STUDY TITLE

Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

DATA REQUIREMENTS

Analytical Method Requirements

STUDY DIRECTOR

Sean Gallagher

ANALYTICAL REPORT DATE

June 2, 2003

PERFORMING LABORATORY / TESTING FACILITY

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PROJECT

Exygen Protocol Number: 01P-023-066
Exygen Study Number: 023-066

Total Pages: 98

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number 023-066, entitled "Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum 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 Exygen Research.

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Date

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QUALITY ASSURANCE STATEMENT

Exygen's Quality Assurance Unit reviewed Exygen Study Number 023-066, entitled, "Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells for Analysis Using HPLC-Electrospray/Mass Spectrometry". All reviewed phases were inspected for conduct according to Exygen's 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 Exygen Management	Date Reported to Study Director and Sponsor Management
1. Protocol Review	5/22/01	6/4/01	6/15/01
2. Extraction, Fortification	5/22/01	6/4/01	6/15/01
3. Raw Data Review	6/4,6,8/01	7/13/01	9/7/01
4. Raw Data Review	8/7-9/01	8/21/01	9/7/01
5. Draft Report Review	11/15,16,19/01	12/05/01	02/21/02
6. Final Report Review	04/11,21/03	06/02/03	06/02/03

Lydia Shaffer

Lydia Shaffer
Quality Assurance Auditor

06/02/03
Date

CERTIFICATION OF AUTHENTICITY

This report, for Exygen Study Number 023-066, is a true and complete representation of the raw data for the study.

Submitted by: Exygen Research
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STUDY IDENTIFICATION

Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

EXYGEN PROTOCOL NUMBER:	01P-023-066
EXYGEN STUDY NUMBER:	023-066
TYPE OF STUDY:	Analytical
SAMPLE MATRIX:	Mallard and Quail Red Blood Cells and Serum
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
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ANALYTICAL PHASE TIMETABLE:		
	Study Initiation Date:	04/23/01
	Experimental Start Date:	05/18/01
	Experimental Termination Date:	07/28/01
	Analytical Report Date:	06/02/03

PROJECT PERSONNEL

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

Name	Title
Emily Decker	Scientist
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Karen Risha	Scientist
Ling Ling Liu	Technician
Marcia Swartzwelder	Technician
Xiaoming Zhu	Technician
Mitra Arjmand	Technician
Lawrence Ord	Sample Custodian
Ed Carnes	Sample Custodian
Shawn Robb	Sample Custodian

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1.0 SUMMARY

Exygen Research (Exygen) extracted mallard and quail red blood cell samples and serum samples for the determination of perfluorooctanesulfonate (PFOS) according to protocol 01P-023-066 (Appendix A).

The limit of quantitation for mallard red blood cells and serum was 10 ng/mL. The limit of quantitation for quail red blood cells and serum was 10 ng/mL. The LOQ for each matrix was determined in a method verification study performed at Centre (Centre Study: 023-065).

PFOS in the mallard red blood cell samples ranged from non-quanitifiable levels to 418 µg/mL. PFOS in the mallard serum samples ranged from non-detected levels to 685 µg/mL.

PFOS in the quail red blood cell samples ranged from non-quantifiable levels to 361 µg/mL. PFOS in the quail serum samples ranged from non-quantifiable levels to 514 µg/mL.

The average percent recoveries ± standard deviation for PFOS in mallard red blood cell samples and serum samples were 90% ± 9% and 99% ± 13%, respectively. The average percent recovery ± standard deviation for PFOS in quail red blood cell samples and serum samples were 100% ± 10% and 102% ± 20%, respectively.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of mallard and quail red blood cell samples and serum samples using the analytical method described in protocol 01P-023-066.

3.0 INTRODUCTION

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

The study was initiated on April 23, 2001, when the study director signed Exygen protocol number 01P-023-066. The experimental start date was May 18, 2001, and the experimental termination date was July 28, 2001.

4.0 TEST SYSTEM

The control mallard and quail red blood cells and serum used for the matrix blanks and matrix fortifications were received chilled on blue ice on May 17, 2001 from Wildlife International Ltd., Easton, MD. They were assigned the following Exygen ID numbers:

Matrix	Exygen ID
Quail Serum	0107375
Quail Blood	0107376
Mallard Serum	0107377
Mallard Blood	0107378

Seventy-seven quail red blood cell samples and seventy-six quail serum samples were received chilled on blue ice at Exygen on May 17, 2001 and logged in by Exygen personnel and placed in frozen storage. Seventy-three mallard red blood cell samples and seventy-two mallard serum samples were received chilled on blue ice at Exygen on May 17, 2001 and logged in by Exygen personnel and placed in frozen storage.

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 Exygen 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 Exygen 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.

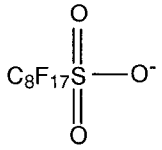
Compound	Exygen Control No.	TCR No.	Purity (%)	Expiration Date
PFOS	00-023-042	TCR 00017-46	97.9	08/31/01

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

6.1 Extraction Procedure

A 100 µL aliquot of the red blood cells and serum was used for the extraction procedure. After fortification of appropriate samples, the samples were vortexed for ~ 15 seconds. An aliquot of 0.5 milliliter of 0.5 M tetrabutylammonium hydrogen sulfate was added to the samples. One milliliter of 0.25 M sodium carbonate/sodium bicarbonate was added to the samples. Five milliliters of MTBE were added to the samples. Each sample was placed on wrist-action shaker for ~ 20 min. and then centrifuged for ~ 15 min. Four milliliters of the organic layer were 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 March 14, 2001 as specified in Exygen protocol 01P-023-066. 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	60	40
11.0	60	40

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 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:

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

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:

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

$$= 0.00976 \text{ µg/mL}$$

From equation 3:

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

$$= 98\%$$

7.0 EXPERIMENTAL DESIGN

Each set of samples (red blood cells 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 amount of PFOS found in the reagent blanks are listed in **Table I**. The amount of PFOS in the mallard red blood cells and serum blanks are given in **Tables II & III**. The amount of PFOS in the quail red blood cells and serum are given in **Tables IV & V**. Peaks were detected in the control matrices corresponding to the analyte retention time. The amounts detected were only significant enough to alter several fortification recoveries and as a result are reported. The rest of the samples were less than the lowest calibration standard (0.0001 µg/mL) and not quantifiable.

Individual recoveries for PFOS in the mallard red blood cell and serum samples are detailed in **Tables VI & VII**. The average percent recoveries ± standard deviations for PFOS in mallard red blood cell and serum samples were 90% ± 9% and 99% ± 13%, respectively. Individual recoveries for PFOS in the quail red blood cell and serum samples are detailed in **Tables VIII & IX**. The average percent recoveries ± standard deviations for PFOS in mallard red blood cell and serum samples were 100% ± 10% and 102% ± 20%, respectively.

PFOS in the mallard red blood cell samples ranged from non-detected levels to 418 µg/mL. Individual results are listed in **Tables X-XVII**. PFOS in the mallard serum

samples ranged from non-detected levels to 685 µg/mL. Individual results are listed in **Tables XVIII-XXV.**

PFOS in the quail red blood cell samples ranged from non-detected levels to 361 µg/mL. Individual results are listed in **Tables XXVI-XXXIII**. PFOS in the quail serum samples ranged from non-detected levels to 514 µg/mL. Individual results are listed in **Tables XXXIV-XLI**.

9.0 CONCLUSIONS

The mallard and quail red blood cell and serum samples were successfully extracted and analyzed according to protocol 01P-023-066.

10.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Exygen 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 Exygen archives for the period of time specified in 40 CFR Part 792. Retained samples of reference substances are archived by the sponsor.

TABLES

Table I. Summary of PFOS in Reagent Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
na	Reagent Blank A	051801A	5/18/01	5/18-19/01	NQ
na	Reagent Blank A*	051801A	5/18/01	5/18-19/01	NQ
na	Reagent Blank A2	051801B	5/18/01	5/19/01	NQ
na	Reagent Blank A2*	051801B	5/18/01	5/19/01	NQ
na	Reagent Blank A	052101A	5/21/01	5/22/01	NQ
na	Reagent Blank A*	052101A	5/21/01	5/22/01	NQ
na	Reagent Blank A	052101B	5/21/01	5/22/01	NQ
na	Reagent Blank A*	052101B	5/21/01	5/22/01	NQ
na	Reagent Blank A	052201A	5/22/01	5/23/01	NQ
na	Reagent Blank A*	052201A	5/22/01	5/23/01	NQ
na	Reagent Blank A	052201B	5/22/01	5/23/01	NQ
na	Reagent Blank A*	052201B	5/22/01	5/23/01	NQ
na	Reagent Blank A	052201C	5/22/01	5/23/01	NQ
na	Reagent Blank A*	052201C	5/22/01	5/23/01	NQ
na	Reagent Blank A3	071901A	7/19/01	7/19-20/01	ND
na	Reagent Blank A3*	071901A	7/19/01	7/19-20/01	ND
na	Reagent Blank A	072001A	7/20/01	7/21/01	ND
na	Reagent Blank A*	072001A	7/20/01	7/21/01	ND
na	Reagent Blank A2	072001B	7/20/01	7/21-22/01	NQ
na	Reagent Blank A2*	072001B	7/20/01	7/21-22/01	ND
na	Reagent Blank A	072301A	7/23/01	7/25/01	NQ
na	Reagent Blank A*	072301A	7/23/01	7/25/01	NQ
na	Reagent Blank A2	072301B	7/23/01	7/24/01	NQ
na	Reagent Blank A2*	072301B	7/23/01	7/24/01	NQ
na	Reagent Blank A3	072301C	7/23/01	7/24-25/01	NQ
na	Reagent Blank A3*	072301C	7/23/01	7/24-25/01	NQ
				AVERAGE:	**NQ**
				STANDARD DEVIATION:	**NQ**

* Duplicate injection

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

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/mL for red blood cells and serum

Table II. Summary of PFOS in Mallard Red Blood Cell Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
control	0107378 Blank A	052101B	5/21/01	5/22/01	NQ
control	0107378 Blank A*	052101B	5/21/01	5/22/01	NQ
control	0107378 Blank A	052201A	5/22/01	5/23/01	NQ
control	0107378 Blank A*	052201A	5/22/01	5/23/01	NQ
control	0107378 Blank A2	052201B	5/22/01	5/23/01	0.00224
control	0107378 Blank A2*	052201B	5/22/01	5/23/01	0.00184
control	0107378 Blank B	052201C	5/22/01	5/23/01	NQ
control	0107378 Blank B*	052201C	5/22/01	5/23/01	NQ
				AVERAGE:	**0.000548**
				STANDARD DEVIATION:	**0.000927**

Table III. Summary of PFOS in Mallard Serum Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
control	0107377 Blank A3	071901A	7/19/01	7/19-20/01	0.00325
control	0107377 Blank A3*	071901A	7/19/01	7/19-20/01	0.00331
control	0107377 Blank A	072001A	7/20/01	7/21/01	0.00277
control	0107377 Blank A*	072001A	7/20/01	7/21/01	0.00312
control	0107377 Blank A2	072001B	7/20/01	7/21-22/01	0.00188
control	0107377 Blank A2*	072001B	7/20/01	7/21-22/01	0.00214
				AVERAGE:	**0.00275**
				STANDARD DEVIATION:	**0.000605**

* 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/mL for red blood cells and 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 in Quail Red Blood Cell Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
control	0107376 Blank A	051801A	5/18/01	5/18-19/01	NQ
control	0107376 Blank A*	051801A	5/18/01	5/18-19/01	NQ
control	0107376 Blank A2	051801B	5/18/01	5/19/01	NQ
control	0107376 Blank A2*	051801B	5/18/01	5/19/01	NQ
control	0107376 Blank A	052101A	5/21/01	5/22/01	NQ
control	0107376 Blank A*	052101A	5/21/01	5/22/01	NQ
control	0107376 Blank A	052201C	5/22/01	5/23/01	NQ
control	0107376 Blank A*	052201C	5/22/01	5/23/01	NQ
				AVERAGE:	**NQ**
				STANDARD DEVIATION:	**NQ**

Table V. Summary of PFOS in Quail Serum Blanks

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
control	0107375 Blank A	072301AD	7/23/01	7/26/01	0.00309
control	0107375 Blank A*	072301AD	7/23/01	7/26/01	0.00272
control	0107375 Blank A2	072301B	7/23/01	7/24/01	NQ
control	0107375 Blank A2*	072301B	7/23/01	7/24/01	NQ
control	0107375 Blank A3	072301C	7/23/01	7/24-25/01	NQ
control	0107375 Blank A3*	072301C	7/23/01	7/24-25/01	NQ
				AVERAGE:	**0.00100**
				STANDARD DEVIATION:	**0.00148**

* 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/mL for red blood cells and 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 VI. Summary of PFOS Recoveries in Mallard Red Blood Cells

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
control	0107378 Spk A	052101B	5/21/01	5/22/01	10	85
control	0107378 Spk A*	052101B	5/21/01	5/22/01	10	88
control	0107378 Spk B	052101B	5/21/01	5/22/01	50000	91
control	0107378 Spk B*	052101B	5/21/01	5/22/01	50000	95
control	0107378 Spk A	052201A	5/22/01	5/23/01	10	84
control	0107378 Spk A*	052201A	5/22/01	5/23/01	10	82
control	0107378 Spk B	052201A	5/22/01	5/23/01	50000	84
control	0107378 Spk B*	052201A	5/22/01	5/23/01	50000	82
control	0107378 Spk A2	052201B	5/22/01	5/23/01	10	80
control	0107378 Spk A2*	052201B	5/22/01	5/23/01	10	76
control	0107378 Spk B2	052201B	5/22/01	5/23/01	50000	101
control	0107378 Spk B2*	052201B	5/22/01	5/23/01	50000	105
control	0107378 Spk B	052201C	5/22/01	5/23/01	50000	102
control	0107378 Spk B*	052201C	5/22/01	5/23/01	50000	101
					AVERAGE:	**90**
					STANDARD DEVIATION:	**9**
					RELATIVE STANDARD DEVIATION:	**11**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table VII. Summary of PFOS Recoveries in Mallard Serum

