19/00	50	85
na	0003804 Spk B*	111700A	11/17/00	11/18-19/00	50	84
na	0003804 Spk A	112000AR	11/20/00	11/22/00	100	114
na	0003804 Spk A*	112000AR	11/20/00	11/22/00	100	115
na	0003804 Spk B	112000A	11/20/00	11/20-21/00	4000	106
na	0003804 Spk B*	112000A	11/20/00	11/20-21/00	4000	107
na	0003804 Spk A	112100A	11/21/00	11/22-23/00	1000	89
na	0003804 Spk A*	112100A	11/21/00	11/22-23/00	1000	88
na	0003804 Spk B	112100AD	11/21/00	11/27/00	20000	90
na	0003804 Spk B*	112100AD	11/21/00	11/27/00	20000	89
na	0003804 Spk A	030801A	3/8/01	3/11/01	20	92
na	0003804 Spk A*	030801A	3/8/01	3/11/01	20	92
na	0003804 Spk B	030801AD	3/8/01	3/11-12/01	200	107
na	0003804 Spk B*	030801AD	3/8/01	3/11-12/01	200	101
					AVERAGE:	**95**
					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 Mallard Serum

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
na	0003803 Spk A	112100B	11/21/00	11/23/00	10	98
na	0003803 Spk A*	112100B	11/21/00	11/23/00	10	98
na	0003803 Spk B	112100B	11/21/00	11/23/00	500	84
na	0003803 Spk B*	112100B	11/21/00	11/23/00	500	86
na	0003803 Spk A	112700A	11/27/00	11/28-29/00	10	81
na	0003803 Spk A*	112700A	11/27/00	11/28-29/00	10	81
na	0003803 Spk B	112700A	11/27/00	11/28-29/00	10000	91
na	0003803 Spk B*	112700A	11/27/00	11/28-29/00	10000	91
na	0003803 Spk A	112700B	11/27/00	11/29/00	500	97
na	0003803 Spk A*	112700B	11/27/00	11/29/00	500	97
na	0003803 Spk B	112700B	11/27/00	11/29/00	100000	89
na	0003803 Spk B*	112700B	11/27/00	11/29/00	100000	89
na	0003803 Spk A	030701A	3/7/01	3/9/01	100	79
na	0003803 Spk A*	030701A	3/7/01	3/9/01	100	77
na	0003803 Spk B	030701A	3/7/01	3/9/01	1000	89
na	0003803 Spk B*	030701A	3/7/01	3/9/01	1000	93
na	0003803 Spk A2	030701B	3/7/01	3/9-10/01	100	90
na	0003803 Spk A2*	030701B	3/7/01	3/9-10/01	100	90
na	0003803 Spk B2	030701B	3/7/01	3/9-10/01	1000	118
na	0003803 Spk B2*	030701B	3/7/01	3/9-10/01	1000	119
					AVERAGE:	**92**
					STANDARD DEVIATION:	**11**
					RELATIVE STANDARD DEVIATION:	**12**

* 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)
1961 M	0008006	111700A	11/17/00	11/18-19/00	0.000813
1961*	0008006	111700A	11/17/00	11/18-19/00	0.000763
1962 F	0008007	111700A	11/17/00	11/18-19/00	0.000509
1962*	0008007	111700A	11/17/00	11/18-19/00	0.000631
1963 M	0008008	111700A	11/17/00	11/18-19/00	0.000937
1963*	0008008	111700A	11/17/00	11/18-19/00	0.000887
1964 F	0008009	111700A	11/17/00	11/18-19/00	0.00155
1964*	0008009	111700A	11/17/00	11/18-19/00	0.00157
1965 M	0008010	111700A	11/17/00	11/18-19/00	0.00110
1965*	0008010	111700A	11/17/00	11/18-19/00	0.00111
1966 F	0008011	111700A	11/17/00	11/18-19/00	0.00446
1966*	0008011	111700A	11/17/00	11/18-19/00	0.00432
1967 M	0008012	111700ADD	11/17/00	11/20/00	0.662
1967*	0008012	111700ADD	11/17/00	11/20/00	0.684
1968 F	0008013	111700AD	11/17/00	11/19/00	0.238
1968*	0008013	111700AD	11/17/00	11/19/00	0.246
1969 M	0008014	111700AD	11/17/00	11/19/00	0.115
1969*	0008014	111700AD	11/17/00	11/19/00	0.106
1970 F	0008015	111700AD	11/17/00	11/19/00	0.355
1970*	0008015	111700AD	11/17/00	11/19/00	0.343
				AVERAGE:	**0.138**
				STANDARD DEVIATION:	**0.219**

* Duplicate injection

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

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)
E01-0263-21065 M	0103870	030801AD	3/8/01	3/11-12/01	0.359
E01-0263-21065*	0103870	030801AD	3/8/01	3/11-12/01	0.350
E01-0263-21066 M	0103871	030801AD	3/8/01	3/11-12/01	0.647
E01-0263-21066*	0103871	030801AD	3/8/01	3/11-12/01	0.631
E01-0263-21067 M	0103872	030801AD	3/8/01	3/11-12/01	0.886
E01-0263-21067*	0103872	030801AD	3/8/01	3/11-12/01	0.880
E01-0263-21068 M	0103873	030801AD	3/8/01	3/11-12/01	2.11
E01-0263-21068*	0103873	030801AD	3/8/01	3/11-12/01	2.04
E01-0263-21069 M	0103874	030801AD	3/8/01	3/11-12/01	0.222
E01-0263-21069*	0103874	030801AD	3/8/01	3/11-12/01	0.232
E01-0263-21075 F	0103880	030801AD	3/8/01	3/11-12/01	0.317
E01-0263-21075*	0103880	030801AD	3/8/01	3/11-12/01	0.326
E01-0263-21076 F	0103881	030801AD	3/8/01	3/11-12/01	0.219
E01-0263-21076*	0103881	030801AD	3/8/01	3/11-12/01	0.230
E01-0263-21077 F	0103882	030801AD	3/8/01	3/11-12/01	0.194
E01-0263-21077*	0103882	030801AD	3/8/01	3/11-12/01	0.197
E01-0263-21078 F	0103883	030801AD	3/8/01	3/11-12/01	0.346
E01-0263-21078*	0103883	030801AD	3/8/01	3/11-12/01	0.342
E01-0263-21079 F	0103884	030801AD	3/8/01	3/11-12/01	0.0893
E01-0263-21079*	0103884	030801AD	3/8/01	3/11-12/01	0.0888
				AVERAGE:	**0.535**
				STANDARD DEVIATION:	**0.574**

* 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)
E01-0263-21070	0103875	030801AD	3/8/01	3/11-12/01	1.04
E01-0263-21070*	0103875	030801AD	3/8/01	3/11-12/01	1.01
E01-0263-21071	0103876	030801AD	3/8/01	3/11-12/01	0.963
E01-0263-21071*	0103876	030801AD	3/8/01	3/11-12/01	0.907
E01-0263-21072	0103877	030801AD	3/8/01	3/11-12/01	1.92
E01-0263-21072*	0103877	030801AD	3/8/01	3/11-12/01	1.81
E01-0263-21073	0103878	030801AD	3/8/01	3/11-12/01	0.791
E01-0263-21073*	0103878	030801AD	3/8/01	3/11-12/01	0.827
E01-0263-21074	0103879	030801AD	3/8/01	3/11-12/01	1.28
E01-0263-21074*	0103879	030801AD	3/8/01	3/11-12/01	1.34
E01-0263-21080	0103885	030801AD	3/8/01	3/11-12/01	1.01
E01-0263-21080*	0103885	030801AD	3/8/01	3/11-12/01	1.03
E01-0263-21081	0103886	030801AD	3/8/01	3/11-12/01	0.0614
E01-0263-21081*	0103886	030801AD	3/8/01	3/11-12/01	0.0578
E01-0263-21082	0103887	030801AD	3/8/01	3/11-12/01	0.473
E01-0263-21082*	0103887	030801AD	3/8/01	3/11-12/01	0.517
E01-0263-21083	0103888	030801AD	3/8/01	3/11-12/01	1.66
E01-0263-21083*	0103888	030801AD	3/8/01	3/11-12/01	1.52
E01-0263-21084	0103889	030801AD	3/8/01	3/11-12/01	0.0862
E01-0263-21084*	0103889	030801AD	3/8/01	3/11-12/01	0.0878
				AVERAGE:	**0.920**
				STANDARD DEVIATION:	**0.576**

