Project / Study Archive Request

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Requestor Name	Phone	Date	Request for:
Kent Lindstrom	651 778-5352	09/22/06	☒ Archival ☐ Retrieval

Report	☒ FC	
LIMS number: E05-0209		
Title/Summary: Interim Report #24 – Analysis of Water, Sludge, and Sediment Samples Study Title: Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program		
Executive Summary		
Group: 3M Decatur		
Group Project Number: Exygen Protocol Number P0000760		
Project Lead/ Study Director: Gary Hohenstein (data management), Mike Santoro (Sponsor), John Flaherty (Principal Investigator), Jaisimha Kesari P.E., DEE (Study Director, Weston Solutions, Inc)		
Contract: Exygen Research		
Contract Project #: P0000760		

Analyte(s): PFOA		
Test System (Sample Matrix): Water, Sludge, Sediment		
Project Type	☒ Analysis ☐ Degradation ☐ Duct Residue Analysis ☐ Ecotox	☐ Method Validation ☐ NA ☐ Phys/Chem Properties ☐ Research/Method Development
Regulatory Status: ☐ Non-GLP ☒ GLP Other:		
Initiation Date: 11/05/04 (Protocol Signed by Study Director), Analytical Start Date for Interim Report: 05/09/06		
Completion Date: Analytical Termination Date for Interim Report #24: 07/07/06 Signed by Study Director: See Report, Signed by Sponsor: See Report		
Electronic Media:		
Data CD: ☐ FTIR Data ☐ Instrument Data ☒ None (raw data files archived at Exygen)		

Project / Study Archive Request

Archive Date	Scan Date	Off-Site Storage Date	

Key Words: Decatur Memorandum of Understanding, MOU, GLP, Protocol P0000760, PFOA, E05-0209, E05-0210, Interim Report #24

Comments:

Note to Archivist. This is Interim Report #24 for this LIMS project (GLP). There will be multiple Interim reports. Please include the Interim Report number in the title of the PDF.

Please send this entire report including the raw data via web to Jai Kesari. Please send a copy of the report without raw data to Michael Santoro and to Gary Hohenstein.

Distribution Point	Date
Gary Hohenstein (report without raw data)	~ 09/22/06
Jai Kesari (report **with** raw data)	~ 09/22/06
Michael Santoro (report without raw data)	~ 09/22/06

INTERIM REPORT #24 - Analysis of Water, Sludge, and Sediment Samples

STUDY TITLE

Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

DATA REQUIREMENTS

EPA TSCA Good Laboratory Practice Standards 40 CFR 792

STUDY DIRECTOR

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.
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Phone: 610-701-3761

INTERIM REPORT COMPLETION DATE

September 13, 2006

PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039

STUDY SPONSOR

3M Company
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: 651-733-6374

PROJECT

Protocol Number: P0000760
Exygen Study Number: P0000760

Total Pages: 140

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number P0000760, entitled "Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," conducted for 3M Company, is being performed in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by Exygen Research.

for John Flaherty — 9/13/06

John Flaherty
Principal Investigator
Exygen Research
Date

9/18/06

Jaisimha Kesari P.E., DEE
Study Director
Weston Solutions, Inc.
Date

9/20/06

Michael A. Santoro
Sponsor Representative
3M Company
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number P0000760, entitled, "Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program". All reviewed phases[1] were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director
34) Raw Data and Interim Report Review	6/26-27/06	07/06/06	07/07/06	07/07/06
36) Raw Data and Interim Report Review	09/12/06	09/13/06	09/13/06	09/13/06

Lydia Shaffer
Technical Lead, Quality Assurance Unit

09/13/06
Date

[1]Note: All in-lab inspections will be documented in the QA statement for the final analytical report at the conclusion of the study. This QA statement involves only the review of the interim report and associated raw data.

CERTIFICATION OF AUTHENTICITY

This interim report, for Exygen Study Number P0000760, is a true and complete representation of the raw data.

Submitted by: Exygen Research
3058 Research Drive
State College, PA 16801
(814) 272-1039

Principal Investigator, Exygen:

Chas Jones for John Flaherty — 9/13/06

John Flaherty
Vice President
Exygen Research

Date

Exygen Research Facility Management:

R A Grazzini — 13-SEP-2006

Richard A. Grazzini
President
Exygen Research

Date

Study Director, Weston Solutions, Inc.