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
control	0107377 Spk A3	071901A	7/19/01	7/19-20/01	10	90
control	0107377 Spk A3*	071901A	7/19/01	7/19-20/01	10	89
control	0107377 Spk B3	071901A	7/19/01	7/19-20/01	500000	111
control	0107377 Spk B3*	071901A	7/19/01	7/19-20/01	500000	112
control	0107377 Spk A	072001A	7/20/01	7/21/01	10	76
control	0107377 Spk A*	072001A	7/20/01	7/21/01	10	82
control	0107377 Spk B	072001A	7/20/01	7/21/01	500000	117
control	0107377 Spk B*	072001A	7/20/01	7/21/01	500000	97
control	0107377 Spk A2	072001B	7/20/01	7/21-22/01	10	107
control	0107377 Spk A2*	072001B	7/20/01	7/21-22/01	10	110
control	0107377 Spk B2	072001B	7/20/01	7/21-22/01	500000	92
control	0107377 Spk B2*	072001B	7/20/01	7/21-22/01	500000	99
					AVERAGE:	**99**
					STANDARD DEVIATION:	**13**
					RELATIVE STANDARD DEVIATION:	**13**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table VIII. Summary of PFOS Recoveries in Quail Red Blood Cells

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
control	0107376 Spk A	051801DR	5/18/01	5/21-22/01	10	102
control	0107376 Spk A*	051801DR	5/18/01	5/21-22/01	10	99
control	0107376 Spk B	051801DR	5/18/01	5/21-22/01	50	111
control	0107376 Spk B*	051801DR	5/18/01	5/21-22/01	50	111
control	0107376 Spk A2	051801DR	5/18/01	5/21-22/01	10	113
control	0107376 Spk A2*	051801DR	5/18/01	5/21-22/01	10	110
control	0107376 Spk B2	051801DR	5/18/01	5/21-22/01	50	109
control	0107376 Spk B2*	051801DR	5/18/01	5/21-22/01	50	104
control	0107376 Spk A	052101A	5/21/01	5/22/01	10	87
control	0107376 Spk A*	052101A	5/21/01	5/22/01	10	89
control	0107376 Spk B	052101A	5/21/01	5/22/01	50000	91
control	0107376 Spk B*	052101A	5/21/01	5/22/01	50000	91
control	0107376 Spk A	052201C	5/22/01	5/23/01	10	90
control	0107376 Spk A*	052201C	5/22/01	5/23/01	10	94
					AVERAGE:	**100**
					STANDARD DEVIATION:	**10**
					RELATIVE STANDARD DEVIATION:	**10**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table IX. Summary of PFOS Recoveries in Quail Serum

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amt Added (ng/mL)	% Recovery
control	0107375 Spk A	072301AR	7/23/01	7/25/01	10	64
control	0107375 Spk A*	072301AR	7/23/01	7/25/01	10	62
control	0107375 Spk B	072301AR	7/23/01	7/25/01	500000	104
control	0107375 Spk B*	072301AR	7/23/01	7/25/01	500000	107
control	0107375 Spk A2	072301D	7/23/01	7/26-27/01	10	112
control	0107375 Spk A2*	072301D	7/23/01	7/26-27/01	10	113
control	0107375 Spk B2	072301B	7/23/01	7/24/01	500000	124
control	0107375 Spk B2*	072301B	7/23/01	7/24/01	500000	126
control	0107375 Spk A3	072301D	7/23/01	7/26-27/01	10	106
control	0107375 Spk A3*	072301D	7/23/01	7/26-27/01	10	105
control	0107375 Spk B3	072301C	7/23/01	7/24-25/01	500000	100
control	0107375 Spk B3*	072301C	7/23/01	7/24-25/01	500000	101
					AVERAGE:	**102**
					STANDARD DEVIATION:	**20**
					RELATIVE STANDARD DEVIATION:	**20**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table X. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk5	0107230	052101B	5/21/01	5/22/01	NQ
454-109-3249-Wk5*	0107230	052101B	5/21/01	5/22/01	NQ
454-109-3251-Wk5	0107231	052101B	5/21/01	5/22/01	0.00197
454-109-3251-Wk5*	0107231	052101B	5/21/01	5/22/01	0.00167
454-109-3252-Wk5	0107232	052101B	5/21/01	5/22/01	NQ
454-109-3252-Wk5*	0107232	052101B	5/21/01	5/22/01	NQ
454-109-3253-Wk5	0107233	052101B	5/21/01	5/22/01	NQ
454-109-3253-Wk5*	0107233	052101B	5/21/01	5/22/01	NQ
454-109-3254-Wk5	0107234	052101B	5/21/01	5/22/01	NQ
454-109-3254-Wk5*	0107234	052101B	5/21/01	5/22/01	NQ
454-109-3255-Wk5	0107235	052101B	5/21/01	5/22/01	0.00143
454-109-3255-Wk5*	0107235	052101B	5/21/01	5/22/01	NQ
454-109-3256-Wk5	0107236	052101B	5/21/01	5/22/01	NQ
454-109-3256-Wk5*	0107236	052101B	5/21/01	5/22/01	NQ
				AVERAGE:	**0.000401**
				STANDARD DEVIATION:	**0.000706**

* 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/mL for red blood cells and 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 Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk5	0107237	052101BD	5/21/01	5/23/01	24.4
454-109-3289-Wk5*	0107237	052101BD	5/21/01	5/23/01	24.7
454-109-3290-Wk5	0107238	052101BD	5/21/01	5/23/01	11.2
454-109-3290-Wk5*	0107238	052101BD	5/21/01	5/23/01	10.0
454-109-3291-Wk5	0107239	052101BD	5/21/01	5/23/01	11.8
454-109-3291-Wk5*	0107239	052101BD	5/21/01	5/23/01	12.3
454-109-3292-Wk5	0107240	052101BD	5/21/01	5/23/01	15.1
454-109-3292-Wk5*	0107240	052101BD	5/21/01	5/23/01	13.1
454-109-3293-Wk5	0107241	052101BD	5/21/01	5/23/01	30.1
454-109-3293-Wk5*	0107241	052101BD	5/21/01	5/23/01	30.8
454-109-3294-Wk5	0107242	052101BD	5/21/01	5/23/01	4.88
454-109-3294-Wk5*	0107242	052101BD	5/21/01	5/23/01	3.95
454-109-3295-Wk5	0107243	052101BD	5/21/01	5/23/01	18.9
454-109-3295-Wk5*	0107243	052101BD	5/21/01	5/23/01	18.8
454-109-3296-Wk5	0107244	052101BD	5/21/01	5/23/01	26.4
454-109-3296-Wk5*	0107244	052101BD	5/21/01	5/23/01	28.5
				AVERAGE:	**17.8**
				STANDARD DEVIATION:	**8.80**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XII. Summary of PFOS in Mallard Red Blood Cell Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3329-Wk5	0107245	052201CD	5/22/01	5/24/01	58.9
454-109-3329-Wk5*	0107245	052201CD	5/22/01	5/24/01	62.2
454-109-3330-Wk5	0107246	052201CD	5/22/01	5/24/01	112
454-109-3330-Wk5*	0107246	052201CD	5/22/01	5/24/01	123
				AVERAGE:	**89**
				STANDARD DEVIATION:	**33**

Table XIII. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk10	0107250	052201A	5/22/01	5/23/01	NQ
454-109-3249-Wk10*	0107250	052201A	5/22/01	5/23/01	NQ
454-109-3250-Wk10	0107251	052201A	5/22/01	5/23/01	NQ
454-109-3250-Wk10*	0107251	052201A	5/22/01	5/23/01	NQ
454-109-3251-Wk10	0107252	052201A	5/22/01	5/23/01	NQ
454-109-3251-Wk10*	0107252	052201A	5/22/01	5/23/01	NQ
454-109-3252-Wk10	0107253	052201A	5/22/01	5/23/01	NQ
454-109-3252-Wk10*	0107253	052201A	5/22/01	5/23/01	NQ
454-109-3253-Wk10	0107254	052201A	5/22/01	5/23/01	NQ
454-109-3253-Wk10*	0107254	052201A	5/22/01	5/23/01	NQ
454-109-3254-Wk10	0107255	052201A	5/22/01	5/23/01	NQ
454-109-3254-Wk10*	0107255	052201A	5/22/01	5/23/01	NQ
454-109-3255-Wk10	0107256	052201A	5/22/01	5/23/01	NQ
454-109-3255-Wk10*	0107256	052201A	5/22/01	5/23/01	NQ
454-109-3256-Wk10	0107257	052201A	5/22/01	5/23/01	NQ
454-109-3256-Wk10*	0107257	052201A	5/22/01	5/23/01	0.00245
				AVERAGE:	**0.000200**
				STANDARD DEVIATION:	**0.000600**

* 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/mL for red blood cells and 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 XIV. Summary of PFOS in Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk10	0107258	052201AD	5/22/01	5/23-24/01	19.3
454-109-3289-Wk10*	0107258	052201AD	5/22/01	5/23-24/01	19.6
454-109-3290-Wk10	0107259	052201AD	5/22/01	5/23-24/01	20.4
454-109-3290-Wk10*	0107259	052201AD	5/22/01	5/23-24/01	20.2
454-109-3291-Wk10	0107260	052201AD	5/22/01	5/23-24/01	14.1
454-109-3291-Wk10*	0107260	052201AD	5/22/01	5/23-24/01	14.9
454-109-3292-Wk10	0107261	052201AD	5/22/01	5/23-24/01	67.3
454-109-3292-Wk10*	0107261	052201AD	5/22/01	5/23-24/01	66.0
454-109-3293-Wk10	0107262	052201AD	5/22/01	5/23-24/01	33.5
454-109-3293-Wk10*	0107262	052201AD	5/22/01	5/23-24/01	34.2
454-109-3294-Wk10	0107263	052201AD	5/22/01	5/23-24/01	60.8
454-109-3294-Wk10*	0107263	052201AD	5/22/01	5/23-24/01	58.9
454-109-3295-Wk10	0107264	052201AD	5/22/01	5/23-24/01	16.1
454-109-3295-Wk10*	0107264	052201AD	5/22/01	5/23-24/01	16.0
454-109-3296-Wk10	0107265	052201AD	5/22/01	5/23-24/01	11.6
454-109-3296-Wk10*	0107265	052201AD	5/22/01	5/23-24/01	11.7
				AVERAGE:	**30.3**
				STANDARD DEVIATION:	**20.7**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XV. Summary of PFOS in Mallard Red Blood Cell Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk15	0107266	052201B	5/22/01	5/23/01	NQ
454-109-3249-Wk15*	0107266	052201B	5/22/01	5/23/01	NQ
454-109-3250-Wk15	0107267	052201B	5/22/01	5/23/01	NQ
454-109-3250-Wk15*	0107267	052201B	5/22/01	5/23/01	NQ
454-109-3251-Wk15	0107268	052201B	5/22/01	5/23/01	0.00179
454-109-3251-Wk15*	0107268	052201B	5/22/01	5/23/01	0.00188
454-109-3252-Wk15	0107269	052201B	5/22/01	5/23/01	NQ
454-109-3252-Wk15*	0107269	052201B	5/22/01	5/23/01	NQ
454-109-3253-Wk15	0107270	052201B	5/22/01	5/23/01	NQ
454-109-3253-Wk15*	0107270	052201B	5/22/01	5/23/01	NQ
454-109-3254-Wk15	0107271	052201B	5/22/01	5/23/01	ND
454-109-3254-Wk15*	0107271	052201B	5/22/01	5/23/01	ND
454-109-3255-Wk15	0107272	052201B	5/22/01	5/23/01	NQ
454-109-3255-Wk15*	0107272	052201B	5/22/01	5/23/01	NQ
454-109-3256-Wk15	0107273	052201B	5/22/01	5/23/01	NQ
454-109-3256-Wk15*	0107273	052201B	5/22/01	5/23/01	NQ
454-109-3236-Wk15	0107274	052201B	5/22/01	5/23/01	NQ
454-109-3236-Wk15*	0107274	052201B	5/22/01	5/23/01	NQ
				AVERAGE:	**0.000243**
				STANDARD DEVIATION:	**0.000613**

* Duplicate injection

ND = No peak detected
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/mL for red blood cells and serum

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

Table XVI. Summary of PFOS in Mallard Red Blood Cell Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk15	0107275	052201BD	5/22/01	5/23-24/01	53.5
454-109-3289-Wk15*	0107275	052201BD	5/22/01	5/23-24/01	48.2
454-109-3290-Wk15	0107276	052201BD	5/22/01	5/23-24/01	3.98
454-109-3290-Wk15*	0107276	052201BD	5/22/01	5/23-24/01	3.44
454-109-3291-Wk15	0107277	052201BD	5/22/01	5/23-24/01	60.9
454-109-3291-Wk15*	0107277	052201BD	5/22/01	5/23-24/01	61.4
454-109-3292-Wk15	0107278	052201BD	5/22/01	5/23-24/01	9.65
454-109-3292-Wk15*	0107278	052201BD	5/22/01	5/23-24/01	8.79
454-109-3293-Wk15	0107279	052201BDR	5/22/01	5/24/01	113
454-109-3293-Wk15*	0107279	052201BDR	5/22/01	5/24/01	116
454-109-3294-Wk15	0107280	052201BDR	5/22/01	5/24/01	1.54
454-109-3294-Wk15*	0107280	052201BDR	5/22/01	5/24/01	1.61
454-109-3295-Wk15	0107281	052201BDR	5/22/01	5/24/01	8.03
454-109-3295-Wk15*	0107281	052201BDR	5/22/01	5/24/01	6.56
454-109-3296-Wk15	0107282	052201BD	5/22/01	5/23-24/01	0.623
454-109-3296-Wk15*	0107282	052201BD	5/22/01	5/23-24/01	0.505
				AVERAGE:	**31.1**
				STANDARD DEVIATION:	**39.8**