* 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)
1991	0008016	111700ADD	11/17/00	11/20/00	15.9
1991*	0008016	111700ADD	11/17/00	11/20/00	16.4
1992	0008017	111700ADD	11/17/00	11/20/00	22.8
1992*	0008017	111700ADD	11/17/00	11/20/00	21.9
1993	0008018	111700ADD	11/17/00	11/20/00	7.02
1993*	0008018	111700ADD	11/17/00	11/20/00	6.69
1994	0008019	111700AD	11/17/00	11/19/00	2.82
1994*	0008019	111700AD	11/17/00	11/19/00	2.93
1995	0008020	111700AD	11/17/00	11/19/00	3.53
1995*	0008020	111700AD	11/17/00	11/19/00	3.60
1996	0008021	111700ADD	11/17/00	11/20/00	13.2
1996*	0008021	111700ADD	11/17/00	11/20/00	13.5
1997	0008022	111700AD	11/17/00	11/19/00	5.62
1997*	0008022	111700AD	11/17/00	11/19/00	5.58
1998	0008023	111700AD	11/17/00	11/19/00	3.72
1998*	0008023	111700AD	11/17/00	11/19/00	3.86
1999	0008024	111700ADD	11/17/00	11/20/00	15.5
1999*	0008024	111700ADD	11/17/00	11/20/00	16.1
2000	0008025	111700AD	11/17/00	11/19/00	5.97
2000*	0008025	111700AD	11/17/00	11/19/00	6.10
				AVERAGE:	**9.64**
				STANDARD DEVIATION:	**6.57**

* 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)
2301	0008086	112000A	11/20/00	11/20-21/00	NQ
2301*	0008086	112000A	11/20/00	11/20-21/00	NQ
2302	0008087	112000A	11/20/00	11/20-21/00	NQ
2302*	0008087	112000A	11/20/00	11/20-21/00	NQ
2303	0008088	112000A	11/20/00	11/20-21/00	NQ
2303*	0008088	112000A	11/20/00	11/20-21/00	NQ
2305	0008089	112000A	11/20/00	11/20-21/00	NQ
2305*	0008089	112000A	11/20/00	11/20-21/00	NQ
2307	0008090	112000A	11/20/00	11/20-21/00	NQ
2307*	0008090	112000A	11/20/00	11/20-21/00	NQ
2308	0008091	112000A	11/20/00	11/20-21/00	NQ
2308*	0008091	112000A	11/20/00	11/20-21/00	NQ
2314	0008092	112000A	11/20/00	11/20-21/00	NQ
2314*	0008092	112000A	11/20/00	11/20-21/00	NQ
2316	0008093	112000A	11/20/00	11/20-21/00	NQ
2316*	0008093	112000A	11/20/00	11/20-21/00	NQ
2317	0008094	112000A	11/20/00	11/20-21/00	NQ
2317*	0008094	112000A	11/20/00	11/20-21/00	NQ
2320	0008095	112000A	11/20/00	11/20-21/00	NQ
2320*	0008095	112000A	11/20/00	11/20-21/00	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))

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

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.

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)
2322	0008096	112000A	11/20/00	11/20-21/00	0.00454
2322*	0008096	112000A	11/20/00	11/20-21/00	0.00458
2323	0008097	112000A	11/20/00	11/20-21/00	0.00124
2323*	0008097	112000A	11/20/00	11/20-21/00	0.00129
2325	0008098	112000A	11/20/00	11/20-21/00	0.00150
2325*	0008098	112000A	11/20/00	11/20-21/00	0.00154
2326	0008099	112000A	11/20/00	11/20-21/00	0.00236
2326*	0008099	112000A	11/20/00	11/20-21/00	0.00245
2328	0008100	112000A	11/20/00	11/20-21/00	0.00167
2328*	0008100	112000A	11/20/00	11/20-21/00	0.00165
2330	0008101	112000A	11/20/00	11/20-21/00	0.00185
2330*	0008101	112000A	11/20/00	11/20-21/00	0.00182
2331	0008102	112000A	11/20/00	11/20-21/00	0.000719
2331*	0008102	112000A	11/20/00	11/20-21/00	0.000763
2332	0008103	112000A	11/20/00	11/20-21/00	0.000425
2332*	0008103	112000A	11/20/00	11/20-21/00	0.000430
2333	0008104	112000A	11/20/00	11/20-21/00	0.000969
2333*	0008104	112000A	11/20/00	11/20-21/00	0.000966
2334	0008105	112000A	11/20/00	11/20-21/00	0.00356
2334*	0008105	112000A	11/20/00	11/20-21/00	0.00355
				AVERAGE:	**0.00189**
				STANDARD DEVIATION:	**0.00126**

* 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)
2335 F	0008106	112100A	11/21/00	11/22-23/00	0.00197
2335*	0008106	112100A	11/21/00	11/22-23/00	0.00200
2336 F	0008107	112100A	11/21/00	11/22-23/00	0.00377
2336*	0008107	112100A	11/21/00	11/22-23/00	0.00378
2337 F	0008108	112100A	11/21/00	11/22-23/00	0.00380
2337*	0008108	112100A	11/21/00	11/22-23/00	0.00363
2338 M	0008109	112100A	11/21/00	11/22-23/00	0.00555
2338*	0008109	112100A	11/21/00	11/22-23/00	0.00535
2339 M	0008110	112100A	11/21/00	11/22-23/00	0.00540
2339*	0008110	112100A	11/21/00	11/22-23/00	0.00518
2341 M	0008111	112100A	11/21/00	11/22-23/00	0.00306
2341*	0008111	112100A	11/21/00	11/22-23/00	0.00313
2342 F	0008112	112100A	11/21/00	11/22-23/00	0.00312
2342*	0008112	112100A	11/21/00	11/22-23/00	0.00320
2343 M	0008113	112100A	11/21/00	11/22-23/00	0.00329
2343*	0008113	112100A	11/21/00	11/22-23/00	0.00332
2344 F	0008114	112100A	11/21/00	11/22-23/00	0.00402
2344*	0008114	112100A	11/21/00	11/22-23/00	0.00405
2346 F	0008115	112100AD	11/21/00	11/27/00	0.0141
2346*	0008115	112100AD	11/21/00	11/27/00	0.0146
				AVERAGE:	**0.00482**
				STANDARD DEVIATION:	**0.00341**

* 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)
2347 F	0008116	112100AD	11/21/00	11/27/00	0.0383
2347*	0008116	112100AD	11/21/00	11/27/00	0.0370
2348 F	0008117	112100AD	11/21/00	11/27/00	0.0456
2348*	0008117	112100AD	11/21/00	11/27/00	0.0465
2353 F	0008118	112100AD	11/21/00	11/27/00	0.0234
2353*	0008118	112100AD	11/21/00	11/27/00	0.0227
2354 M	0008119	112100AD	11/21/00	11/27/00	0.0184
2354*	0008119	112100AD	11/21/00	11/27/00	0.0189
2355 M	0008120	112100A	11/21/00	11/22-23/00	0.0110
2355* M	0008120	112100A	11/21/00	11/22-23/00	0.0105
2357 M	0008121	112100AD	11/21/00	11/27/00	0.0616
2357*	0008121	112100AD	11/21/00	11/27/00	0.0629
2358 F	0008122	112100AD	11/21/00	11/27/00	0.0175
2358*	0008122	112100AD	11/21/00	11/27/00	0.0189
2359 F	0008123	112100AD	11/21/00	11/27/00	0.0211
2359*	0008123	112100AD	11/21/00	11/27/00	0.0211
2360 M	0008124	112100A	11/21/00	11/22-23/00	0.00623
2360*	0008124	112100A	11/21/00	11/22-23/00	0.00628
2363 M	0008125	112100AD	11/21/00	11/27/00	0.0172
2363*	0008125	112100AD	11/21/00	11/27/00	0.0191
				AVERAGE:	**0.0262**
				STANDARD DEVIATION:	**0.0168**

* Duplicate injection

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

Table XIV. Summary of PFOS in Control Adult Serum Samples

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0263-21005	0103810	030701A	3/7/01	3/9/01	0.0463
E01-0263-21005*	0103810	030701A	3/7/01	3/9/01	0.0455
E01-0263-21006	0103811	030701A	3/7/01	3/9/01	0.00828
E01-0263-21006*	0103811	030701A	3/7/01	3/9/01	0.00928
E01-0263-21007	0103812	030701A	3/7/01	3/9/01	0.00954
E01-0263-21007*	0103812	030701A	3/7/01	3/9/01	0.0103
E01-0263-21008	0103813	030701A	3/7/01	3/9/01	0.00319
E01-0263-21008*	0103813	030701A	3/7/01	3/9/01	0.00452
E01-0263-21009	0103814	030701A	3/7/01	3/9/01	0.00423
E01-0263-21009*	0103814	030701A	3/7/01	3/9/01	0.00616
E01-0263-21025	0103830	030701B	3/7/01	3/9-10/01	0.00285
E01-0263-21025*	0103830	030701B	3/7/01	3/9-10/01	0.00207
E01-0263-21026	0103831	030701B	3/7/01	3/9-10/01	NQ
E01-0263-21026*	0103831	030701B	3/7/01	3/9-10/01	NQ
E01-0263-21027	0103832	030701B	3/7/01	3/9-10/01	NQ
E01-0263-21027*	0103832	030701B	3/7/01	3/9-10/01	NQ
E01-0263-21028	0103833	030701B	3/7/01	3/9-10/01	0.00428
E01-0263-21028*	0103833	030701B	3/7/01	3/9-10/01	0.00265
E01-0263-21029	0103834	030701B	3/7/01	3/9-10/01	NQ
E01-0263-21029*	0103834	030701B	3/7/01	3/9-10/01	NQ
				AVERAGE:	**0.00797**
				STANDARD DEVIATION:	**0.0134**