J Kesari — 9/18/06

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.

Date

Sponsor Representative, 3M Company:

Michael A Santoro — 9/20/06

Michael A. Santoro
Director of Regulatory Affairs

Date

STUDY IDENTIFICATION

Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

PROTOCOL NUMBER:	P0000760
EXYGEN STUDY NUMBER:	P0000760
TYPE OF STUDY:	Residue
SAMPLE MATRIX:	Water, Sludge, and Sediment
TEST SUBSTANCE:	Perfluorooctanoic acid (PFOA)
SPONSOR:	3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
STUDY DIRECTOR:	Jaisimha Kesari P.E., DEE Weston Solutions, Inc. 1400 Weston Way West Chester, PA 19380
STUDY MONITOR:	Michael A. Santoro 3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
PERFORMING LABORATORY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 11/05/04 Interim Analytical Start Date: 05/09/06 Interim Analytical Termination Date: 07/06/06 Interim Report Completion Date: 09/13/06

PROJECT PERSONNEL

The Study Director for this project is Jaisimha Kesari at Weston Solutions, Inc. The following personnel from Exygen Research were associated with various phases of this interim portion of the study:

Name	Title
John Flaherty	Vice President
Karen Risha	Laboratory Supervisor
Chrissy Edwards	Technician
Mark Ammerman	Sample Custodian
Amy Sheehan	Associate Scientist
Eric Edwards	Sample Custodian
Mindy Cressley	Technician
Brian McAllister	Sample Custodian
Frances Crespi	Technician
Sharareh Zolghadr	Technician
Scott Crain	Technician
Krista Gallant	Technician
Brittany Kravets	Technician
Kim Hall	Technician

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1.0 SUMMARY

Exygen Research extracted and analyzed water, sludge, and sediment samples for the determination of perfluorooctanoic acid (PFOA) according to Exygen Methods V0001780, V0001781, and V0001782, respectively (**Appendix A**).

The limit of quantitation for PFOA in water was 25 ng/L. The limit of quantitation for PFOA in sludge and sediment was 0.2 ng/g (wet weight).

Analytical results for the analysis of PFOA in water samples are summarized in **Table I**. Analytical results for the analysis of PFOA in re-extracted water samples are summarized in **Table II**. Analytical results for the analysis of PFOA in sludge samples are summarized in **Table III**. Analytical results for the analysis of PFOA in sediment samples are summarized in **Table IV**. Quantitative results were obtained for all samples and analytes except for samples from sites DAL-GW-138R-0-060412, DAL-GW-138S-0-060412, DAL-SD-OSP01-0-0000, and DAL-SD-OSP02-0-0000 that are not reported (NR) due to quality control failures.

Fortification recoveries for PFOA in the water samples are detailed in **Table V**. The average percent recovery ± standard deviation for PFOA in the water samples was 75 ± 22%. Fortification recoveries for PFOA in the re-extracted water samples are detailed in **Table VI**. The average percent recovery ± standard deviation for PFOA in the re-extracted water samples was 99 ± 16%. Fortification recoveries for PFOA in the sludge samples are detailed in **Table VII**. The average percent recovery for PFOA in the sludge sample was 69%. A standard deviation was not calculated for PFOA in sludge because only one sample was analyzed. Fortification recoveries for PFOA in the sediment samples are listed as NR in **Table VIII**. The average percent recovery ± standard deviation for PFOA in the sediment samples was not calculated due to quality control failures.

Fortification recoveries for ^{13}C PFOA in the water samples are detailed in **Table IX**. The average percent recovery ± standard deviation for ^{13}C PFOA in the water samples was 75 ± 26%. Fortification recoveries for ^{13}C PFOA in the re-extracted water samples are detailed in **Table X**. The average percent recovery ± standard deviation for ^{13}C PFOA in the re-extracted water samples was 97 ± 34%. Fortification recoveries for ^{13}C PFOA in the sludge samples are detailed in **Table XI**. The average percent recovery ± standard deviation for ^{13}C PFOA in the sludge samples was 49 ± 16%. Fortification recoveries for ^{13}C PFOA in the sediment samples are listed as NR in **Table XII**. The average percent recovery ± standard deviation for ^{13}C PFOA in the sediment samples was not calculated due to quality control failures. Percent recoveries have not been reported for samples that contained analyte levels three times or greater than the fortification level. Percent solids for sludge and sediment samples are detailed in **Table XIII**.