Table XVII. Summary of PFOS in Mallard Red Blood Cell Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3297-TERM	0107283	052201CD	5/22/01	5/24/01	275
454-109-3297-TERM*	0107283	052201CD	5/22/01	5/24/01	272
454-109-3298-TERM	0107284	052201BDR	5/22/01	5/24/01	406
454-109-3298-TERM*	0107284	052201BDR	5/22/01	5/24/01	418
				AVERAGE:	**343**
				STANDARD DEVIATION:	**80**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XVIII. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk5	0107303	072001A	7/20/01	7/21/01	0.00414
454-109-3249-Wk5*	0107303	072001A	7/20/01	7/21/01	0.00417
454-109-3251-Wk5	0107304	072001A	7/20/01	7/21/01	0.00566
454-109-3251-Wk5*	0107304	072001A	7/20/01	7/21/01	0.00599
454-109-3252-Wk5	0107305	072001A	7/20/01	7/21/01	0.00878
454-109-3252-Wk5*	0107305	072001A	7/20/01	7/21/01	0.00943
454-109-3253-Wk5	0107306	072001A	7/20/01	7/21/01	0.00516
454-109-3253-Wk5*	0107306	072001A	7/20/01	7/21/01	0.00499
454-109-3254-Wk5	0107307	072001A	7/20/01	7/21/01	0.00432
454-109-3254-Wk5*	0107307	072001A	7/20/01	7/21/01	0.00444
454-109-3255-Wk5	0107308	072001A	7/20/01	7/21/01	0.00462
454-109-3255-Wk5*	0107308	072001A	7/20/01	7/21/01	0.00392
454-109-3256-Wk5	0107309	072001A	7/20/01	7/21/01	0.00544
454-109-3256-Wk5*	0107309	072001A	7/20/01	7/21/01	0.00516
				AVERAGE:	**0.00544**
				STANDARD DEVIATION:	**0.00167**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XIX. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk5	0107310	072001AD	7/20/01	7/22/01	128
454-109-3289-Wk5*	0107310	072001AD	7/20/01	7/22/01	120
454-109-3290-Wk5	0107311	072001AD	7/20/01	7/22/01	79.6
454-109-3290-Wk5*	0107311	072001AD	7/20/01	7/22/01	94.2
454-109-3291-Wk5	0107312	072001AD	7/20/01	7/22/01	104
454-109-3291-Wk5*	0107312	072001AD	7/20/01	7/22/01	110
454-109-3292-Wk5	0107313	072001AD	7/20/01	7/22/01	83.4
454-109-3292-Wk5*	0107313	072001AD	7/20/01	7/22/01	85.8
454-109-3293-Wk5	0107314	072001AD	7/20/01	7/22/01	59.4
454-109-3293-Wk5*	0107314	072001AD	7/20/01	7/22/01	76.6
454-109-3294-Wk5	0107315	072001AD	7/20/01	7/22/01	60.6
454-109-3294-Wk5*	0107315	072001AD	7/20/01	7/22/01	57.5
454-109-3295-Wk5	0107316	072001AD	7/20/01	7/22/01	73.3
454-109-3295-Wk5*	0107316	072001AD	7/20/01	7/22/01	76.9
				AVERAGE:	**86.4**
				STANDARD DEVIATION:	**22.2**

Table XX. Summary of PFOS in Mallard Serum Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3329-Wk5	0107317	072001AD	7/20/01	7/22/01	376
454-109-3329-Wk5*	0107317	072001AD	7/20/01	7/22/01	400
454-109-3330-Wk5	0107318	072001AD	7/20/01	7/22/01	320
454-109-3330-Wk5*	0107318	072001AD	7/20/01	7/22/01	290
				AVERAGE:	**347**
				STANDARD DEVIATION:	**50**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXI. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3249-Wk10	0107322	072001B	7/20/01	7/21-22/01	0.00271
454-109-3249-Wk10*	0107322	072001B	7/20/01	7/21-22/01	0.00372
454-109-3250-Wk10	0107323	072001B	7/20/01	7/21-22/01	0.00726
454-109-3250-Wk10*	0107323	072001B	7/20/01	7/21-22/01	0.00666
454-109-3251-Wk10	0107324	072001B	7/20/01	7/21-22/01	0.00659
454-109-3251-Wk10*	0107324	072001B	7/20/01	7/21-22/01	0.00530
454-109-3252-Wk10	0107325	072001B	7/20/01	7/21-22/01	0.00899
454-109-3252-Wk10*	0107325	072001B	7/20/01	7/21-22/01	0.00940
454-109-3253-Wk10	0107326	072001B	7/20/01	7/21-22/01	0.00517
454-109-3253-Wk10*	0107326	072001B	7/20/01	7/21-22/01	0.00482
454-109-3254-Wk10	0107327	072001B	7/20/01	7/21-22/01	ND
454-109-3254-Wk10*	0107327	072001B	7/20/01	7/21-22/01	ND
454-109-3255-Wk10	0107328	072001B	7/20/01	7/21-22/01	0.00337
454-109-3255-Wk10*	0107328	072001B	7/20/01	7/21-22/01	0.00403
454-109-3256-Wk10	0107329	072001B	7/20/01	7/21-22/01	0.00419
454-109-3256-Wk10*	0107329	072001B	7/20/01	7/21-22/01	0.00384
				AVERAGE:	**0.00476**
				STANDARD DEVIATION:	**0.00266**

* Duplicate injection

ND = No peak detected
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/mL for red blood cells and serum

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

Table XXII. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk10	0107330	072001BD	7/20/01	7/22/01	145
454-109-3289-Wk10*	0107330	072001BD	7/20/01	7/22/01	147
454-109-3290-Wk10	0107331	072001BD	7/20/01	7/22/01	32.7
454-109-3290-Wk10*	0107331	072001BD	7/20/01	7/22/01	32.5
454-109-3291-Wk10	0107332	072001BD	7/20/01	7/22/01	93.5
454-109-3291-Wk10*	0107332	072001BD	7/20/01	7/22/01	102
454-109-3292-Wk10	0107333	072001BD	7/20/01	7/22/01	107
454-109-3292-Wk10*	0107333	072001BD	7/20/01	7/22/01	111
454-109-3293-Wk10	0107334	072001BD	7/20/01	7/22/01	132
454-109-3293-Wk10*	0107334	072001BD	7/20/01	7/22/01	136
454-109-3294-Wk10	0107335	072001BD	7/20/01	7/22/01	85.3
454-109-3294-Wk10*	0107335	072001BD	7/20/01	7/22/01	86.4
454-109-3295-Wk10	0107336	072001BD	7/20/01	7/22/01	123
454-109-3295-Wk10*	0107336	072001BD	7/20/01	7/22/01	120
454-109-3296-Wk10	0107337	072001BD	7/20/01	7/22/01	114
454-109-3296-Wk10*	0107337	072001BD	7/20/01	7/22/01	112
				AVERAGE:	**105**
				STANDARD DEVIATION:	**33.8**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXIII. Summary of PFOS in Mallard Serum Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3236-Wk15	0107338	071901A	7/19/01	7/19-20/01	ND
454-109-3236-Wk15*	0107338	071901A	7/19/01	7/19-20/01	ND
454-109-3249-Wk15	0107339	071901A	7/19/01	7/19-20/01	0.00729
454-109-3249-Wk15*	0107339	071901A	7/19/01	7/19-20/01	0.0102
454-109-3250-Wk15	0107340	071901A	7/19/01	7/19-20/01	ND
454-109-3250-Wk15*	0107340	071901A	7/19/01	7/19-20/01	ND
454-109-3251-Wk15	0107341	071901A	7/19/01	7/19-20/01	0.00466
454-109-3251-Wk15*	0107341	071901A	7/19/01	7/19-20/01	0.00441
454-109-3252-Wk15	0107342	071901A	7/19/01	7/19-20/01	ND
454-109-3252-Wk15*	0107342	071901A	7/19/01	7/19-20/01	ND
454-109-3253-Wk15	0107343	071901A	7/19/01	7/19-20/01	0.00649
454-109-3253-Wk15*	0107343	071901A	7/19/01	7/19-20/01	0.00723
454-109-3254-Wk15	0107344	071901A	7/19/01	7/19-20/01	ND
454-109-3254-Wk15*	0107344	071901A	7/19/01	7/19-20/01	ND
454-109-3255-Wk15	0107345	071901A	7/19/01	7/19-20/01	0.0233
454-109-3255-Wk15*	0107345	071901A	7/19/01	7/19-20/01	0.00699
454-109-3256-Wk15	0107346	071901A	7/19/01	7/19-20/01	0.0195
454-109-3256-Wk15*	0107346	071901A	7/19/01	7/19-20/01	ND
				AVERAGE:	**0.000503**
				STANDARD DEVIATION:	**0.00616**

* Duplicate injection

ND = No peak detected
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/mL for red blood cells and serum

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

Table XXIV. Summary of PFOS in Mallard Serum Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3289-Wk15	0107347	071901AD	7/19/01	7/20-21/01	152
454-109-3289-Wk15*	0107347	071901AD	7/19/01	7/20-21/01	162
454-109-3290-Wk15	0107348	071901AD	7/19/01	7/20-21/01	10.1
454-109-3290-Wk15*	0107348	071901AD	7/19/01	7/20-21/01	8.75
454-109-3291-Wk15	0107349	071901AD	7/19/01	7/20-21/01	152
454-109-3291-Wk15*	0107349	071901AD	7/19/01	7/20-21/01	142
454-109-3292-Wk15	0107350	071901AD	7/19/01	7/20-21/01	12.1
454-109-3292-Wk15*	0107350	071901AD	7/19/01	7/20-21/01	11.6
454-109-3293-Wk15	0107351	071901AD	7/19/01	7/20-21/01	128
454-109-3293-Wk15*	0107351	071901AD	7/19/01	7/20-21/01	132
454-109-3294-Wk15	0107352	071901AD	7/19/01	7/20-21/01	6.73
454-109-3294-Wk15*	0107352	071901AD	7/19/01	7/20-21/01	4.52
454-109-3295-Wk15	0107353	071901AD	7/19/01	7/20-21/01	117
454-109-3295-Wk15*	0107353	071901AD	7/19/01	7/20-21/01	116
454-109-3296-Wk15	0107354	071901AD	7/19/01	7/20-21/01	9.52
454-109-3296-Wk15*	0107354	071901AD	7/19/01	7/20-21/01	11.3
				AVERAGE:	**73.5**
				STANDARD DEVIATION:	**67.3**

Table XXV. Summary of PFOS in Mallard Serum Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-109-3297-TERM	0107355	072001AD	7/20/01	7/22/01	685
454-109-3297-TERM*	0107355	072001AD	7/20/01	7/22/01	601
454-109-3298-TERM	0107356	072001AD	7/20/01	7/22/01	664
454-109-3298-TERM*	0107356	072001AD	7/20/01	7/22/01	601
				AVERAGE:	**638**
				STANDARD DEVIATION:	**43**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXVI. Summary of PFOS in Quail Red Blood Cell Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk5	0107077	051801A	5/18/01	5/18-19/01	NQ
454-108-361-Wk5*	0107077	051801A	5/18/01	5/18-19/01	NQ
454-108-362-Wk5	0107078	051801A	5/18/01	5/18-19/01	NQ
454-108-362-Wk5*	0107078	051801A	5/18/01	5/18-19/01	NQ
454-108-363-Wk5	0107079	051801A	5/18/01	5/18-19/01	NQ
454-108-363-Wk5*	0107079	051801A	5/18/01	5/18-19/01	NQ
454-108-364-Wk5	0107080	051801A	5/18/01	5/18-19/01	NQ
454-108-364-Wk5*	0107080	051801A	5/18/01	5/18-19/01	NQ
454-108-365-Wk5	0107081	051801A	5/18/01	5/18-19/01	NQ
454-108-365-Wk5*	0107081	051801A	5/18/01	5/18-19/01	NQ
454-108-366-Wk5	0107082	051801A	5/18/01	5/18-19/01	0.00292
454-108-366-Wk5*	0107082	051801A	5/18/01	5/18-19/01	0.00264
454-108-368-Wk5	0107083	051801A	5/18/01	5/18-19/01	NQ
454-108-368-Wk5*	0107083	051801A	5/18/01	5/18-19/01	NQ
				AVERAGE:	**0.000440**
				STANDARD DEVIATION:	**0.000993**