* 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/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 XV. 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)
1961 M	0008026	112100B	11/21/00	11/23/00	0.00605
1961*	0008026	112100B	11/21/00	11/23/00	0.00594
1962 F	0008027	112100B	11/21/00	11/23/00	0.00494
1962* M	0008027	112100B	11/21/00	11/23/00	0.00501
1963	0008028	112100B	11/21/00	11/23/00	0.00844
1963*	0008028	112100B	11/21/00	11/23/00	0.00825
1964 F	0008029	112100B	11/21/00	11/23/00	0.00798
1964*	0008029	112100B	11/21/00	11/23/00	0.00761
1965 M	0008030	112100B	11/21/00	11/23/00	0.0104
1965*	0008030	112100B	11/21/00	11/23/00	0.0103
1966 F	0008031	112100B	11/21/00	11/23/00	0.0358
1966*	0008031	112100B	11/21/00	11/23/00	0.0357
1967 M	0008032	112100BD	11/21/00	11/27-28/00	6.11
1967*	0008032	112100BD	11/21/00	11/27-28/00	6.02
1968 F	0008033	112100BD	11/21/00	11/27-28/00	3.80
1968*	0008033	112100BD	11/21/00	11/27-28/00	3.81
1969 M	0008034	112100BD	11/21/00	11/27-28/00	0.843
1969*	0008034	112100BD	11/21/00	11/27-28/00	0.849
1970 F	0008035	112100BD	11/21/00	11/27-28/00	1.41
1970*	0008035	112100BD	11/21/00	11/27-28/00	1.38
				AVERAGE:	**1.22**
				STANDARD DEVIATION:	**2.03**

* 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 1.8 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0263-21010	0103815	030701AD	3/7/01	3/10/01	12.8
E01-0263-21010*	0103815	030701AD	3/7/01	3/10/01	12.1
E01-0263-21011	0103816	030701AD	3/7/01	3/10/01	18.2
E01-0263-21011*	0103816	030701AD	3/7/01	3/10/01	20.1
E01-0263-21012	0103817	030701AD	3/7/01	3/10/01	22.5
E01-0263-21012*	0103817	030701AD	3/7/01	3/10/01	24.0
E01-0263-21013	0103818	030701AD	3/7/01	3/10/01	20.4
E01-0263-21013*	0103818	030701AD	3/7/01	3/10/01	20.4
E01-0263-21014	0103819	030701AD	3/7/01	3/10/01	10.6
E01-0263-21014*	0103819	030701AD	3/7/01	3/10/01	10.6
E01-0263-21030	0103835	030701BD	3/7/01	3/10-11/01	0.870
E01-0263-21030*	0103835	030701BD	3/7/01	3/10-11/01	0.854
E01-0263-21031	0103836	030701BD	3/7/01	3/10-11/01	6.70
E01-0263-21031*	0103836	030701BD	3/7/01	3/10-11/01	6.43
E01-0263-21032	0103837	030701BD	3/7/01	3/10-11/01	0.443
E01-0263-21032*	0103837	030701BD	3/7/01	3/10-11/01	0.468
E01-0263-21033	0103838	030701BD	3/7/01	3/10-11/01	8.88
E01-0263-21033*	0103838	030701BD	3/7/01	3/10-11/01	8.38
E01-0263-21034	0103839	030701BD	3/7/01	3/10-11/01	1.68
E01-0263-21034*	0103839	030701BD	3/7/01	3/10-11/01	1.74
				AVERAGE:	**10.4**
				STANDARD DEVIATION:	**8.15**

* 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 6.2 ppm a.i. (Test Termination 6 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0263-21015	0103820	030701AD	3/7/01	3/10/01	47.1
E01-0263-21015*	0103820	030701AD	3/7/01	3/10/01	44.7
E01-0263-21016	0103821	030701AD	3/7/01	3/10/01	40.4
E01-0263-21016*	0103821	030701AD	3/7/01	3/10/01	35.5
E01-0263-21017	0103822	030701AD	3/7/01	3/10/01	45.5
E01-0263-21017*	0103822	030701AD	3/7/01	3/10/01	44.4
E01-0263-21018	0103823	030701AD	3/7/01	3/10/01	23.5
E01-0263-21018*	0103823	030701AD	3/7/01	3/10/01	24.7
E01-0263-21019	0103824	030701AD	3/7/01	3/10/01	20.3
E01-0263-21019*	0103824	030701AD	3/7/01	3/10/01	20.8
E01-0263-21035	0103840	030701BD	3/7/01	3/10-11/01	17.8
E01-0263-21035*	0103840	030701BD	3/7/01	3/10-11/01	17.3
E01-0263-21036	0103841	030701BD	3/7/01	3/10-11/01	2.83
E01-0263-21036*	0103841	030701BD	3/7/01	3/10-11/01	2.87
E01-0263-21037	0103842	030701BD	3/7/01	3/10-11/01	3.48
E01-0263-21037*	0103842	030701BD	3/7/01	3/10-11/01	4.12
E01-0263-21038	0103843	030701BD	3/7/01	3/10-11/01	61.9
E01-0263-21038*	0103843	030701BD	3/7/01	3/10-11/01	60.3
E01-0263-21039	0103844	030701BD	3/7/01	3/10-11/01	3.69
E01-0263-21039*	0103844	030701BD	3/7/01	3/10-11/01	2.79
				AVERAGE:	**26.2**
				STANDARD DEVIATION:	**20.0**

* Duplicate injection

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

Table XVIII. 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)
1991 M	0008036	112100BDD	11/21/00	11/28/00	41.0
1991*	0008036	112100BDD	11/21/00	11/28/00	41.1
1992 F	0008037	112100BDD	11/21/00	11/28/00	47.0
1992*	0008037	112100BDD	11/21/00	11/28/00	49.5
1993 M	0008038	112100BDD	11/21/00	11/28/00	199
1993*	0008038	112100BDD	11/21/00	11/28/00	198
1994 F	0008039	112100BDD	11/21/00	11/28/00	93.9
1994*	0008039	112100BDD	11/21/00	11/28/00	93.0
1995 M	0008040	112100BDD	11/21/00	11/28/00	182
1995*	0008040	112100BDD	11/21/00	11/28/00	180
1996 F	0008041	112100BDD	11/21/00	11/28/00	218
1996*	0008041	112100BDD	11/21/00	11/28/00	217
1997 M	0008042	112100BDD	11/21/00	11/28/00	214
1997*	0008042	112100BDD	11/21/00	11/28/00	212
1998 F	0008043	112100BDD	11/21/00	11/28/00	156
1998*	0008043	112100BDD	11/21/00	11/28/00	156
1999 M	0008044	112100BDD	11/21/00	11/28/00	156
1999*	0008044	112100BDD	11/21/00	11/28/00	161
2000 F	0008045	112100BDD	11/21/00	11/28/00	200
2000*	0008045	112100BDD	11/21/00	11/28/00	205
				AVERAGE:	**151**
				STANDARD DEVIATION:	**65.0**

* Duplicate injection

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

Table XIX. Summary of PFOS in Adult Serum Samples at 17.6 ppm a.i. (6 Week Samples)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
E01-0263-21020	0103825	030801AD	3/7/01	3/11-12/01	18.6
E01-0263-21020*	0103825	030801AD	3/7/01	3/11-12/01	18.1
E01-0263-21021	0103826	030701AD	3/7/01	3/10/01	152
E01-0263-21021*	0103826	030701AD	3/7/01	3/10/01	151
E01-0263-21022	0103827	030701AD	3/7/01	3/10/01	156
E01-0263-21022*	0103827	030701AD	3/7/01	3/10/01	157
E01-0263-21023	0103828	030701AD	3/7/01	3/10/01	137
E01-0263-21023*	0103828	030701AD	3/7/01	3/10/01	138
E01-0263-21024	0103829	030801AD	3/7/01	3/11-12/01	42.2
E01-0263-21024*	0103829	030801AD	3/7/01	3/11-12/01	39.1
E01-0263-21040	0103845	030701BD	3/7/01	3/10-11/01	55.9
E01-0263-21040*	0103845	030701BD	3/7/01	3/10-11/01	62.1
E01-0263-21041	0103846	030701BD	3/7/01	3/10-11/01	49.8
E01-0263-21041*	0103846	030701BD	3/7/01	3/10-11/01	43.2
E01-0263-21042	0103847	030701BD	3/7/01	3/10-11/01	15.8
E01-0263-21042*	0103847	030701BD	3/7/01	3/10-11/01	17.7
E01-0263-21043	0103848	030701BD	3/7/01	3/10-11/01	18.5
E01-0263-21043*	0103848	030701BD	3/7/01	3/10-11/01	17.3
E01-0263-21044	0103849	030701BD	3/7/01	3/10-11/01	14.1
E01-0263-21044*	0103849	030701BD	3/7/01	3/10-11/01	14.4
				AVERAGE:	**65.9**
				STANDARD DEVIATION:	**57.5**