2.0 OBJECTIVE

The objective of the analytical part of this study was to determine level of perfluorooctanoic acid (PFOA) in water, sludge, and sediment samples according to Protocol P0000760 (**Appendix A**).

3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFOA, in water samples using the analytical method entitled, "V0001780: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS," in sludge using the analytical method entitled, "V0001781: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS," and in sediment using the analytical method entitled, "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS."

The study was initiated on November 05, 2004, when the study director signed protocol number P0000760. The analytical start date for this interim report was May 9, 2006 and the analytical termination date for this interim report was July 07, 2006.

4.0 ANALYTICAL TEST SAMPLES

In total, one hundred and sixty water samples, one sludge sample, and two sediment samples were received from the client. Thirty-eight water samples (Exygen ID: C0169333 – C0169370) were received on wet ice on March 13, 2006 from Tim Frinak at Weston Solutions, Inc. One hundred and twenty-two water samples, one sludge sample, and two sediment samples (Exygen ID: C0170984 – C0171108) were received on wet ice on March 18, 2006 from Tim Frinak at Weston Solutions, Inc. All samples were logged in by Exygen personnel and placed in refrigerated storage.

Sample log-in and chain of custody information is located in the raw data package associated with this interim report. Storage records will be kept at Exygen Research.

5.0 REFERENCE MATERIAL

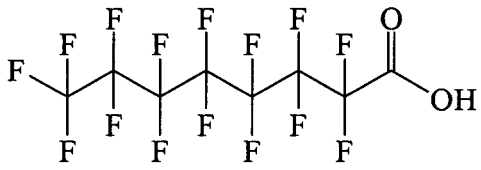
The analytical standard, PFOA, was purchased from Oakwood Products, Inc. and was received at Exygen on March 17, 2005. The surrogate spiking standard, ^{13}C labeled perfluorooctanoic acid (^{13}C PFOA SP0004184), was received at Exygen on April 15, 2004 from the 3M Company.

The available information for the reference materials is listed below. PFOA was stored ambient and ^{13}C PFOA was stored frozen.

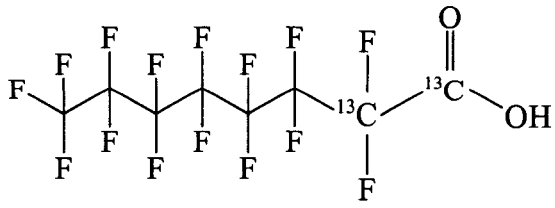
Compound	Exygen Inventory No.	Lot #	Purity (%)	Expiration Date
PFOA	SP0005444	203	99	03/31/07
^{13}C PFOA	SP0004184	3507-195	97	03/29/09

The molecular structures of PFOA and ^{13}C PFOA are given on the following pages:

PFOA
Chemical Name: Perfluorooctanoic acid
Molecular Weight: 414
Transitions Monitored: 413 → 369 (for quantification) and
413 → 219 (for confirmation)
Structure:



^{13}C PFOA
Chemical Name: 1,2-13C perfluorooctanoic acid
Molecular Weight: 416
Transition Monitored: 415 → 370
Structure:



6.0 DESCRIPTION OF ANALYTICAL METHOD

The analytical methods "V0001780: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS," "V0001781: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS," and "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS" were used for this study.

6.1 Extraction Procedure for Water

A 40 mL aliquot of the water sample was used for the extraction procedure. After fortification of appropriate samples, the samples were loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.2 Extraction Procedure for Sludge

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorous shaking the sample container. A 5-gram portion of sludge was weighed into a fifty-milliliter centrifuge tube for the extraction. After fortification of appropriate samples, 5 mL of methanol was added to the samples. The samples were allowed to shake on a wrist action shaker for ~15 minutes and were then sonicated in an ultrasonic bath for ~15 minutes. The volume was taken to 40 mL with water and the samples were then centrifuged for ~10 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.3 Extraction Procedure for Sediment