* 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/mL for red blood cells and 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 XXVII. Summary of PFOS in Quail Red Blood Cell Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk5	0107084	051801DR	5/18/01	5/21-22/01	113
454-108-401-Wk5*	0107084	051801DR	5/18/01	5/21-22/01	107
454-108-402-Wk5	0107085	051801DRD	5/18/01	5/22/01	353
454-108-402-Wk5*	0107085	051801DRD	5/18/01	5/22/01	351
454-108-403-Wk5	0107086	051801DR	5/18/01	5/21-22/01	30.5
454-108-403-Wk5*	0107086	051801DR	5/18/01	5/21-22/01	30.4
454-108-404-Wk5	0107087	051801DR	5/18/01	5/21-22/01	26.9
454-108-404-Wk5*	0107087	051801DR	5/18/01	5/21-22/01	25.8
454-108-405-Wk5	0107088	051801DRD	5/18/01	5/22/01	415
454-108-405-Wk5*	0107088	051801DRD	5/18/01	5/22/01	447
454-108-406-Wk5	0107089	051801DRD	5/18/01	5/22/01	1050
454-108-406-Wk5*	0107089	051801DRD	5/18/01	5/22/01	1040
454-108-407-Wk5	0107090	051801DRD	5/18/01	5/22/01	495
454-108-407-Wk5*	0107090	051801DRD	5/18/01	5/22/01	501
454-108-408-Wk5	0107091	051801DRD	5/18/01	5/22/01	451
454-108-408-Wk5*	0107091	051801DRD	5/18/01	5/22/01	471
				AVERAGE:	**369**
				STANDARD DEVIATION:	**324**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXVIII. Summary of PFOS in Quail Red Blood Cell Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-441-Wk5	0107092	052201CD	5/22/01	5/24/01	166
454-108-441-Wk5*	0107092	052201CD	5/22/01	5/24/01	163
454-108-442-Wk5	0107093	052201CD	5/22/01	5/24/01	108
454-108-442-Wk5*	0107093	052201CD	5/22/01	5/24/01	103
				AVERAGE:	**135**
				STANDARD DEVIATION:	**34**

Table XXIX. Summary of PFOS in Quail Red Blood Cell Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk10	0107100	051801B	5/18/01	5/19/01	NQ
454-108-361-Wk10*	0107100	051801B	5/18/01	5/19/01	NQ
454-108-362-Wk10	0107101	051801B	5/18/01	5/19/01	NQ
454-108-362-Wk10*	0107101	051801B	5/18/01	5/19/01	NQ
454-108-363-Wk10	0107102	051801B	5/18/01	5/19/01	NQ
454-108-363-Wk10*	0107102	051801B	5/18/01	5/19/01	NQ
454-108-364-Wk10	0107103	051801B	5/18/01	5/19/01	NQ
454-108-364-Wk10*	0107103	051801B	5/18/01	5/19/01	NQ
454-108-365-Wk10	0107104	051801B	5/18/01	5/19/01	NQ
454-108-365-Wk10*	0107104	051801B	5/18/01	5/19/01	NQ
454-108-366-Wk10	0107105	051801B	5/18/01	5/19/01	NQ
454-108-366-Wk10*	0107105	051801B	5/18/01	5/19/01	NQ
454-108-367-Wk10	0107106	051801B	5/18/01	5/19/01	NQ
454-108-367-Wk10*	0107106	051801B	5/18/01	5/19/01	NQ
454-108-368-Wk10	0107107	051801B	5/18/01	5/19/01	NQ
454-108-368-Wk10*	0107107	051801B	5/18/01	5/19/01	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))

LOQ = 0.01 µg/mL for red blood cells and 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 XXX.Summary of PFOS in Quail Red Blood Cell Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk10	0107108	051801DRD	5/18/01	5/22/01	672
454-108-401-Wk10*	0107108	051801DRD	5/18/01	5/22/01	671
454-108-402-Wk10	0107109	051801DRD	5/18/01	5/22/01	203
454-108-402-Wk10*	0107109	051801DRD	5/18/01	5/22/01	190
454-108-403-Wk10	0107110	051801DR	5/18/01	5/21-22/01	23.0
454-108-403-Wk10*	0107110	051801DR	5/18/01	5/21-22/01	20.7
454-108-404-Wk10	0107111	051801DR	5/18/01	5/21-22/01	19.0
454-108-404-Wk10*	0107111	051801DR	5/18/01	5/21-22/01	21.2
454-108-405-Wk10	0107112	051801DR	5/18/01	5/21-22/01	18.0
454-108-405-Wk10*	0107112	051801DR	5/18/01	5/21-22/01	20.0
454-108-406-Wk10	0107113	051801DR	5/18/01	5/21-22/01	48.9
454-108-406-Wk10*	0107113	051801DR	5/18/01	5/21-22/01	50.5
454-108-407-Wk10	0107114	051801DR	5/18/01	5/21-22/01	22.8
454-108-407-Wk10*	0107114	051801DR	5/18/01	5/21-22/01	22.3
454-108-408-Wk10	0107115	051801DR	5/18/01	5/21-22/01	30.4
454-108-408-Wk10*	0107115	051801DR	5/18/01	5/21-22/01	30.2
				AVERAGE:	**129**
				STANDARD DEVIATION:	**220**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXI. Summary of PFOS in Quail Red Blood Cell Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk15	0107116	052101A	5/21/01	5/22/01	NQ
454-108-361-Wk15*	0107116	052101A	5/21/01	5/22/01	NQ
454-108-362-Wk15	0107117	052101A	5/21/01	5/22/01	NQ
454-108-362-Wk15*	0107117	052101A	5/21/01	5/22/01	NQ
454-108-363-Wk15	0107118	052101A	5/21/01	5/22/01	0.00226
454-108-363-Wk15*	0107118	052101A	5/21/01	5/22/01	0.00328
454-108-364-Wk15	0107119	052101A	5/21/01	5/22/01	0.00127
454-108-364-Wk15*	0107119	052101A	5/21/01	5/22/01	NQ
454-108-365-Wk15	0107120	052101A	5/21/01	5/22/01	0.00705
454-108-365-Wk15*	0107120	052101A	5/21/01	5/22/01	0.00391
454-108-366-Wk15	0107121	052101A	5/21/01	5/22/01	NQ
454-108-366-Wk15*	0107121	052101A	5/21/01	5/22/01	NQ
454-108-367-Wk15	0107122	052101A	5/21/01	5/22/01	0.00795
454-108-367-Wk15*	0107122	052101A	5/21/01	5/22/01	0.00816
454-108-368-Wk15	0107123	052101A	5/21/01	5/22/01	NQ
454-108-368-Wk15*	0107123	052101A	5/21/01	5/22/01	NQ
454-108-333-Wk15	0107124	052101A	5/21/01	5/22/01	0.00130
454-108-333-Wk15*	0107124	052101A	5/21/01	5/22/01	NQ
				AVERAGE:	**0.00198**
				STANDARD DEVIATION:	**0.00304**

* Duplicate injection

ND = No peak detected
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/mL for red blood cells and serum

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

Table XXXII. Summary of PFOS in Quail Red Blood Cell Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk15	0107125	052101AD	5/21/01	5/22-23/01	72.2
454-108-401-Wk15*	0107125	052101AD	5/21/01	5/22-23/01	69.1
454-108-402-Wk15	0107126	052101AD	5/21/01	5/22-23/01	2.36
454-108-402-Wk15*	0107126	052101AD	5/21/01	5/22-23/01	1.65
454-108-403-Wk15	0107127	052101AD	5/21/01	5/22-23/01	21.2
454-108-403-Wk15*	0107127	052101AD	5/21/01	5/22-23/01	18.8
454-108-404-Wk15	0107128	052101AD	5/21/01	5/22-23/01	1.30
454-108-404-Wk15*	0107128	052101AD	5/21/01	5/22-23/01	1.22
454-108-405-Wk15	0107129	052101AD	5/21/01	5/22-23/01	15.8
454-108-405-Wk15*	0107129	052101AD	5/21/01	5/22-23/01	17.9
454-108-406-Wk15	0107130	052101AD	5/21/01	5/22-23/01	3.61
454-108-406-Wk15*	0107130	052101AD	5/21/01	5/22-23/01	3.23
454-108-407-Wk15	0107131	052101AD	5/21/01	5/22-23/01	13.3
454-108-407-Wk15*	0107131	052101AD	5/21/01	5/22-23/01	9.60
454-108-408-Wk15	0107132	052201CD	5/21/01	5/24/01	1.24
454-108-408-Wk15*	0107132	052201CD	5/21/01	5/24/01	1.55
454-108-383-Wk15	0107133	052101AD	5/21/01	5/22-23/01	56.7
454-108-383-Wk15*	0107133	052101AD	5/21/01	5/22-23/01	54.1
				AVERAGE:	**20.3**
				STANDARD DEVIATION:	**24.8**

Table XXXIII. Summary of PFOS in Quail Red Blood Cell Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-409-TERM	0107134	052201CD	5/22/01	5/24/01	361
454-108-409-TERM*	0107134	052201CD	5/22/01	5/24/01	356
454-108-410-TERM	0107135	052201CD	5/22/01	5/24/01	106
454-108-410-TERM*	0107135	052201CD	5/22/01	5/24/01	114
				AVERAGE:	**234**
				STANDARD DEVIATION:	**144**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXIV. Summary of PFOS in Quail Serum Samples at 0 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk5	0107154	072301AR	7/23/01	7/25/01	0.0282
454-108-361-Wk5*	0107154	072301AR	7/23/01	7/25/01	0.0295
454-108-362-Wk5	0107155	072301AR	7/23/01	7/25/01	0.0149
454-108-362-Wk5*	0107155	072301AR	7/23/01	7/25/01	0.0134
454-108-363-Wk5	0107156	072301AR	7/23/01	7/25/01	0.0198
454-108-363-Wk5*	0107156	072301AR	7/23/01	7/25/01	0.0166
454-108-364-Wk5	0107157	072301AR	7/23/01	7/25/01	0.00510
454-108-364-Wk5*	0107157	072301AR	7/23/01	7/25/01	0.00535
454-108-366-Wk5	0107159	072301AR	7/23/01	7/25/01	0.0135
454-108-366-Wk5*	0107159	072301AR	7/23/01	7/25/01	0.0120
454-108-368-Wk5	0107160	072301AR	7/23/01	7/25/01	0.0138
454-108-368-Wk5*	0107160	072301AR	7/23/01	7/25/01	0.0156
				AVERAGE:	**0.0156**
				STANDARD DEVIATION:	**0.00746**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXV. Summary of PFOS in Quail Serum Samples at 10 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-402-Wk5	0107162	072301AD	7/23/01	7/26/01	45.8
454-108-402-Wk5*	0107162	072301AD	7/23/01	7/26/01	45.6
454-108-403-Wk5	0107163	072301AD	7/23/01	7/26/01	115
454-108-403-Wk5*	0107163	072301AD	7/23/01	7/26/01	128
454-108-404-Wk5	0107164	072301AD	7/23/01	7/26/01	104
454-108-404-Wk5*	0107164	072301AD	7/23/01	7/26/01	98.3
454-108-405-Wk5	0107165	072301AD	7/23/01	7/26/01	138
454-108-405-Wk5*	0107165	072301AD	7/23/01	7/26/01	129
454-108-406-Wk5	0107166	072301AD	7/23/01	7/26/01	106
454-108-406-Wk5*	0107166	072301AD	7/23/01	7/26/01	107
454-108-407-Wk5	0107167	072301AD	7/23/01	7/26/01	42.4
454-108-407-Wk5*	0107167	072301AD	7/23/01	7/26/01	36.3
454-108-408-Wk5	0107168	072301AD	7/23/01	7/26/01	77.6
454-108-408-Wk5*	0107168	072301AD	7/23/01	7/26/01	84.4
				AVERAGE:	**89.8**
				STANDARD DEVIATION:	**35.0**

Table XXXVI. Summary of PFOS in Quail Serum Samples at 50 ppm a.i. for Week 5

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-441-Wk5	0107092	072301AD	7/23/01	7/26/01	551
454-108-441-Wk5*	0107092	072301AD	7/23/01	7/26/01	562
454-108-442-Wk5	0107093	072301AD	7/23/01	7/26/01	529
454-108-442-Wk5*	0107093	072301AD	7/23/01	7/26/01	520
				AVERAGE:	**541**
				STANDARD DEVIATION:	**19.4**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXVII. Summary of PFOS in Quail Serum Samples at 0 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk10	0107177	072301B	7/23/01	7/24/01	0.00483
454-108-361-Wk10*	0107177	072301B	7/23/01	7/24/01	0.00489
454-108-362-Wk10	0107178	072301B	7/23/01	7/24/01	0.00295
454-108-362-Wk10*	0107178	072301B	7/23/01	7/24/01	0.00249
454-108-363-Wk10	0107179	072301B	7/23/01	7/24/01	0.00467
454-108-363-Wk10*	0107179	072301B	7/23/01	7/24/01	0.00521
454-108-364-Wk10	0107180	072301B	7/23/01	7/24/01	0.00390
454-108-364-Wk10*	0107180	072301B	7/23/01	7/24/01	0.00375
454-108-365-Wk10	0107181	072301B	7/23/01	7/24/01	0.00271
454-108-365-Wk10*	0107181	072301B	7/23/01	7/24/01	0.00292
454-108-366-Wk10	0107182	072301B	7/23/01	7/24/01	0.00439
454-108-366-Wk10*	0107182	072301B	7/23/01	7/24/01	0.00437
454-108-367-Wk10	0107183	072301B	7/23/01	7/24/01	0.00563
454-108-367-Wk10*	0107183	072301B	7/23/01	7/24/01	0.00542
454-108-368-Wk10	0107184	072301B	7/23/01	7/24/01	0.00537
454-108-368-Wk10*	0107184	072301B	7/23/01	7/24/01	0.00526
				AVERAGE:	**0.00430**
			STANDARD DEVIATION:		**0.00105**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXVIII. Summary of PFOS in Quail Serum Samples at 10 ppm a.i. for Week 10