* 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 0 ppm a.i. (Test Termination 12 Weeks)

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
2301 C	0008126	112700A	11/27/00	11/28-29/00	NQ
2301*	0008126	112700A	11/27/00	11/28-29/00	NQ
2302 F	0008127	112700A	11/27/00	11/28-29/00	NQ
2302*	0008127	112700A	11/27/00	11/28-29/00	NQ
2303 F	0008128	112700A	11/27/00	11/28-29/00	0.00458
2303*	0008128	112700A	11/27/00	11/28-29/00	0.00453
2305 M	0008129	112700A	11/27/00	11/28-29/00	NQ
2305*	0008129	112700A	11/27/00	11/28-29/00	NQ
2307 M	0008130	112700A	11/27/00	11/28-29/00	0.00278
2307*	0008130	112700A	11/27/00	11/28-29/00	0.00272
2308 M	0008131	112700A	11/27/00	11/28-29/00	NQ
2308*	0008131	112700A	11/27/00	11/28-29/00	NQ
2314 M	0008132	112700A	11/27/00	11/28-29/00	NQ
2314*	0008132	112700A	11/27/00	11/28-29/00	NQ
2316 M	0008133	112700A	11/27/00	11/28-29/00	0.00381
2316*	0008133	112700A	11/27/00	11/28-29/00	0.00379
2317 F	0008134	112700A	11/27/00	11/28-29/00	0.00626
2317*	0008134	112700A	11/27/00	11/28-29/00	0.00601
2320 F	0008135	112700A	11/27/00	11/28-29/00	0.00141
2320*	0008135	112700A	11/27/00	11/28-29/00	0.00153
				AVERAGE:	**0.00190**
				STANDARD DEVIATION:	**0.00222**

* 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/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 XXI. 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)
2322 F	0008136	112700A	11/27/00	11/28-29/00	0.0464
2322*	0008136	112700A	11/27/00	11/28-29/00	0.0472
2323 M	0008137	112700A	11/27/00	11/28-29/00	0.0274
2323*	0008137	112700A	11/27/00	11/28-29/00	0.0281
2325 F	0008138	112700A	11/27/00	11/28-29/00	0.0497
2325*	0008138	112700A	11/27/00	11/28-29/00	0.0487
2326 F	0008139	112700A	11/27/00	11/28-29/00	0.0160
2326*	0008139	112700A	11/27/00	11/28-29/00	0.0156
2328 M	0008140	112700D	11/27/00	11/29-30/00	0.249
2328*	0008140	112700D	11/27/00	11/29-30/00	0.250
2330 F	0008141	112700A	11/27/00	11/28-29/00	0.00788
2330*	0008141	112700A	11/27/00	11/28-29/00	0.00747
2331 M	0008142	112700A	11/27/00	11/28-29/00	0.0101
2331*	0008142	112700A	11/27/00	11/28-29/00	0.00998
2332 F	0008143	112700A	11/27/00	11/28-29/00	0.0113
2332*	0008143	112700A	11/27/00	11/28-29/00	0.0110
2333 F	0008144	112700A	11/27/00	11/28-29/00	0.0339
2333*	0008144	112700A	11/27/00	11/28-29/00	0.0339
2334 M	0008145	112700A	11/27/00	11/28-29/00	0.0554
2334*	0008145	112700A	11/27/00	11/28-29/00	0.0555
				AVERAGE:	**0.0507**
				STANDARD DEVIATION:	**0.0701**

* Duplicate injection

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

Table XXII. 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)
2335 F	0008146	112700B	11/27/00	11/29/00	0.0159
2335*	0008146	112700B	11/27/00	11/29/00	0.0162
2336 F	0008147	112700D	11/27/00	11/29-30/00	0.118
2336*	0008147	112700D	11/27/00	11/29-30/00	0.124
2337 F	0008148	112700D	11/27/00	11/29-30/00	0.181
2337*	0008148	112700D	11/27/00	11/29-30/00	0.183
2338 M	0008149	112700D	11/27/00	11/29-30/00	0.0584
2338*	0008149	112700D	11/27/00	11/29-30/00	0.0592
2339 M	0008150	112700D	11/27/00	11/29-30/00	0.0889
2339*	0008150	112700D	11/27/00	11/29-30/00	0.0840
2341 M	0008151	112700D	11/27/00	11/29-30/00	0.0556
2341*	0008151	112700D	11/27/00	11/29-30/00	0.0558
2342 F	0008152	112700B	11/27/00	11/29/00	0.0380
2342*	0008152	112700B	11/27/00	11/29/00	0.0383
2343 M	0008153	112700D	11/27/00	11/29-30/00	0.0700
2343*	0008153	112700D	11/27/00	11/29-30/00	0.0717
2344 F	0008154	112700D	11/27/00	11/29-30/00	0.0676
2344*	0008154	112700D	11/27/00	11/29-30/00	0.0688
2346 F	0008155	112700B	11/27/00	11/29/00	0.0371
2346*	0008155	112700B	11/27/00	11/29/00	0.0373
				AVERAGE:	**0.0734**
				STANDARD DEVIATION:	**0.0466**

* Duplicate injection

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

Table XXIII. 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)
2347 F	0008156	112700D	11/27/00	11/29-30/00	0.490
2347*	0008156	112700D	11/27/00	11/29-30/00	0.496
2348 F	0008157	112700D	11/27/00	11/29-30/00	0.150
2348*	0008157	112700D	11/27/00	11/29-30/00	0.156
2353 F	0008158	112700D	11/27/00	11/29-30/00	0.894
2353*	0008158	112700D	11/27/00	11/29-30/00	0.908
2354 M	0008159	112700D	11/27/00	11/29-30/00	0.591
2354*	0008159	112700D	11/27/00	11/29-30/00	0.621
2355 M	0008160	112700D	11/27/00	11/29-30/00	0.399
2355*	0008160	112700D	11/27/00	11/29-30/00	0.384
2357 M	0008161	112700D	11/27/00	11/29-30/00	0.547
2357*	0008161	112700D	11/27/00	11/29-30/00	0.547
2358 F	0008162	112700D	11/27/00	11/29-30/00	0.512
2358*	0008162	112700D	11/27/00	11/29-30/00	0.517
2359 F	0008163	112700D	11/27/00	11/29-30/00	0.402
2359*	0008163	112700D	11/27/00	11/29-30/00	0.404
2360 M	0008164	112700D	11/27/00	11/29-30/00	0.521
2360*	0008164	112700D	11/27/00	11/29-30/00	0.514
2363 M	0008165	112700D	11/27/00	11/29-30/00	0.439
2363*	0008165	112700D	11/27/00	11/29-30/00	0.434
				AVERAGE:	**0.496**
				STANDARD DEVIATION:	**0.184**

* Duplicate injection

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

Table XXIV. Summary of Percent Moistures for Mallard Liver Samples

Sponsor ID	Centre ID	Average % Moisture
1961	0008006	69.7
1962	0008007	73.4
1963	0008008	75.7
1964	0008009	69.2
1965	0008010	71.9
1966	0008011	75.0
1967	0008012	72.8
1968	0008013	70.4
1969	0008014	69.5
1970	0008015	72.9
1991	0008016	75.3
1992	0008017	71.8
1993	0008018	71.9
1994	0008019	71.8
1995	0008020	71.5
1996	0008021	72.4
1997	0008022	70.5
1998	0008023	71.3
1999	0008024	69.8
2000	0008025	72.0
2301	0008086	76.6
2302	0008087	77.3
2303	0008088	72.7
2305	0008089	72.5
2307	0008090	72.3
2308	0008091	72.0
2314	0008092	75.0
2316	0008093	69.6
2317	0008094	71.8
2320	0008095	71.6
2322	0008096	71.6
2323	0008097	72.5
2325	0008098	73.2
2326	0008099	72.6
2328	0008100	72.0
2330	0008101	68.9
2331	0008102	71.4
2332	0008103	71.7
2333	0008104	69.8
2334	0008105	68.1
2335	0008106	71.7