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A 5-gram portion of sediment was weighed into a fifty-milliliter centrifuge tube for the extraction. After fortification of appropriate samples, 35 mL of 1% acetic acid in water was added to the samples. The samples were vortexed and allowed to shake on a wrist action shaker for ~60 minutes. The samples were centrifuged for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Twenty milliliters of methanol was added to the sediment samples left in the centrifuge tube. The samples were vortexed and allowed to shake on a wrist action shaker for another 30 minutes. The samples were centrifuged again for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto the same C_{18} SPE cartridge. The eluate was collected into a 500 mL Nalgene Bottle. The column was washed with 4 mL of methanol. The wash was collected in the same bottle as the eluate. Approximately two hundred milliliters of water was added to the bottles. The samples were mixed by shaking and loaded onto another C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a

graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.4 Percent Solids Determination For Sludge and Sediment

Percent solids were determined using the procedure indicated in Exygen method V0000427. Approximately 20 grams of sample was weighed into a pan. The weight of the sample plus the pan was recorded. The sample was then dried in an oven overnight at 104 ± 2 °C. Then the sample was transferred to a dessicator and allowed to cool for ~15 minutes. Each sample was then weighed again, including the weight of the pan. The percent solid for each sample was then calculated.

6.5 Preparation of Standards and Fortification Solutions

A mixed stock standard solution of PFOA and ^{13}C PFOA was prepared at a concentration of 1000 µg/mL by dissolving 100 mg of each of the standards (corrected for purity and salt content) in 100 mL of methanol. From this solution, the following fortification standards were prepared:

Conc. of Stock or Fort. Solution (µg/mL)[1]	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Fortification Std. (µg/mL)
1000	10	100	100
100	10	100	10
10	10	100	1.0
1.0	10	100	0.1
0.1	10	100	0.01

[1] of PFOA and ^{13}C PFOA

A stock solution of only ^{13}C PFOA was also prepared at a concentration of 100 µg/mL by dissolving 10 mg the standard (corrected for purity and salt content) in 100 mL of methanol. From this solution, the following fortification standards were prepared:

Conc. of Stock or Fort. Solution (µg/mL)[1]	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Fortification Std. (µg/mL)
100	10	100	10
10	10	100	1.0
1.0	10	100	0.1

[1] of ^{13}C PFOA

A set of non-extracted calibration standards containing PFOA and ^{13}C PFOA (for the sediment samples) was prepared in methanol, as specified in Exygen method V0001782. The following concentrations were prepared:

Conc. of Fort Solution (ng/mL)[1]	Fort Volume (mL)	Volume of Fortified Sample (mL)	Final Conc. of Calibration Std. (ng/mL)
100	1	10	10.0
100	0.5	10	5.0
100	0.2	10	2.0
10	1	10	1.0
5	1	10	0.5
2	1	10	0.2

[1] of PFOA and ^{13}C PFOA

A set of extracted calibration standards containing PFOA and ^{13}C PFOA (for the sludge and water samples) was prepared in water before extraction, as specified in Exygen methods V0001780 and V0001781.

Conc. of Fort Solution (ng/mL)[1]	Fort Volume (mL)	Volume of Fortified Sample (mL)	Final Conc. of Calibration Std. (ng/L)
-	-	40	0
10	0.1	40	25
10	0.2	40	50
10	0.4	40	100
100	0.1	40	250
100	0.2	40	500
100	0.4	40	1000

[1] of PFOA and ^{13}C PFOA

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 is located in the raw data package associated with this interim report.

6.6 Chromatography

Quantification of PFOA and ^{13}C PFOA was accomplished by LC/MS/MS electrospray. The retention time of PFOA and ^{13}C PFOA was ~11.5 min. Peaks above the LOQ were not detected in any of the reagent blank samples corresponding to the analyte retention time.

6.7 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 25 ng/L of PFOA and ^{13}C PFOA for water samples and a concentration of 0.2 ng/mL of PFOA and ^{13}C PFOA for sludge and sediment samples.

6.8 Description of LC/MS/MS Instrument and Operating Conditions

Instrument: API 4000 Biomolecular Mass Analyzer
Interface: Turbo Ion Spray Liquid Introduction Interface
Computer: DELL OptiPlex GX400
Software: Windows NT, Analyst 1.4.1
HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column: Thermo Fluophase RP, 50 mm x 2.1 mm
Column Temp.: 30° C
Injection Vol.: 15 µL
Mobile Phase (A): 2 mM Ammonium Acetate in water
Mobile Phase (B): Methanol

Time (min)	% A	% B
0.0	65	35
1.0	65	35
8.0	25	75
10.0	25	75
11.0	65	35
18.0	65	35

Total run time: ~18 min
Flow Rate: 0.3 mL/min
Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFOA	negative	413 → 369	~11.5 min.
PFOA Confirm Ion	negative	413 → 219	~11.5 min.
^{13}C PFOA	negative	415 → 370	~11.5 min.