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk10	0107185	072301DD	7/23/01	7/27-28/01	213
454-108-401-Wk10*	0107185	072301DD	7/23/01	7/27-28/01	207
454-108-402-Wk10	0107186	072301DD	7/23/01	7/27-28/01	53.6
454-108-402-Wk10*	0107186	072301DD	7/23/01	7/27-28/01	53.7
454-108-403-Wk10	0107187	072301DD	7/23/01	7/27-28/01	148
454-108-403-Wk10*	0107187	072301DD	7/23/01	7/27-28/01	177
454-108-404-Wk10	0107188	072301D	7/23/01	7/27-28/01	19.4
454-108-404-Wk10*	0107188	072301D	7/23/01	7/27-28/01	20.3
454-108-405-Wk10	0107189	072301DD	7/23/01	7/27-28/01	157
454-108-405-Wk10*	0107189	072301DD	7/23/01	7/27-28/01	147
454-108-407-Wk10	0107190	072301DD	7/23/01	7/27-28/01	105
454-108-407-Wk10*	0107190	072301DD	7/23/01	7/27-28/01	110
454-108-408-Wk10	0107191	072301DD	7/23/01	7/27-28/01	97.0
454-108-408-Wk10*	0107191	072301DD	7/23/01	7/27-28/01	93.7
				AVERAGE:	**114**
				STANDARD DEVIATION:	**63.4**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

Table XXXIX. Summary of PFOS in Quail Serum Samples at 0 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-361-Wk15	0107192	072301C	7/23/01	7/24-25/01	NQ
454-108-361-Wk15*	0107192	072301C	7/23/01	7/24-25/01	NQ
454-108-362-Wk15	0107193	072301C	7/23/01	7/24-25/01	NQ
454-108-362-Wk15*	0107193	072301C	7/23/01	7/24-25/01	NQ
454-108-363-Wk15	0107194	072301C	7/23/01	7/24-25/01	0.00154
454-108-363-Wk15*	0107194	072301C	7/23/01	7/24-25/01	0.00160
454-108-364-Wk15	0107195	072301C	7/23/01	7/24-25/01	NQ
454-108-364-Wk15*	0107195	072301C	7/23/01	7/24-25/01	NQ
454-108-365-Wk15	0107196	072301C	7/23/01	7/24-25/01	NQ
454-108-365-Wk15*	0107196	072301C	7/23/01	7/24-25/01	NQ
454-108-366-Wk15	0107197	072301C	7/23/01	7/24-25/01	NQ
454-108-366-Wk15*	0107197	072301C	7/23/01	7/24-25/01	NQ
454-108-367-Wk15	0107198	072301C	7/23/01	7/24-25/01	0.00227
454-108-367-Wk15*	0107198	072301C	7/23/01	7/24-25/01	0.00249
454-108-368-Wk15	0107199	072301C	7/23/01	7/24-25/01	0.00253
454-108-368-Wk15*	0107199	072301C	7/23/01	7/24-25/01	0.00255
454-108-333-Wk15	0107200	072301C	7/23/01	7/24-25/01	0.00162
454-108-333-Wk15*	0107200	072301C	7/23/01	7/24-25/01	0.00160
				AVERAGE:	**0.000928**
				STANDARD DEVIATION:	**0.00105**

* 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/mL for red blood cells and serum

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

Table XL. Summary of PFOS in Quail Serum Samples at 10 ppm a.i. for Week 15

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-401-Wk15	0107201	072301DD	7/23/01	7/27-28/01	181
454-108-401-Wk15*	0107201	072301DD	7/23/01	7/27-28/01	180
454-108-402-Wk15	0107202	072301DD	7/23/01	7/27-28/01	8.21
454-108-402-Wk15*	0107202	072301DD	7/23/01	7/27-28/01	8.45
454-108-403-Wk15	0107203	072301DD	7/23/01	7/27-28/01	246
454-108-403-Wk15*	0107203	072301DD	7/23/01	7/27-28/01	239
454-108-404-Wk15	0107204	072301DD	7/23/01	7/27-28/01	11.1
454-108-404-Wk15*	0107204	072301DD	7/23/01	7/27-28/01	10.7
454-108-405-Wk15	0107205	072301DD	7/23/01	7/27-28/01	141
454-108-405-Wk15*	0107205	072301DD	7/23/01	7/27-28/01	156
454-108-406-Wk15	0107206	072301DD	7/23/01	7/27-28/01	7.04
454-108-406-Wk15*	0107206	072301DD	7/23/01	7/27-28/01	6.98
454-108-407-Wk15	0107207	072301DD	7/23/01	7/27-28/01	107
454-108-407-Wk15*	0107207	072301DD	7/23/01	7/27-28/01	114
454-108-408-Wk15	0107208	072301DD	7/23/01	7/27-28/01	10.2
454-108-408-Wk15*	0107208	072301DD	7/23/01	7/27-28/01	8.91
454-108-383-Wk15	0107209	072301DD	7/23/01	7/27-28/01	131
454-108-383-Wk15*	0107209	072301DD	7/23/01	7/27-28/01	143
				AVERAGE:	**95.0**
				STANDARD DEVIATION:	**86.6**

Table XLI. Summary of PFOS in Quail Serum Samples at 50 ppm a.i. for TERM

Sponsor ID	Centre ID	Set Number	Extraction Date	Analysis Date	Analyte Found (µg/mL)
454-108-410-TERM	0107211	072301DD	7/23/01	7/27-28/01	476
454-108-410-TERM*	0107211	072301DD	7/23/01	7/27-28/01	514
				AVERAGE:	**495**
				STANDARD DEVIATION:	**26.9**