Table XXIV (cont'). Summary of Percent Moistures for Mallard Liver Samples

Sponsor ID	Centre ID	Average % Moisture
2336	0008107	69.7
2337	0008108	69.7
2338	0008109	69.8
2339	0008110	70.6
2341	0008111	72.6
2342	0008112	78.2
2343	0008113	70.9
2344	0008114	69.2
2346	0008115	70.9
2347	0008116	69.7
2348	0008117	70.3
2353	0008118	73.2
2354	0008119	68.5
2355	0008120	67.8
2357	0008121	74.5
2358	0008122	72.0
2359	0008123	73.6
2360	0008124	69.0
2363	0008125	74.1
E01-0263-21065	0103870	73.8
E01-0263-21066	0103871	81.0
E01-0263-21067	0103872	82.4
E01-0263-21068	0103873	75.8
E01-0263-21069	0103874	78.2
E01-0263-21070	0103875	77.4
E01-0263-21071	0103876	77.5
E01-0263-21072	0103877	74.8
E01-0263-21073	0103878	77.5
E01-0263-21074	0103879	82.7
E01-0263-21075	0103880	71.7
E01-0263-21076	0103881	77.9
E01-0263-21077	0103882	77.5
E01-0263-21078	0103883	76.0
E01-0263-21079	0103884	63.8
E01-0263-21080	0103885	78.2
E01-0263-21081	0103886	73.6
E01-0263-21082	0103887	76.7
E01-0263-21083	0103888	81.4
E01-0263-21084	0103889	72.3

FIGURES

Figure 1. Typical Calibration Curve for PFOS

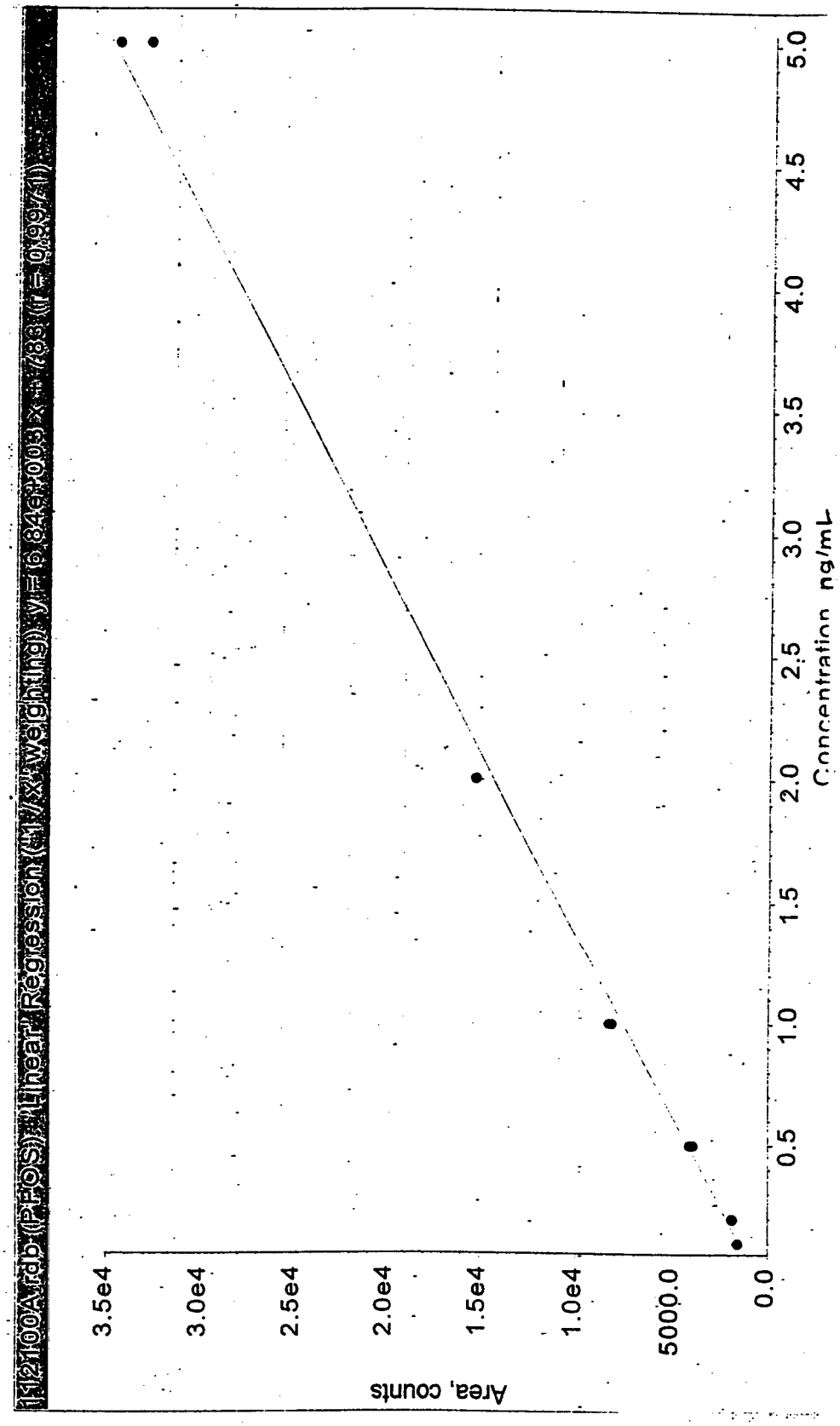


Figure 2. Chromatogram Representing a 0.1 ng/mL standard for PFOS

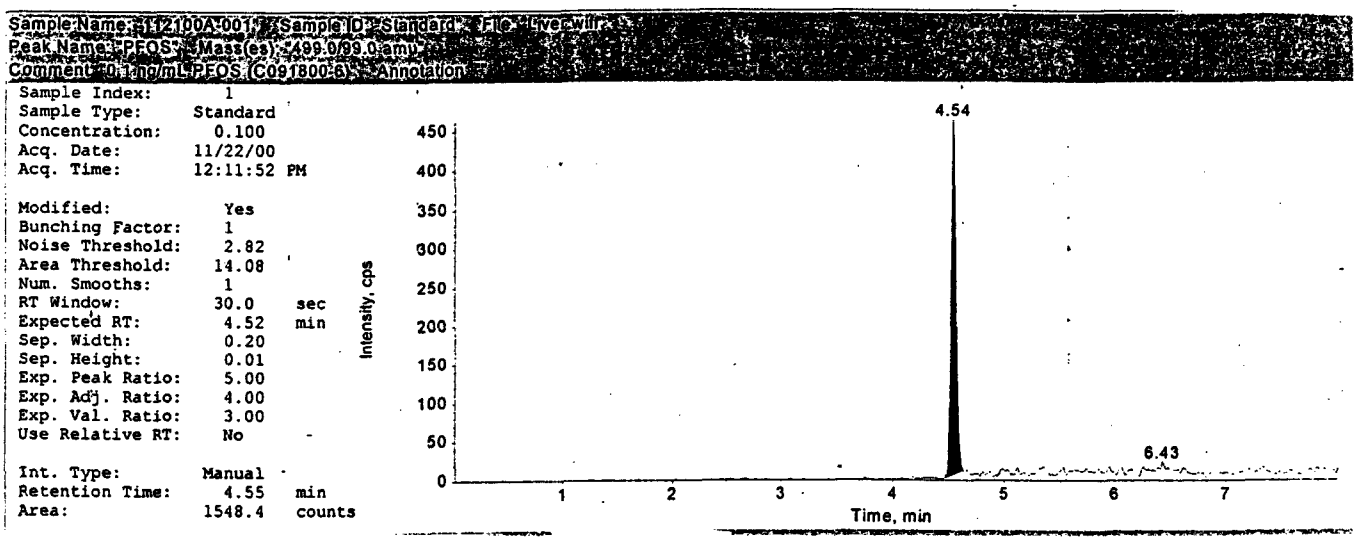


Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS

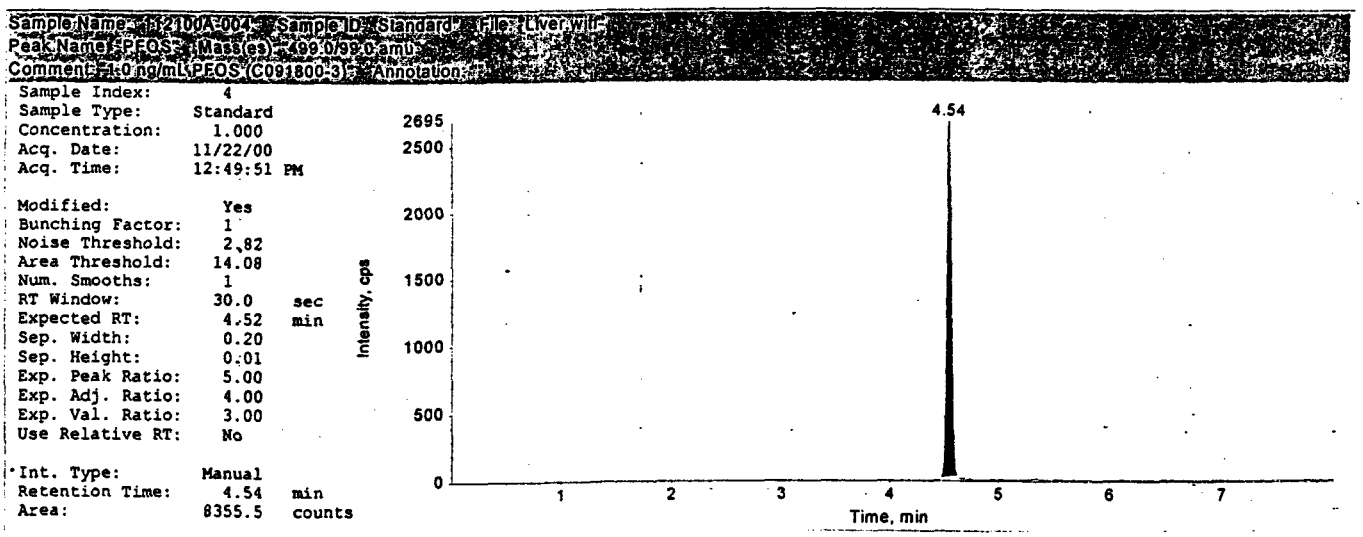


Figure 4. Chromatogram Representing a Reagent Blank for PFOS (Centre ID Reagent Blank A, Set: 112000A)