6.9 Quantitation and Example Calculation

Fifteen 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.

For water and sludge samples:

Equation 1 calculated the amount of analyte found in sludge and water (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

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

Where: DF = Dilution Factor, factor by which the final volume was diluted, if necessary.

For samples fortified with known amounts of PFOA and ^{13}C PFOA prior to extraction, Equation 2 was used to calculate the percent recovery.

Equation 2:

$$\text{Recovery (\%)} = \frac{(\text{analyte found (ng/L) - analyte in control (ng/L)})}{\text{amount added (ng/L)}} \times 100\%$$

Note: For the PFOA recovery calculation, the "control" is the unspiked aliquot of the primary field sample.

Equation 3 was used to convert the amount of sludge PFOA found in ng/L to ng/g (ppb).

Equation 3:

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/L) x volume extracted (0.04L)}]}{\text{sample weight (5 g)}}$$

Equation 4 was then used to calculate the amount of PFOA found in ppb based on dry weight.

Equation 4:

PFOA found (ppb) dry weight = PFOA found (ppb) x [100% / total solids(%)]

NOTE: Total solids (%) = [wet weight (g) / dry weight (g)] × 100%

An example of a calculation using an actual sample follows:

Sludge sample Exygen ID C0171106 Spk D (Set: 050906B), fortified at 50,000 ng/L with PFOA where:

peak area	=	406453
intercept	=	6660
slope	=	456
dilution factor	=	100
ng/L PFOA added (fort level)	=	50,000
ng/L in corresponding sample (ng/L)	=	53000
volume extracted (L)	=	0.04
sample weight (g)	=	5
total solids (%)	=	22.83

From equation 1:

$$\text{Analyte found (ng/L)} = \frac{[406453 - 6660]}{456} \times 100$$

$$= 87700 \text{ ng/L}$$

From equation 2:

$$\text{\% Recovery} = \frac{(87700 \text{ ng/L} - 53000 \text{ ng/L})}{50000 \text{ ng/L}} \times 100\%$$

$$= 69\%$$

From equation 3:

$$\text{PFOA found (ppb)} = \frac{(87700 \text{ ng/L} \times 0.04\text{L})}{5 \text{ g}}$$

$$= 702 \text{ ppb}$$

From equation 4:

$$\text{PFOA found (ppb) dry weight} = 702 \text{ ppb} \times (100\% / 22.83\%)$$

$$= 3070 \text{ ppb}$$

Note: Numbers may vary slightly due to rounding.

For sediment samples:

Equation 5 calculated the amount of analyte found in sediment (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 5:

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

Where: DF = Dilution Factor, factor by which the final volume was diluted, if necessary.

For samples fortified with known amounts of PFOA and ^{13}C PFOA prior to extraction, Equation 6 was used to calculate the percent recovery.

Equation 6:

$$\text{Recovery (\%)} = \frac{(\text{analyte found (ng/mL) - analyte in control (ng/mL)}) \times 100\%}{\text{amount added (ng/mL)}}$$

Note: For the PFOA recovery calculation, the "control" is the unspiked aliquot of the primary field sample.

Equation 7 was used to convert the amount of sediment PFOA found in ng/mL to ng/g (ppb).

Equation 7:

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL) x volume extracted (5 mL)}]}{\text{sample weight (5 g)}}$$

Equation 8 was then used to calculate the amount of PFOA found in ppb based on dry weight.