* Duplicate injection

LOQ = 0.01 µg/mL for red blood cells and serum

FIGURES

Figure 1. Typical Calibration Curve for PFOS

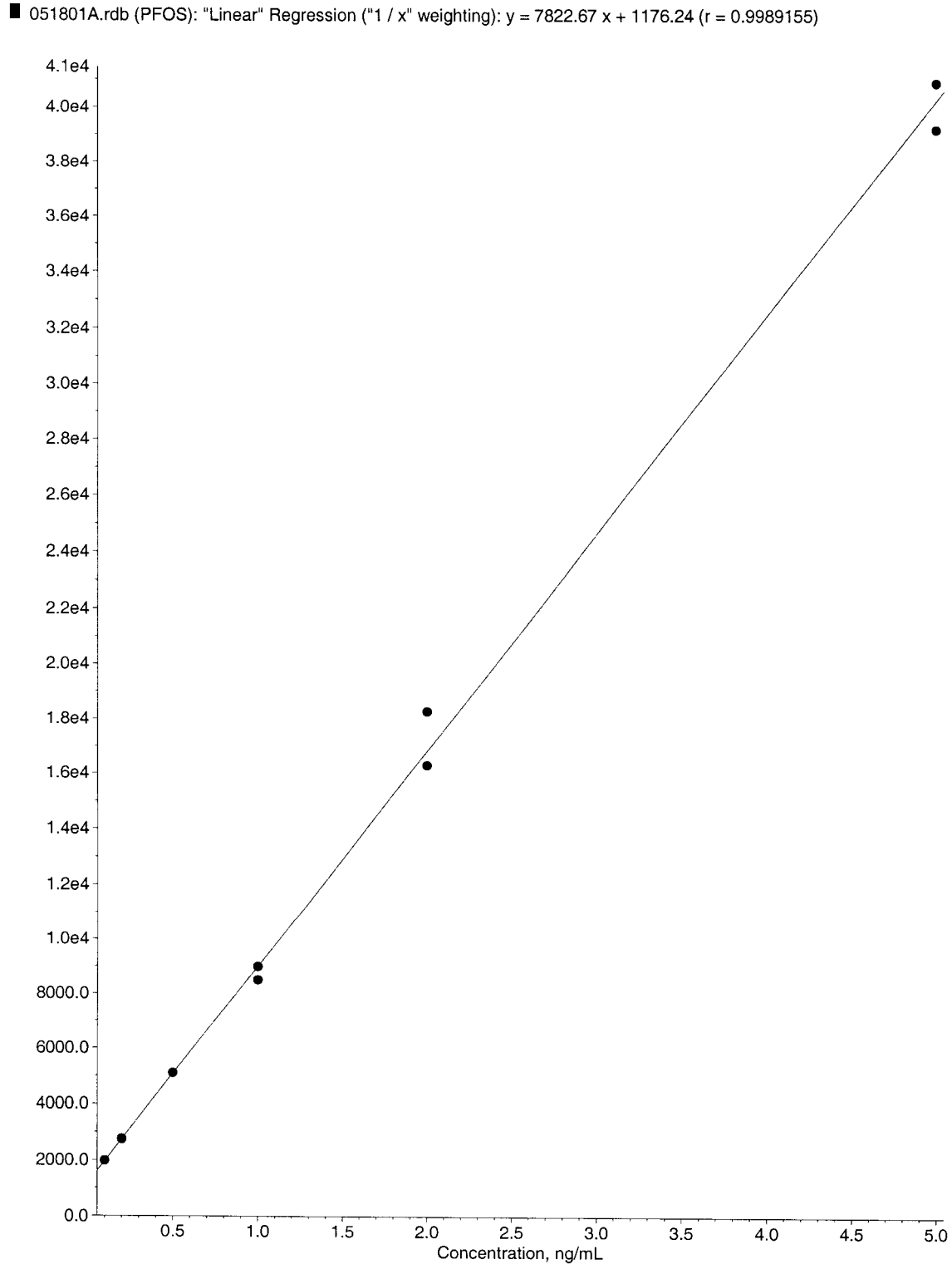


Figure 2. Chromatogram Representing a 0.1 ng/mL standard for PFOS

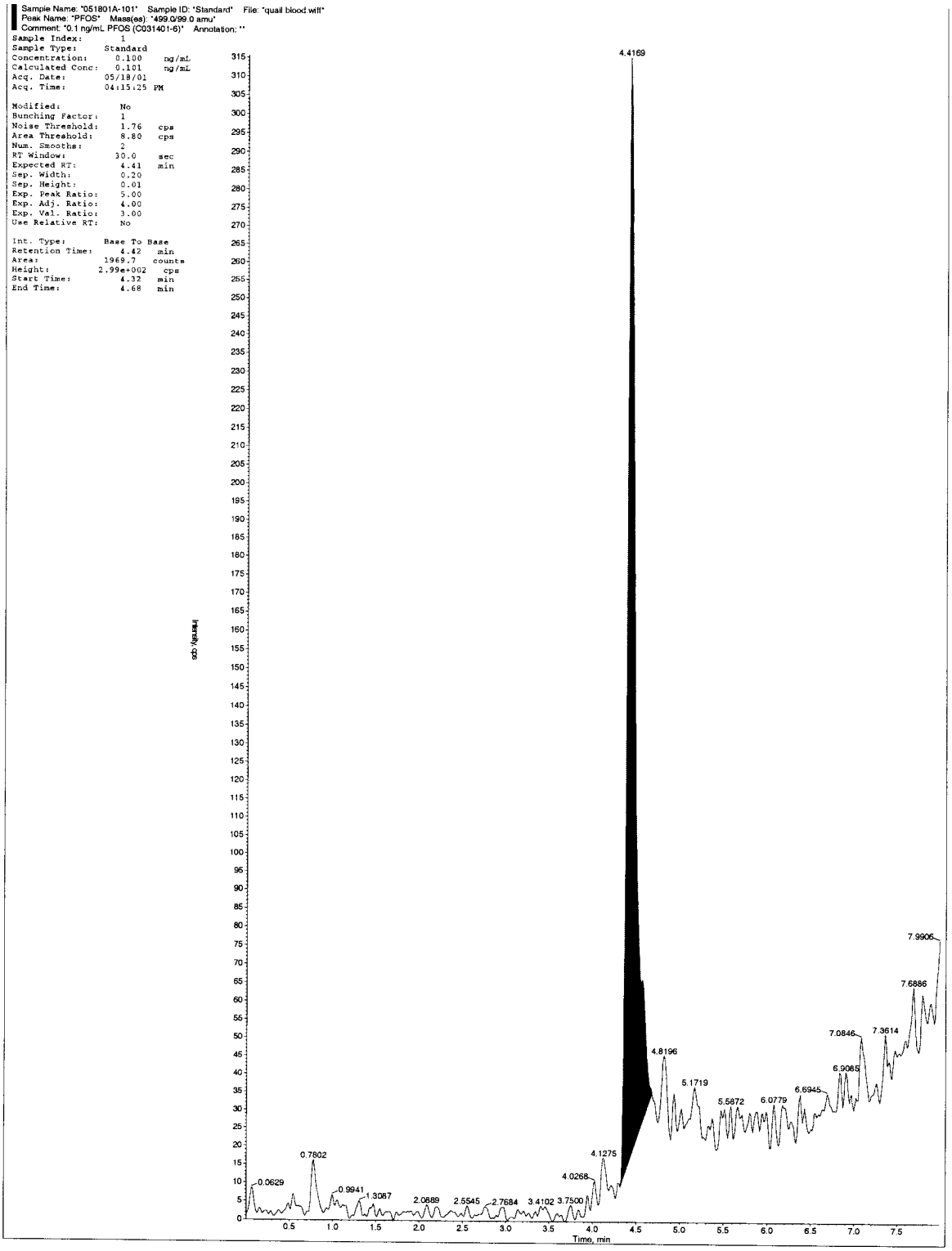


Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS

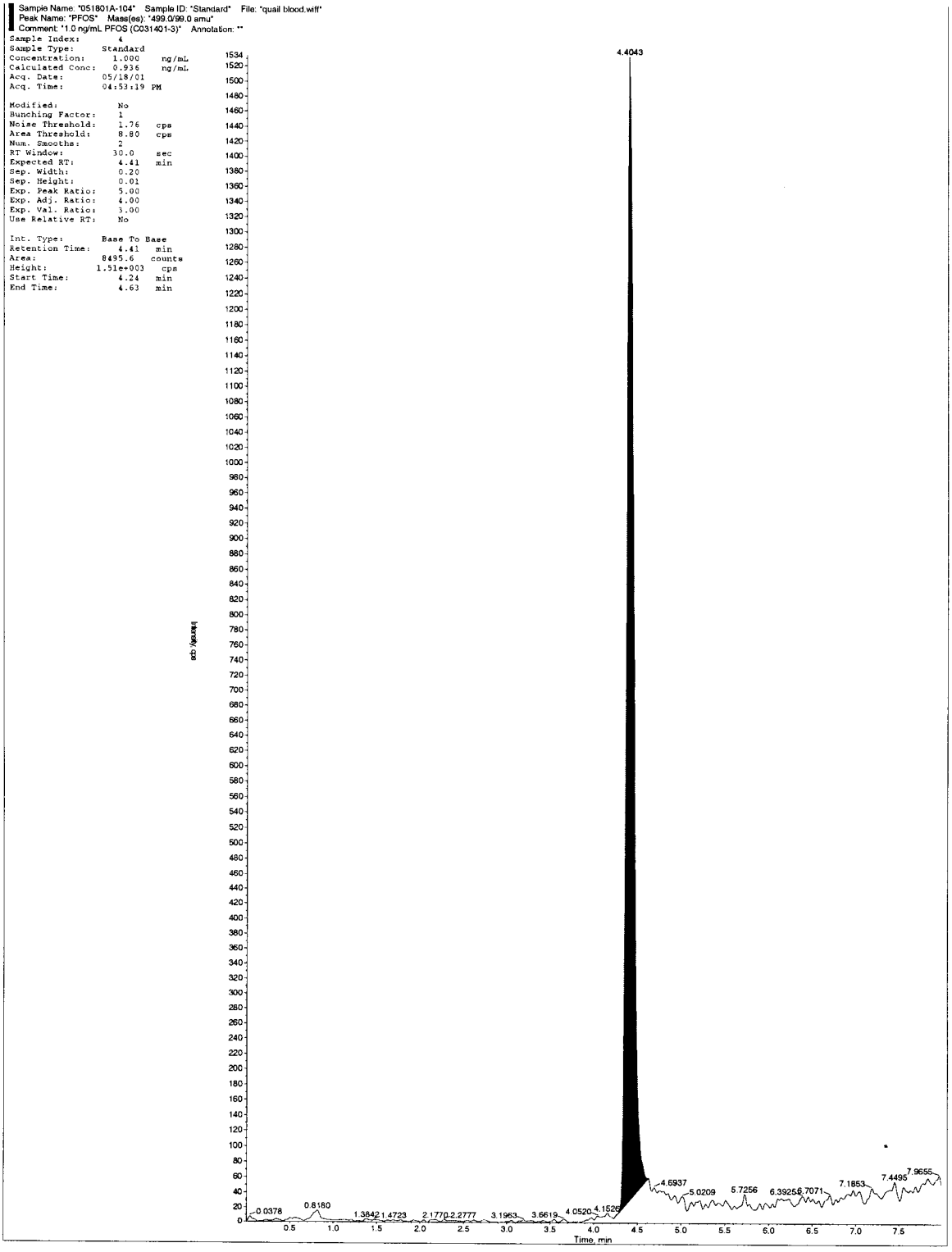


Figure 4. Chromatogram Representing a Reagent Blank for PFOS (Exygen ID Reagent Blank A, Set: 051801A)

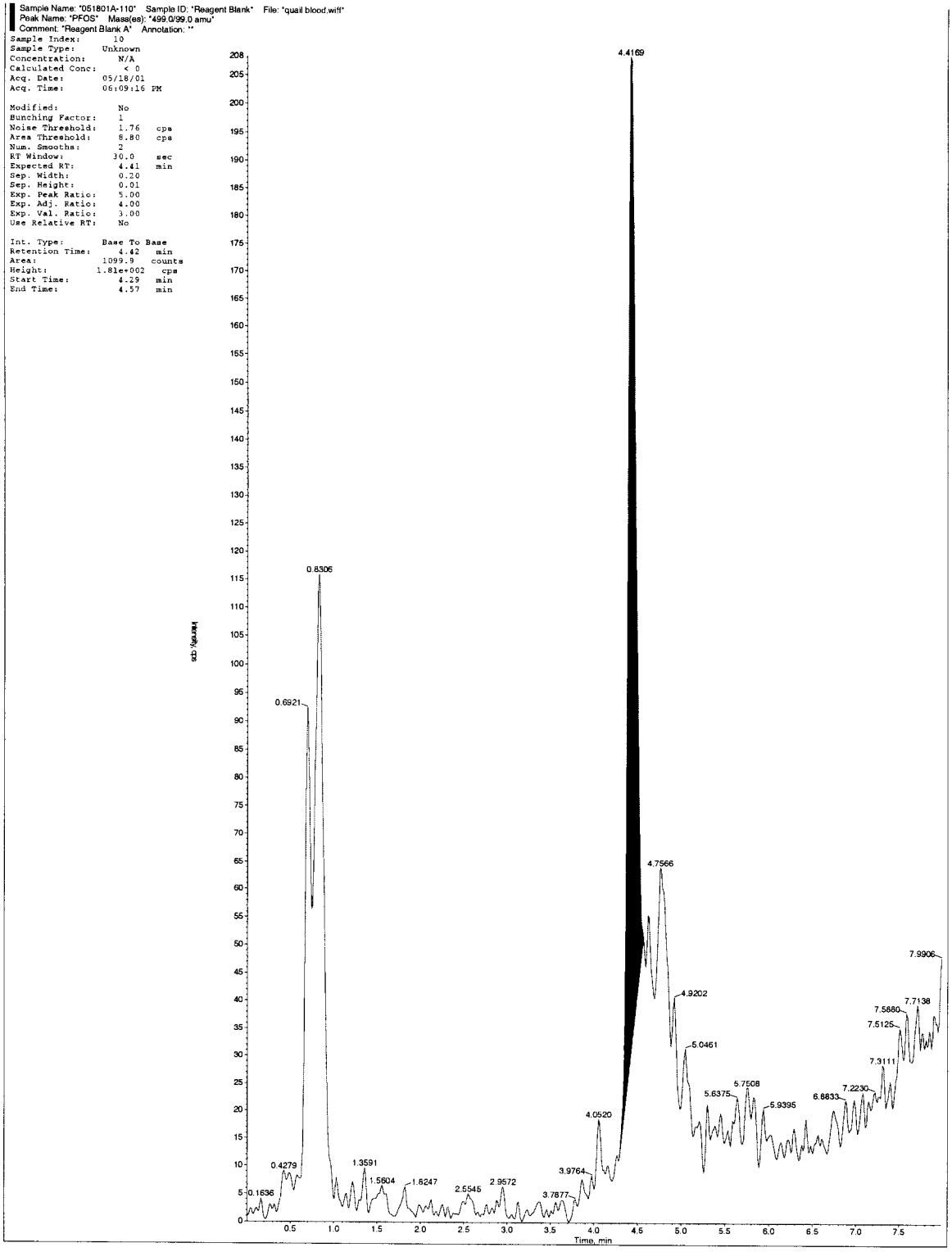


Figure 5. Chromatogram Representing Control Quail Red Blood Cells for PFOS (Exygen ID: 0107376 Blank A, Set: 051801A)

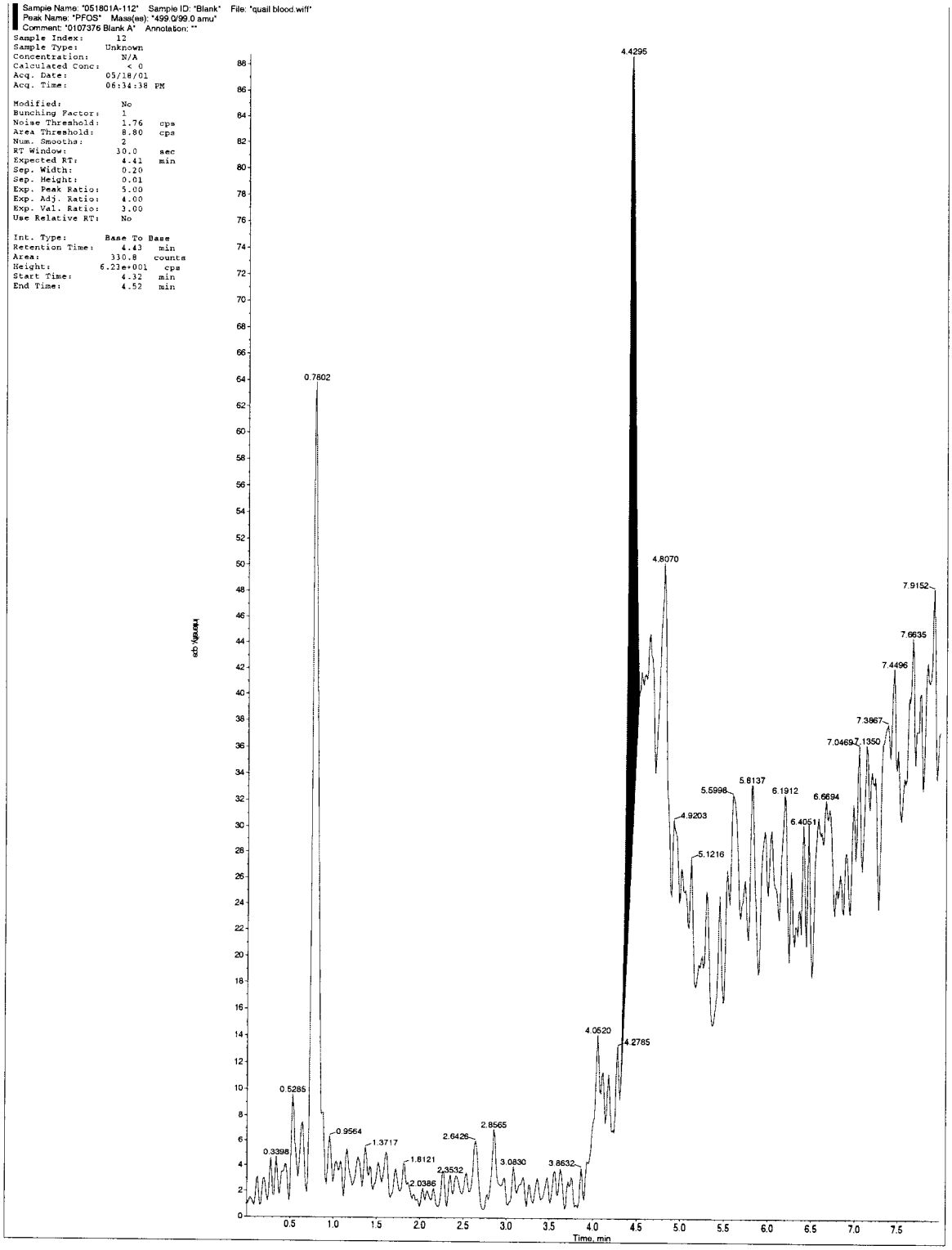


Figure 6. Chromatogram Representing Control Quail Serum for PFOS, (Exygen ID: 0107375 Blank A, Set: 072301C)

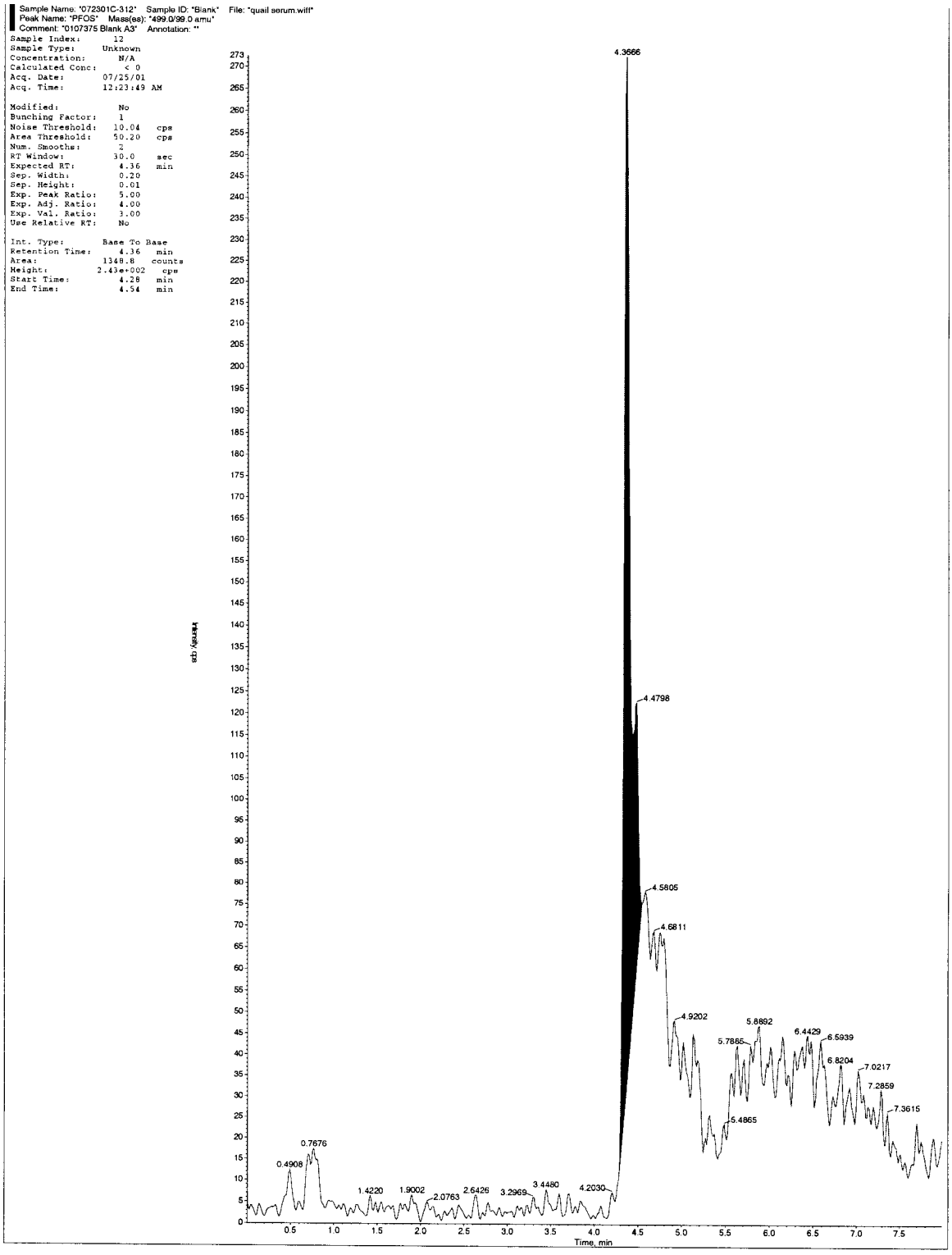


Figure 7. Chromatogram Representing Control Quail Red Blood Cells fortified with 10 ng/mL of PFOS (Exygen ID: 0107376 Spk A, Set: 051801DR)