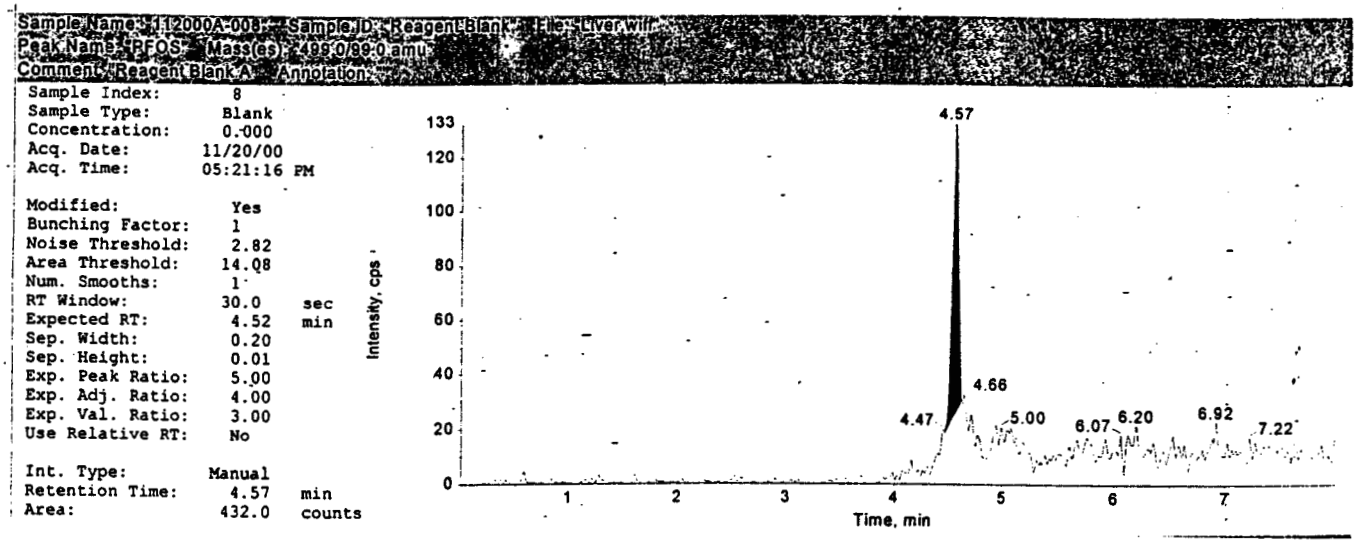


Figure 5. Chromatogram Representing Control Mallard Serum for PFOS (Centre ID: 0003803 Blank A, Set: 112100B)

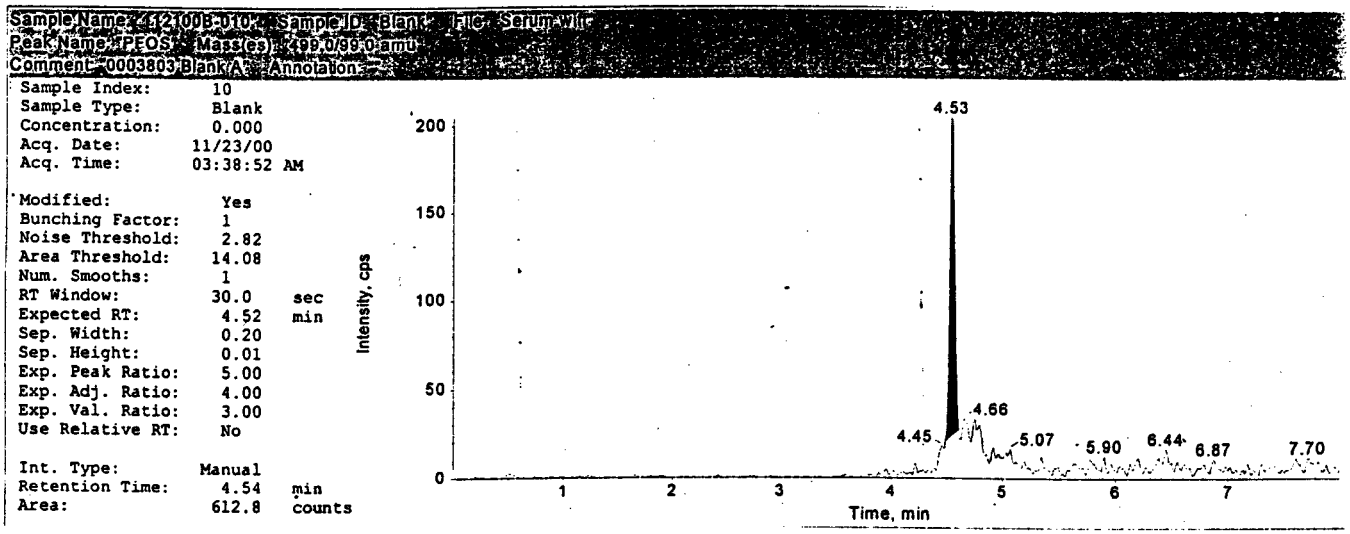


Figure 6. Chromatogram Representing Control Mallard Liver for PFOS, (Centre ID: 0003804 Blank A, Set: 112000A)

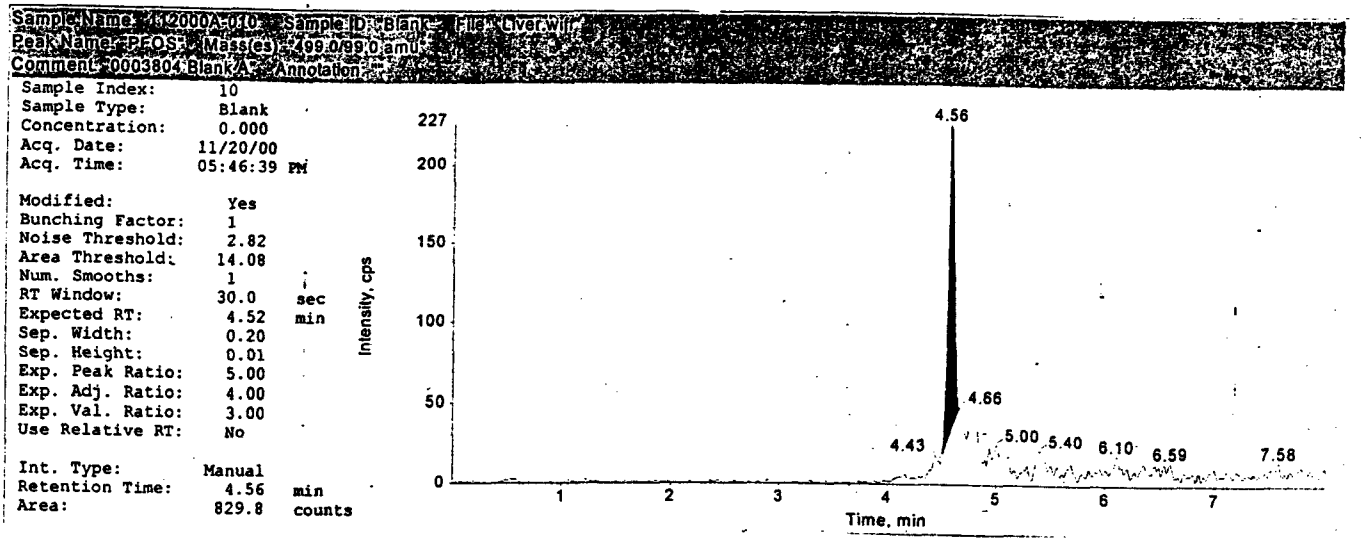


Figure 7. Chromatogram Representing Control Mallard Serum fortified with 10 ppb of PFOS (Centre ID: 0003803 Spk A, Set: 112100B)