Equation 8:

PFOA found (ppb) dry weight = PFOA found (ppb) x [100% / total solids(%)]

NOTE: Total solids (%) = [wet weight (g) / dry weight (g)] × 100%

An example of a calculation using an actual sample follows:

Sediment sample Exygen ID C0171107 Spk C (Set: 051106A), fortified at 0.5 ng/mL with PFOA where:

peak area	=	327495
intercept	=	1130
slope	=	190000
dilution factor	=	1
ng/mL PFOA added (fort level)	=	4.0
ng/mL in corresponding sample (ng/mL)	=	0.440
volume extracted (L)	=	5
sample weight (g)	=	5
total solids (%)	=	58.39

From equation 5:

$$\text{Analyte found (ng/mL)} = \frac{[327495 - 1130]}{190000}$$

$$= 1.72 \text{ ng/mL}$$

From equation 6:

$$\text{\% Recovery} = \frac{(1.72 \text{ ng/mL} - 0.440 \text{ ng/mL})}{4 \text{ ng/mL}} \times 100\%$$

$$= 32\%$$

From equation 7:

$$\text{PFOA found (ppb)} = \frac{(1.72 \text{ ng/mL} \times 5 \text{ mL})}{5 \text{ g}}$$

$$= 1.72 \text{ ppb}$$

From equation 8:

$$\text{PFOA found (ppb) dry weight} = 1.72 \text{ ppb} \times (100\% / 58.39\%)$$

$$= 2.95 \text{ ppb}$$

Note: Numbers may vary slightly due to rounding.

7.0 EXPERIMENTAL DESIGN

^{13}C PFOA was used as a surrogate for all the samples except the rinse blanks. ^{13}C PFOA was added to the sludge and sediment samples and sample replicates in the laboratory after collection. ^{13}C PFOA was added to the water sample collection bottles in the laboratory before being shipped to the field for sampling. For water samples designated as field matrix spikes, PFOA was also added at a known concentration to the bottles in the laboratory before being shipped to the field. The water sample bottles were filled to a 200 mL volumetric fill line in the field except for C0169347 and C0169354, which were filled to 250 mL, and C0171088, which was filled to 230 mL.

The water samples and trip blank samples were analyzed in thirteen sets. Each set included one reagent blank and two reagent blanks fortified at known concentrations. The first water set contained two sample sites and one trip blank and its associated spikes. Water sets two through twelve contained three sample sites each. The thirteenth water set contained one sample site and two trip blanks and their associated spikes. For each site, a sample, a field duplicate and two or three matrix field spikes were collected. For each site, a laboratory duplicate of the primary sample was extracted and two laboratory matrix

spikes were also extracted. For the two laboratory matrix spikes, two 40 mL portions of the primary sample collected for the site was poured from the bottle and fortified. Not only was PFOA added in the laboratory prior to extraction, but ^{13}C PFOA was also added. The additional ^{13}C PFOA was added because the levels of PFOA spiked into the samples were known to exceed the calibration ranges and were not analyzed without dilution; therefore, ^{13}C PFOA levels were adjusted to require the same dilution as the other analytes.

The re-extracted water samples were analyzed in three sets. Each set included one reagent blank and two reagent blanks fortified at known concentrations. The first set contained eighteen samples with appropriate spiking levels. The second set contained fourteen samples with appropriate spiking levels. The last set contained six samples and the appropriate spiking levels. For the two laboratory matrix spikes, two 40 mL portions of the primary sample collected for the site was poured from the bottle and fortified. Not only was PFOA added in the laboratory prior to extraction, but ^{13}C PFOA was also added. The additional ^{13}C PFOA was added because the levels of PFOA spiked into the samples were known to exceed the calibration ranges and were not analyzed without dilution; therefore, ^{13}C PFOA levels were adjusted to require the same dilution as the other analytes.

The single sludge sample was extracted in one set. The set included one reagent blank and two reagent blanks fortified at known concentrations. For each sample, a laboratory duplicate of the sample and two laboratory matrix spikes were also extracted. The laboratory spikes were fortified with known concentrations of PFOA and ^{13}C PFOA.

The two sediment samples were extracted in one set. The set included one reagent blank and two reagent blanks fortified at known concentrations. For each sample, a laboratory duplicate of the sample and two laboratory matrix spikes were also extracted. The laboratory spikes were fortified with known concentrations PFOA and ^{13}C PFOA.

8.0 RESULTS

Analytical results for the analysis of PFOA in water samples are summarized in **Table I**. Analytical results for the analysis of PFOA in re-extracted water samples are summarized in **Table II**. Analytical results for the analysis of PFOA in sludge samples are summarized in **Table III**. Analytical results for the analysis of PFOA in sediment samples are summarized in **Table IV**. Quantitative results were obtained for all samples and analytes except for samples from sites DAL-GW-138R-0-060412, DAL-GW-138S-0-060412, DAL-SD-OSP01-0-0000, and DAL-SD-OSP02-0-0000 that are not reported (NR) due to quality control failures.