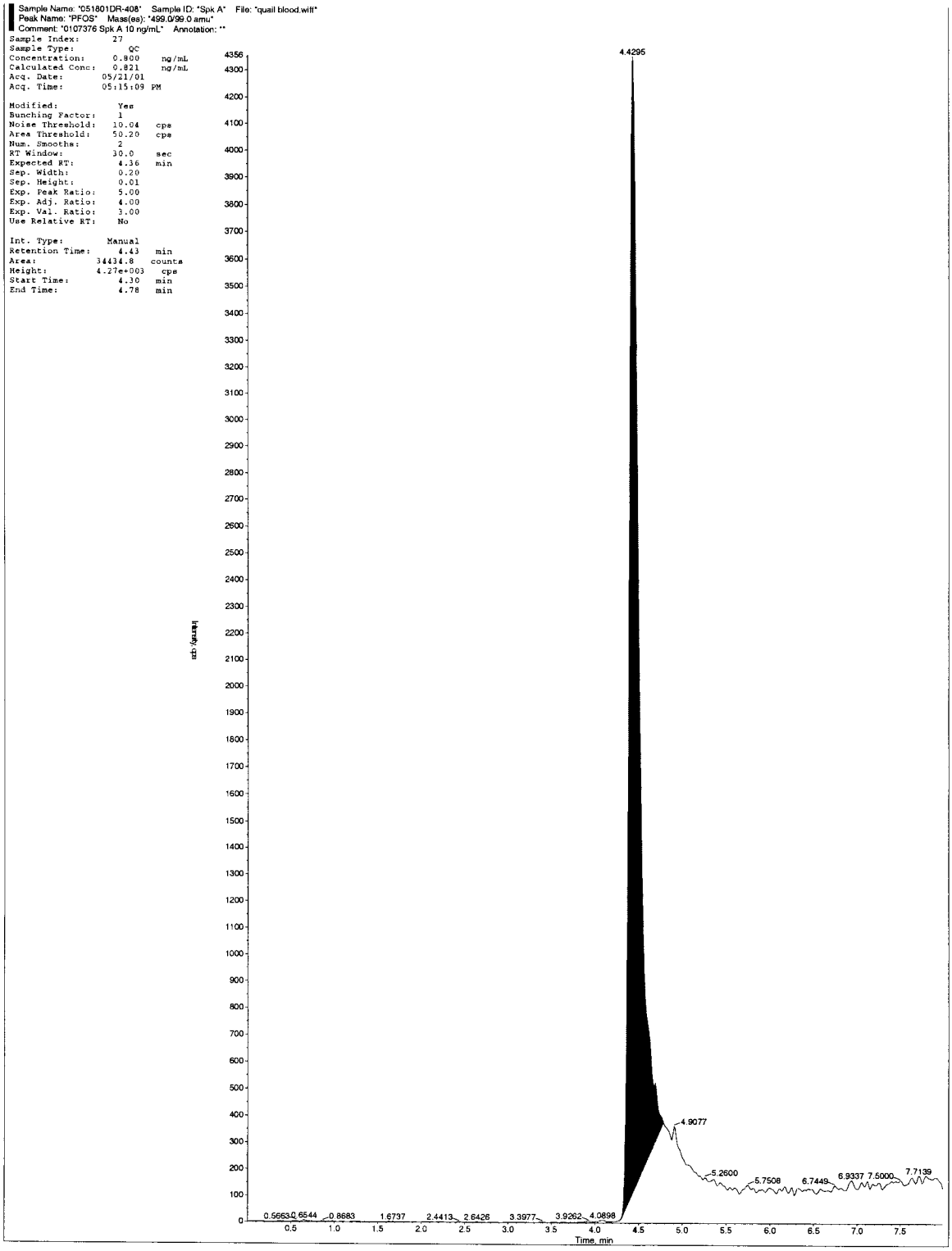


Figure 8. Chromatogram Representing Control Quail Serum fortified with 10 ng/mL of PFOS (Exygen ID: 0107375 Spk A, Set: 072301D)

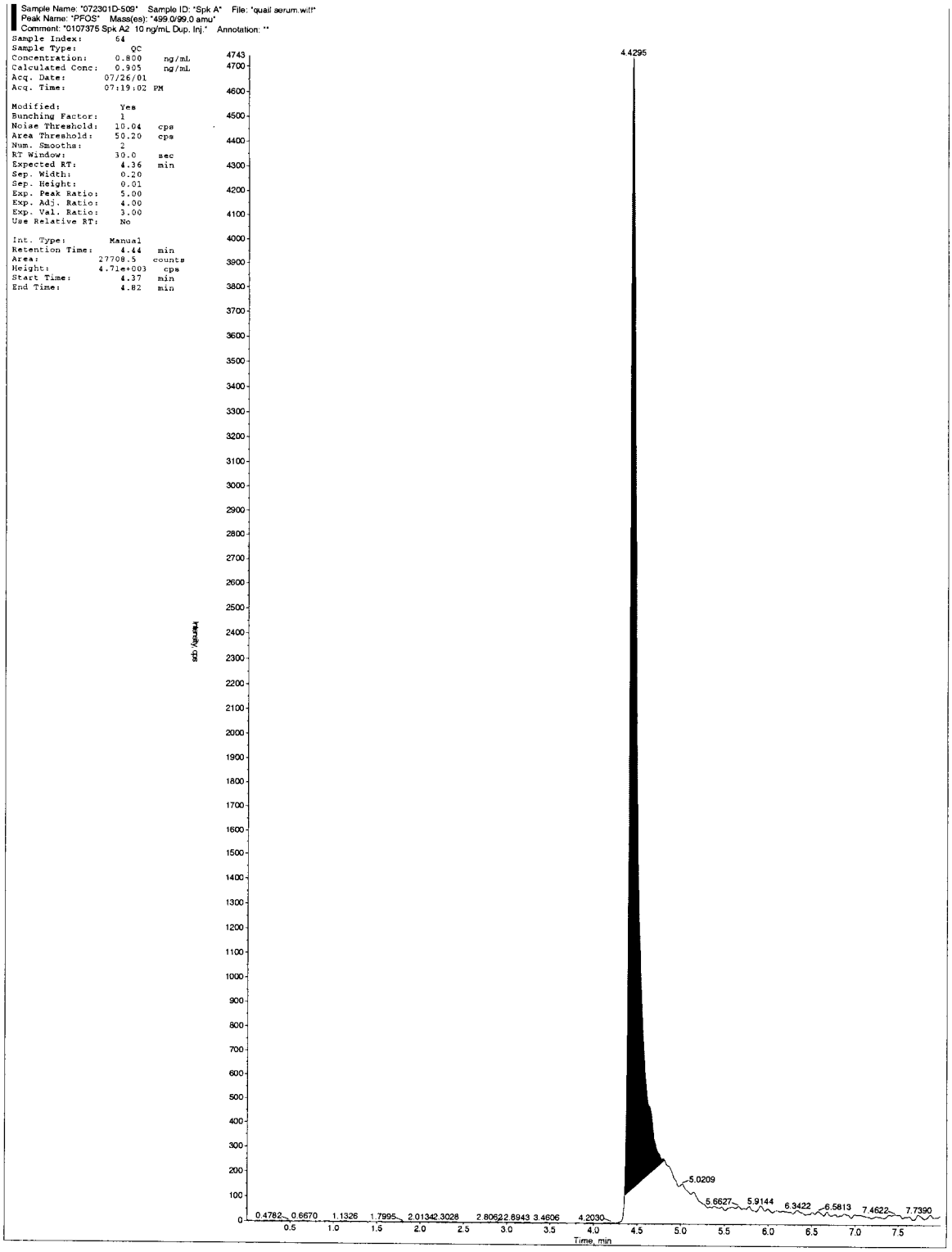


Figure 9. Chromatogram Representing Quail Red Blood Cell Sample (Exygen ID: 0107084, Sponsor ID: 454-108-401 Wk5, Set: 051801DR)

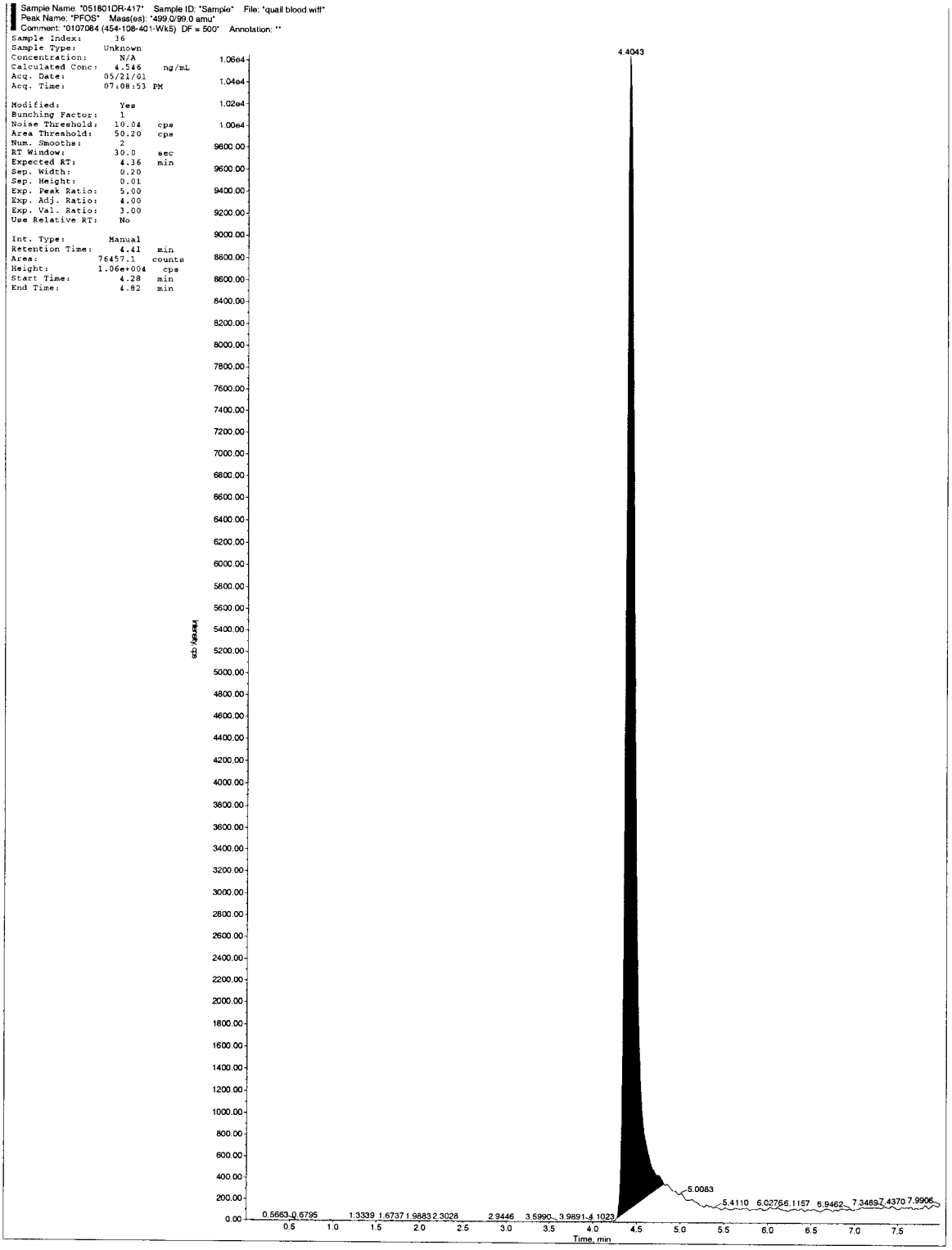
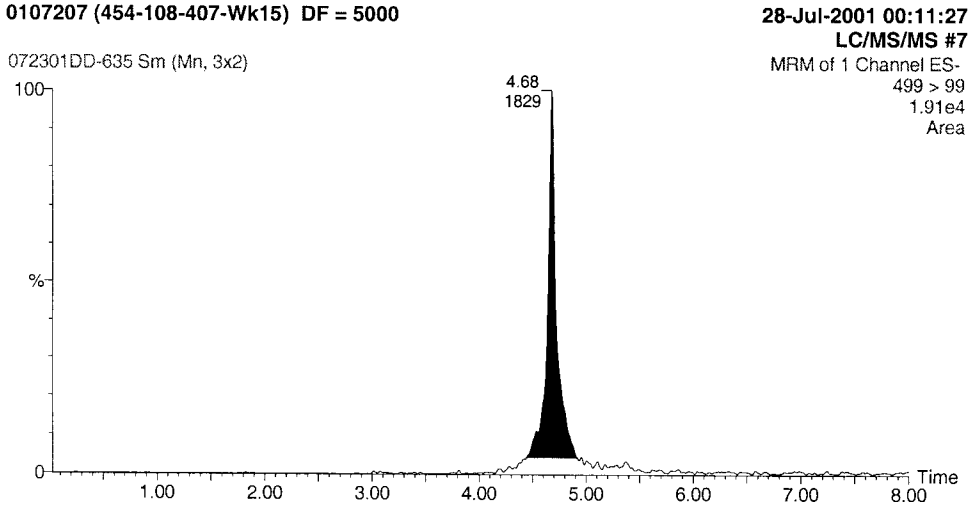


Figure 10. Chromatogram Representing Quail Serum Sample (Exygen ID: 0107207, Sponsor ID: 454-108-407-Wk15, Set: 072301DD)



APPENDIX A

Study Protocol
01P-023-066
(Centre Study No. 023-066)
and
Amendments and Deviations

Centre Protocol No. 01P-023-066

ANALYTICAL PHASE PROTOCOL

ANALYTICAL PHASE TITLE

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM RED BLOOD CELLS 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

PERFORMING LABORATORY

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

PROTOCOL IDENTIFICATION NUMBER

01P-023-066

Centre Protocol No. 01P-023-066

Title: EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM RED BLOOD CELLS FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

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Title:
EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM RED BLOOD CELLS FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

1. PURPOSE

The purpose of this study is to analyze mallard and quail red blood cells samples for residues of perfluorooctanesulfonate (PFOS) using method 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) with the modifications listed in Section 9.