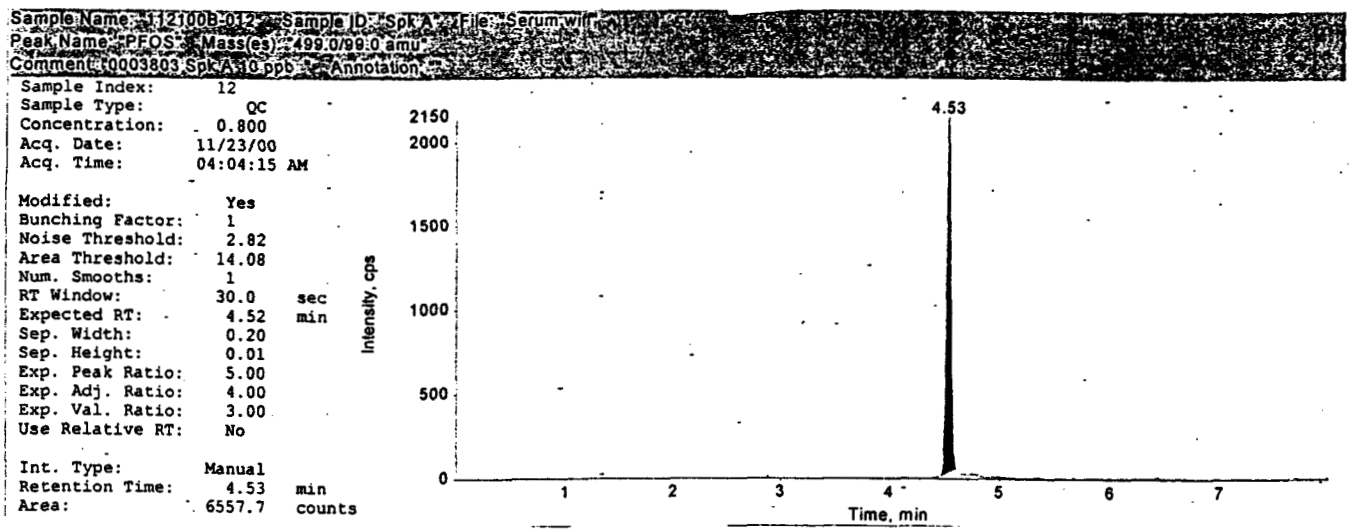


Figure 8. Chromatogram Representing Control Mallard Liver Fortified at 1000 ppb with PFOS (Centre ID: 0003804 Spk A, Set: 112100A)

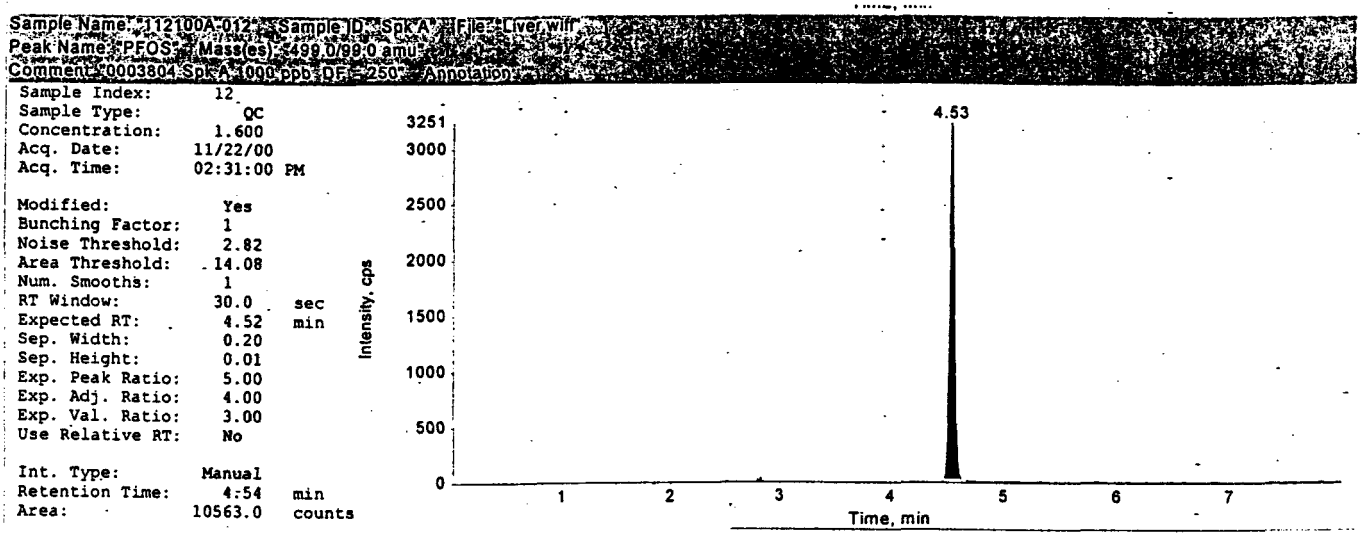


Figure 9. Chromatogram Representing Juvenile Mallard Serum Sample (Centre ID: 0008143, Sponsor ID: 2332, Set: 112700A)

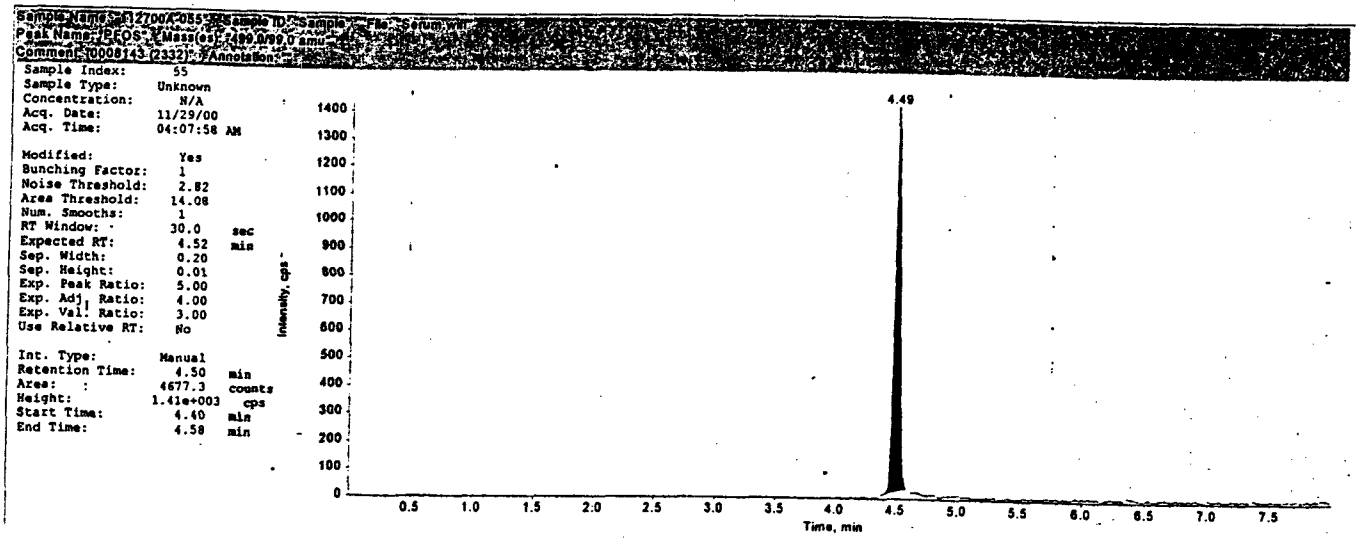
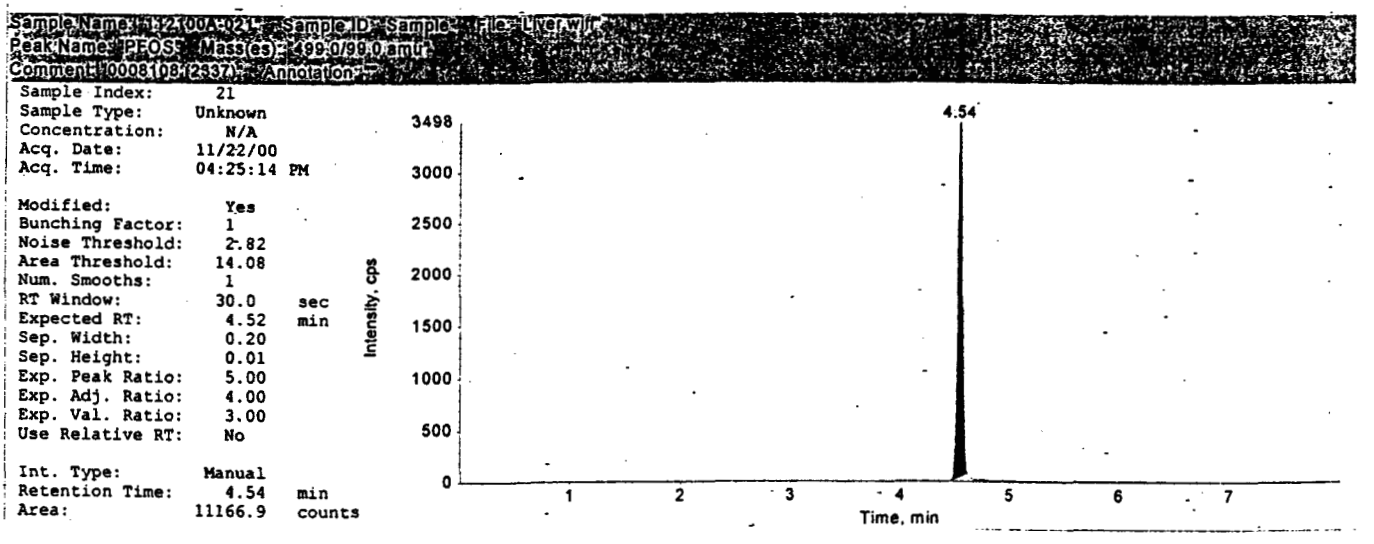