Accuracies were assessed for each sample by reviewing the individual QC results obtained for each sample site. For the water samples, there were two laboratory and two or three field spike recovery results available for each sample site that were used to assess

the accuracy. In instances of failed laboratory or field spikes, recoveries associated with other spikes were used to assess sample accuracy. For the sludge and sediment samples, there were two laboratory spike recovery results available for each sample site that were used to assess the accuracy. In instances of failed laboratory spike recoveries, the samples were not reported due to the quality control failure.

Fortification recoveries for PFOA in the water samples are detailed in **Table V**. The average percent recovery ± standard deviation for PFOA in the water samples was 75 ± 22%. Fortification recoveries for PFOA in the re-extracted water samples are detailed in **Table VI**. The average percent recovery ± standard deviation for PFOA in the re-extracted water samples was 99 ± 16%. Fortification recoveries for PFOA in the sludge samples are detailed in **Table VII**. The average percent recovery for PFOA in the sludge sample was 69%. A standard deviation was not calculated for PFOA in sludge because only one sample was analyzed. Fortification recoveries for PFOA in the sediment samples are listed as NR in **Table VIII**. The average percent recovery ± standard deviation for PFOA in the sediment samples was not calculated due to quality control failures.

Fortification recoveries for ^{13}C PFOA in the water samples are detailed in **Table IX**. The average percent recovery ± standard deviation for ^{13}C PFOA in the water samples was 75 ± 26%. Fortification recoveries for ^{13}C PFOA in the re-extracted water samples are detailed in **Table X**. The average percent recovery ± standard deviation for ^{13}C PFOA in the re-extracted water samples was 97 ± 34%. Fortification recoveries for ^{13}C PFOA in the sludge samples are detailed in **Table XI**. The average percent recovery ± standard deviation for ^{13}C PFOA in the sludge samples was 49 ± 16%. Fortification recoveries for ^{13}C PFOA in the sediment samples are listed as NR in **Table XII**. The average percent recovery ± standard deviation for ^{13}C PFOA in the sediment samples was not calculated due to quality control failures. Percent recoveries have not been reported for samples that contained analyte levels three times or greater than the fortification level. Percent solids for sludge and sediment samples are detailed in **Table XIII**.

9.0 CONCLUSIONS

Except as noted above, the ground water, sludge, and sediment samples were successfully extracted and analyzed for PFOA according to analytical methods V0001780, V0001781, and V0001782 respectively. There were no circumstances that may have affected the data quality or integrity.

10.0 RETENTION OF DATA AND SAMPLES

All original paper data generated by Exygen Research that pertains to this interim report will be shipped to the study director. This does not include facility-specific raw data such as instrument or temperature logs. Exact copies of all raw data, as well as a signed copy of the interim analytical report and all original facility-specific raw data, will be retained in the Exygen Research archives for the period of time specified in EPA TSCA Good Laboratory Practice Standards 40 CFR 792.