The analytical phase will be conducted under EPA TSCA Good Laboratory Practice standards 40 CFR Part 792 (1).

2. REFERENCE MATERIAL

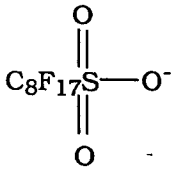
The following analytical standard will be used:

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 from which the PFOS (anion) is obtained 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

Sponsor Representative:
Rochelle Robideau
TELEPHONE: 651-778-7065
FAX NUMBER: 651-778-6176

4. TESTING FACILITY

Wildlife International, Ltd.
8598 Commerce Drive
Easton, MD 21601

STUDY DIRECTOR:
Sean Gallagher
TELEPHONE: 410-822-8600
FAX NUMBER: 410-822-0632

5. 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

6. PROPOSED EXPERIMENTAL TIME-FRAME

Analytical Start Date	April 23, 2001
Analytical Termination Date	April 30, 2001
Report Issued	May 31, 2001

7. JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

Mallard and quail red blood cells samples collected by Wildlife International, Ltd. will be used as the analytical samples for this phase. Mallards and quails represent wild bird populations and they are EPA recommended species because they do well in a laboratory environment. Quail habitats include bushy pastures, grassy roadsides, farmlands and open woodlands, and they are considered land grazers. 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 whole blood of mating adults that were fed PFOS.

A complete description of the test system along with all of the in-life parameters is documented in Mallard Protocol No. 454/010600/MP/SUB454, Project No. 454-105 and Quail Protocol No. 454/010600/QP/SUB454, Project No. 454-104. This project is the analytical portion of those studies.

8. SAMPLE PROCESSING, STORAGE AND IDENTIFICATION

No processing will be required for the any of the samples.

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.

9. ANALYTICAL METHOD

All 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) with the following modifications:

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 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.

3. The sample volume for the red blood cells samples will be 100 µL. The amount of 0.5 M TBA added to the sample will be 0.5 mL and the amount of 0.25 M sodium carbonate/sodium bicarbonate buffer added will be 1.0 mL. The samples will only be filtered with 0.2 µm nylon mesh filter if necessary.

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: Dell UltraScan P1110

Software: PE SciexAnalyst 1.1
Windows NT

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

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

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

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	4.40

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.

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.

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

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, may be excluded from the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 20% 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. Each set must also include at least one methanol blank and one ASTM Type I H_2O blank.

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 (10 ng/mL) and 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 the response of the lowest standard (0.0001 µg/mL) 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.

10. EXPERIMENTAL DESIGN

Samples obtained by Wildlife International Ltd. will be shipped to Centre Analytical Laboratories, Inc. for analysis. There are no known contaminants expected in the test system that would affect the results of this study.

Only those samples designated by the Sponsor will be extracted and analyzed at Centre according to method, "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) with the modifications listed in Section 9.

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. Fortification will be made to the matrix prior to extraction at levels ranging from the LOQ to above the highest residue found. The fortification solution containing the test/reference substance will be applied to the designated samples via a Hamilton syringe, micropipette, pipet, or equivalent, to maintain consistency and accuracy.

The average recovery and relative standard deviation of the fortified samples will be calculated. If necessary, apply a standard test for outliers. If an outlier exists, then it may be excluded from the statistical analysis. Also, the average residue found and standard deviation for each matrix will be calculated.

The following equations will be used for the calculations.
Equation 1:

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

Equation 2:

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

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

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

Equation 3:

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

11. 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 and Sponsor Representative. Planned changes to this protocol or to the analytical method will be made in writing as an amendment and approved by the Principal Investigator, the Study Director, and the Sponsor Representative. 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, Study Director, and Sponsor Representative. 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, Study Director, and Sponsor Representative and reviewed by QAU.

12. 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.

13. 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 GLP standards are met. 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.

14. 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 report will be issued by Centre, approved and signed by the Study Director and the Sponsor Representative 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. Reference materials identified by name, lot, purity, and other characteristics
   4. Name of performing laboratory and analytical start and termination 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 and Sponsor Representative, who will return the report with comments. Centre Analytical will make revisions and finalize the report with the approval of the Study Director and Sponsor Representative. The Centre QA unit will conduct an audit of the final draft report and raw data package to assure accuracy and GLP compliance. The

report must be approved by the Study Director and Sponsor Representative 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, Study Director and Sponsor Representative.

REPORT DISTRIBUTION:

Original:	Study Director
Copy	Sponsor Representative
Copy:	Centre Archives

15. 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.

16. REFERENCES

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

17. PROTOCOL APPROVAL

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

Quality Assurance Reviewer, Centre

Angela L. Morgan
Quality Assurance Auditor — Date: 9/6/01

Principal Investigator, Centre

Emily Decker
Scientist — Date: 9/6/01

Facility Management, Centre

John Flaherty
Operations Manager — Date: 9/10/01

Study Director, Wildlife International, Ltd.

Sean Gallagher
Senior Biologist — Date: 4/23/01

Sponsor, 3M

Rochelle Robideau
Environmental Technologist — Date: 5/1/01

APPENDIX: ANALYTICAL METHOD

A. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum 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:

4/27/99
Laboratory Manager Date

4/26/99
Group Leader Date

04/26/99
Technical Reviewer Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see ***3.0 Definitions***). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 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 1 L Nalgene bottle.

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

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

8.11 **Standards preparation**

8.11.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.12 **Surrogate stock standard preparation**

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_6F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

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

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 **Solvent Blanks, Method blanks and matrix blanks**

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

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

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

10.2 **Matrix spikes**

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

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

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

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

10.3 **Continuing calibration checks**

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

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

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

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

11.0 Calibration and Standardization

11.1 Prepare matrix calibration standards

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

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

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

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

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

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

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

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

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

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

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

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

12.4 Record the initial volume on the extraction worksheet.

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

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

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

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

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

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

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

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

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

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

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

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

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

12.18 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

Blank	Std #	amount =	mL
Serum Extraction Method			Date & Initials
Vortex 15 sec.			
Pipette Matrix	Volume	mL	
Pipette 1 mL of 0.5 M TBA, pH 10. pH =	Std. #		
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #		
Dispense 5 mL of methyl-t-butyl ether	TN-A-		
Shake 20 min.	Shaker speed:		
Centrifuge 20-25 min.	Centrifuge speed:		
Remove a 4 mL aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Temperature:		
Add methanol	Volume mL	TN-A-	
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2um filter into a 1.5 mL autosample vial			

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

MDL/LOQ values for rabbit serum

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Fluids

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

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

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All 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	Surrogate Final conc ng/mL 500	
49.3	50.0	52.4	49.4	50.1	51.3		
97.6	98.9	104	97.8	99.3	102		
248	251	263	248	252	258		
493	500	524	494	501	513		
735	746	782	737	749	766		
976	989	1038	978	993	1017		

Validated ranges – approximate concentrations

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

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PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 06/27/01

Centre Study Number: 023-066 Centre Protocol Number: 01P-023-066

DESCRIPTION OF AMENDED SECTION

1. § 7: Justification for the Selection of the Test System

AMENDED TO

Change the first sentence to read:

Mallard and quail red blood cells and serum samples collected by Wildlife International, Ltd. will be used as the analytical samples for this phase.

RATIONALE

1. At the request of the sponsor, all serum samples are to be analyzed with this study.

IMPACT ON THE STUDY

1. No negative impact on the study.

Sean P. Gallagher 7/12/01
Study Director Signature Date

4/14/01
Sponsor Representative Signature Date

Centre QAU Review 7/10/01

February 12, 1998/1

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Page 1 of 1

PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 03/24/03

Exygen Study Number: 023-066 Protocol Number: 01P-023-066

DESCRIPTION OF AMENDED SECTION

1. Study Title

2. Sponsor Representative

AMENDED TO

1. Title should read "Extraction of Potassium Perfluorooctanesulfonate from Red Blood Cells and Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

2. Sponsor Representative is: John Newsted, Entrix, Inc. (517) 381-1434.

RATIONALE

1-2. Changed as per sponsor request.

IMPACT ON STUDY

1-2. No negative impact on study.

Emily R Decker — Principal Investigator Signature — Date 5/8/03

Sean P. Mallagher — Study Director Signature — Date 5/12/03

John Newsted — Management Signature — Date 5/15/03

(Sponsor) — Date

Exygen QAU Review — LJS 04/11/03

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PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: (1,2) 05/23/01, (3) 05/24/01

Centre Study Number: 023-066 Centre Protocol Number: 01P-023-066

DESCRIPTION OF DEVIATION

1. § 9 Analytical Method, Sample Analysis d.: Quantified Centre sample 0107261 and its duplicate injection when the concentrations were greater than that of the highest standard in Set 052201AD.

2. § 9 Analytical Method Sample Analysis e.: Accepted recovery of 76% for the duplicate injection of Centre sample 0107378 Spk A2 in Set 052201B. for CC ERD 9/4/02

3. 1. § 9 Analytical Method, Sample Analysis d.: Quantified Centre samples 0107092 and 0107134 and their duplicate injections when the concentrations were greater than that of the highest standard in Set 052201CD.

ACTIONS TAKEN

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

1-3. Protocol deviation issued.

Recorded By/Date: _____

IMPACT ON THE STUDY

1,3. No negative impact because the areas were < 15% greater than the highest standard.

2. No negative impact because the overall average of all of the fortifications were within the acceptable range.

Principal Investigator Signature — Date: 5/8/03

Study Director Signature — Date: 5/12/03

Sponsor Management Signature — Date: 5/15/03

CAL QAU Review NCL 9/4/02

February 12, 1998/2

This deviation is being re-issued due to suspected loss of the original draft ERD 9/4/02

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PROTOCOL DEVIATION

Deviation Number: 2

Date of Occurrence: (1) 5/18/01

Centre Study Number: 023-066 Centre Protocol Number: 01P-023-066

DESCRIPTION OF DEVIATION

1. § 9 Analytical Method, 3. Sample volumes used for Centre samples 0107080, 0107081, 0107084, and 0107085 were 0.05 µL, 0.03 µL, 0.025 µL, and 0.025 µL, respectively.
m① m① m① m①

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

1. Protocol deviation issued.

Recorded By/Date: _____

IMPACT ON THE STUDY

1. No negative impact because the actual volume was used in the calculations.

Kelly R. Decker — Principal Investigator Signature — 5/8/03 — Date

Lea P. Gallagher — Study Director Signature — 5/12/03 — Date

John Newstic — Sponsor Management Signature — Date — 5/12/03

CAL QAU Review NCC 9/4/02

February 12, 1998/2

This deviation is being re-issued due to suspected loss of the original draft KRD 9/4/02

①ⓒ KRD 9/4/02

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PROTOCOL DEVIATION

Deviation Number: 3

Date of Occurrence: (1) 7/23/01

Centre Study Number: 023-066 Centre Protocol Number: 01P-023-066

DESCRIPTION OF DEVIATION

1. § 9 Analytical Method, 3. Sample volumes used for Centre samples 0107159, 0107167, 0107168, and 0107209 were 0.05 mL.

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

1. Protocol deviation issued.

Recorded By/Date: _____

IMPACT ON THE STUDY

1. No negative impact because the actual volume was used in the calculations.

Timothy R Decker — 5/8/03
Principal Investigator Signature — Date

Dean P. Gallagher — 5/12/03
Study Director Signature — Date

John Newstead — 5/12/03
Sponsor Management Signature — Date

CAL QAU Review NEC 9/4/02

February 12, 1998/2

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Page 1 of 1

PROTOCOL DEVIATION

Deviation Number: 4
Date of Occurrence: 07/24/01

Exygen Study Number: 023-066 Protocol Number: 01P-023-066

DESCRIPTION OF DEVIATION

Section 9. Sample Analysis e. Accepted recoveries of 124% and 126% in Set 072301B.

ACTIONS TAKEN
for example - deviation issued, SOP revision, etc.

Protocol deviation issued.

Recorded By: Emily R Decker Date: 4/10/03

IMPACT ON STUDY

No negative impact on study because average recoveries within the set were within acceptable range.

Emily R Decker — Principal Investigator Signature — Date: 5/8/03

Sean P. Gallagher — Study Director Signature — Date: 5/12/03

John Newell — Management Signature — Date: 5/15/03

(Sponsor) — Date:

Exygen QAU Review LJS 04/11/03

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