Figure 10. Chromatogram Representing Juvenile Mallard Liver Sample (Centre ID: 0008108, Sponsor ID: 2337, Set: 112100A)



APPENDIX A

Study Protocol
00P-023-042
(Centre Study No. 023-042)
and
Amendments and Deviation

Centre Protocol No. 00P-023-042

STUDY PROTOCOL

STUDY TITLE

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM MALLARD SERUM AND MALLARD 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-023-042

Centre Protocol No. 00P-023-042

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

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1. PURPOSE

The purpose of this study is to analyze mallard serum samples and mallard 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

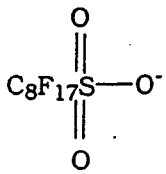
The following analytical standards will be used:

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	Nov. 6, 2000
Experimental Termination Date	Nov. 24, 2000
Report Issued	Dec. 30, 2000

6. JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

Mallard serum samples and liver samples collected by Wildlife International, Ltd. will be used as the test systems for this study. Mallards represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. Mallard habitats include ponds, lakes and marshes, and

they are considered aquatic 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.

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 lying 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.

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 11/15/00

Principal Investigator, Centre

Emily Stauffer
Scientist — Date 11/15/00

Facility Management, Centre

John Flaherty
Laboratory Manager — Date 11/15/00

Richard A. Grazzini, Ph.D.
President — Date 15-NOV-00

Study Director, 3M

Rochelle Robideau
Environmental Technologist — Date 11/10/00

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:

[signature] 4/27/99
Laboratory Manager Date

[signature] 4/26/99
Group Leader Date

[signature] 04/26/99
Technical Reviewer Date

CONFIDENTIAL

1.0 SCOPE AND APPLICATION

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

1.2 Applicable compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

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

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

3.0 Definitions

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

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

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

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

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

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

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

4.0 Warnings and Cautions

4.1 Health and safety warnings

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Vortex mixer, VWR-Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Eppendorf or disposable pipettes

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

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser, 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 REAGENTS AND STANDARDS

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 Fluorochemical standards

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

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

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

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

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

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

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 **Reagent preparation**

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

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

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

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

Blank	Std #	amount =	mL
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.

Attachment A ETS-8-4.1 Page 10 of 14
Extraction of PFOS from Serum

MDL/LOQ values for rabbit serum

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

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

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

Compound: PFOS

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

Compound: PFOSA

Rabbit Serum	Prepared range of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD Range
Full Range	0.993 - 976	4.93 - 976	88-103	5.10-14.7
Low Curve	4.93 - 97.6	4.93 - 97.6	87-105	9.85-14.7
High curve	24.8 - 976	24.8 - 978	93-102	5.08-13.9
1/X	0.993 - 976	4.93 - 976	94-103	5.10-14.5

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

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Attachment B: MDL/LOQ Summary — ETS-8-4.1 Extraction of PFOS from Serum — Page 13 of 14

Ion Pair Standard Curves – Fluids

Prep date(s): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Method/revision: **Blank fluid/identifier:**
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Surrogate
Final conc ng/mL
500

Validated ranges – approximate concentrations

	PFOS	PFOSA	PFOSAA	EtFOSE-OH	PFOSEA	M556
Serum						
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.					

Attachment C: Ion Pair Standard Curves ETS-2-1.1 Page 14 of 14
Extraction of PFOS from Serum

3M ENVIRONMENTAL LABORATORY

METHOD

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

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

Revision Date: NA

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

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

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

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

8.10.8 Other fluorochemicals, as appropriate

8.11 **Reagent preparation**

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

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

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

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

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

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

8.12 **Standards preparation**

8.12.1 Prepare PFOS standards for the standard curve.

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

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

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (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, $C_8F_{17}SO_3H$ 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 sample vial with the study number, weight, liver ID, date and analyst initials.

12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the 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.

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:

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

*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.

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 = g

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 0.2um SRI filter into autosample vial	

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

Attachment B: MDL/LOQ Values ETS-8-6.0 Page 11 of 16

Extraction of PFOS from Liver

MDL/LOQ values for rabbit liver

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

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

Prep date(s): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Blank liver/identifier:
Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm:
FC mix std approx. 5.00 ppm:
FC mix std approx. 50.0 ppm:
Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

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Page 1 of 1

PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 11/21/00

Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

DESCRIPTION OF AMENDED SECTION

1. § 6: Justification for the Selection of the Test System

AMENDED TO

1. Add the following:
   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/MP/SUB454, Project No. 454-105 that is associated with this analytical protocol.

RATIONALE

1. At the request of the sponsor, a description of the in-life parameters for the test system was not to be included in this protocol that covers the analytical phase for the analysis of mallard liver and serum.

IMPACT ON THE STUDY

1. No negative impact on the study.

Emily R Stauffer
Principal Investigator Signature

11/30/00
Date

Sponsor Representative (Study Director) Signature

12/8/00
Date

Centre QAU Review VLL 11/30/00

February 12, 1998/1

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Page 1 of 1

PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 11/29/00

Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

DESCRIPTION OF AMENDED SECTION

1. § 9: Experimental Design

AMENDED TO

1. Add the following:
   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 ~10 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.

Principal Investigator Signature — Date: 9/6/01

Sponsor Representative (Study Director) Signature — Date: 9/6/01

Centre QAU Review 9/6/01

February 12, 1998/1

NOTE: THIS AMENDMENT WAS RE-ISSUED ON 9/6/01 BECAUSE ORIGINAL WAS LOST SEE PHONE LOGS DATED 9/6/01 FRD 9/6/01

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PROTOCOL AMENDMENT
Amendment Number: 3
Effective Date: 03/22/01

Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

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

   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 P. Decker
Principal Investigator Signature 9/6/01 Date

Lisa P. Hallbauer
Study Director Signature 9/6/01 Date

Centre QAU Review 9/6/01

February 12, 1998/1

NOTE: THIS AMENDMENT WAS RE-ISSUED ON 9/6/01 BECAUSE ORIGINAL WAS LOST SEE PHONE LOGS DATED 9/6/01 AND 9/6/01

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PROTOCOL AMENDMENT
Amendment Number: 4
Date of Occurrence: 08/24/01

Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

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 Hakert — 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

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PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 11/19/00 (2) 11/19/00, (3) 11/23/00 (4) 11/29-30/00 (5) 3/10/01
Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

DESCRIPTION OF DEVIATION

1. § 8 Sample Analysis (a) Page 9: Accepted recoveries of 79% and 77% for Centre sample 0003804 Spk A and its duplicate injection in Set 111700AD.

2. § 8 Calibration Procedures (b) Pages 8-9: Included a standard in the calibration curve that fell outside ± 30% of its calculated concentration in Set 111700AD (C091800-5).

3. § 8 Calibration Procedures (b) Pages 8-9: Excluded a standard from the calibration curve that fell within ± 30% of its calculated concentration in Set 112100B (C091800-3).

4. § 8 Calibration Procedures (b) Pages 8-9: Included a standard in the calibration curve that fell outside ± 30% of its calculated concentration in Set 112700D (C091800-6).

5. § 8 Sample Analysis (a) Page 9: Accepted recoveries of 79% and 77% for Centre sample 0003803 Spk A and its duplicate injection in Set 030701A.

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

5 REVISED 9/6/01
1. Protocol deviation issued.

Recorded By/Date: Emily R Decker 9/6/01

IMPACT ON THE STUDY

1,5. No negative impact on the study because the overall average of the spike recoveries for the study fell within the acceptable range of 80%-120%.
2,4. No negative impact on the study because the curve was still within acceptable linear requirements.
3. No negative impact on the study because the total number of standards excluded from the curve was less than 20% of the total number of standards injected.

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

Dean P. Dallaire 9/6/01
Study Director Signature Date

NA NA
Sponsor Management Signature Date

Centre REVISED 9/6/01 CAL QAU Review 9/6/01
DEVIATION REVISED 9/6/01

February 12, 1998/2

NOTE: THIS AMENDMENT WAS RE-ISSUED ON 9/6/01 BECAUSE ORIGINAL WAS LOST SEE PHONE LOGS DATED 9/6/01 ERD 9/6/01