TABLES

Table I. Summary of PFOA in Water Samples

Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L) C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0169333	DAL-GW-TRIP1-0-060411	ND	30
C0169336	DAL-GW-138L-0-060411	219	50
C0169336 Rep	DAL-GW-138L-0-060411*	239	50
C0169337	DAL-GW-138L-DB-060411	238	50
C0169341	DAL-GW-138R-0-060412	NR	NR
C0169341 Rep	DAL-GW-138R-0-060412*	NR	NR
C0169342	DAL-GW-138R-DB-060412	NR	NR
C0169346	DAL-GW-138S-0-060412	NR	NR
C0169346 Rep	DAL-GW-138S-0-060412*	NR	NR
C0169347	DAL-GW-138S-DB-060412^	NR	NR
C0169351	DAL-GW-605R-0-060412	92.1	50
C0169351 Rep	DAL-GW-605R-0-060412*	106	50
C0169352	DAL-GW-605R-DB-060412	66.1	50
C0169355	DAL-GW-605L-0-060412	ND	50
C0169355 Rep	DAL-GW-605L-0-060412*	ND	50
C0169356	DAL-GW-605L-DB-060412	ND	50
C0169359	DAL-GW-603S-0-060412	317	30
C0169359 Rep	DAL-GW-603S-0-060412*	321	30
C0169360	DAL-GW-603S-DB-060412	392	30
C0169363	DAL-GW-604L-0-060412	1580	40
C0169363 Rep	DAL-GW-604L-0-060412*	1490	40
C0169364	DAL-GW-604L-DB-060412	1710	40
C0169367	DAL-GW-604S-0-060412	913	30
C0169367 Rep	DAL-GW-604S-0-060412*	937	30
C0169368	DAL-GW-604S-DB-060412	1070	30
C0170984	DAL-GW-601R-0-060413	NR	NR
C0170984 Rep	DAL-GW-601R-0-060413*	NR	NR
C0170985	DAL-GW-601R-DB-060413	NR	NR
C0170988	DAL-GW-601S-0-060413	NR	NR
C0170988 Rep	DAL-GW-601S-0-060413*	NR	NR
C0170989	DAL-GW-601S-DB-060413	NR	NR
C0170992	DAL-GW-602R-0-060413	584	30
C0170992 Rep	DAL-GW-602R-0-060413*	563	30
C0170993	DAL-GW-602R-DB-060413	627	30
C0170996	DAL-GW-601L-0-060413	NR	NR
C0170996 Rep	DAL-GW-601L-0-060413*	NR	NR
C0170997	DAL-GW-601L-DB-060413	NR	NR
C0171000	DAL-GW-602S-0-060413	557	50
C0171000 Rep	DAL-GW-602S-0-060413*	523	50
C0171001	DAL-GW-602S-0-060413	563	50

*Laboratory Duplicate
^ Sample was bottle was filled to 250 mL.
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table II for re-extraction data

Table I. Summary of PFOA in Water Samples Continued

Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L) C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (%)
C0171004	DAL-GW-602L-0-060413	2820	30
C0171004 Rep	DAL-GW-602L-0-060413*	2940	30
C0171005	DAL-GW-602L-DB-060413	3070	30
C0171008	DAL-GW-604R-0-060413	699	40
C0171008 Rep	DAL-GW-604R-0-060413*	694	40
C0171009	DAL-GW-604R-DB-060413	737	40
C0171012	DAL-GW-603R-0-060413	545	50
C0171012 Rep	DAL-GW-603R-0-060413*	551	50
C0171013	DAL-GW-603R-DB-060413	574	50
C0171016	DAL-GW-603L-0-060413	145	30
C0171016 Rep	DAL-GW-603L-0-060413*	148	30
C0171017	DAL-GW-603L-DB-060413	145	30
C0171020	DAL-SW-BPP01-0-060413	NR	NR
C0171020 Rep	DAL-SW-BPP01-0-060413*	NR	NR
C0171021	DAL-SW-BPP01-0-060413	NR	NR
C0171025	DAL-SW-BPP02-0-060413	248000	30
C0171025 Rep	DAL-SW-BPP02-0-060413*	250000	30
C0171026	DAL-SW-BPP02-DB-060413	249000	30
C0171030	DAL-SW-BPP03-0-060413	231000	30
C0171030 Rep	DAL-SW-BPP03-0-060413*	248000	30
C0171031	DAL-SW-BPP03-DB-060413	352000	30
C0171035	DAL-SW-BCT01-0-060412	NR	NR
C0171035 Rep	DAL-SW-BCT01-0-060412*	NR	NR
C0171036	DAL-SW-BCT01-DB-060412	NR	NR
C0171039	DAL-SW-BCT02-0-060412	132000	40
C0171039 Rep	DAL-SW-BCT02-0-060412*	126000	40
C0171040	DAL-SW-BCT02-DB-060412	125000	40
C0171043	DAL-SW-BCT03-0-060412	24700	50
C0171043 Rep	DAL-SW-BCT03-0-060412*	24700	50
C0171044	DAL-SW-BCT03-DB-060412	28000	50
C0171047	DAL-SW-BC01-0-060412	103	50
C0171047 Rep	DAL-SW-BC01-0-060412*	104	50
C0171048	DAL-SW-BC01-DB-060412	111	50
C0171051	DAL-SW-BC02-0-060412	126	50
C0171051 Rep	DAL-SW-BC02-0-060412*	133	50
C0171052	DAL-SW-BC02-DB-060412	140	50
C0171055	DAL-SW-BC03-0-060414	2370	30
C0171055 Rep	DAL-SW-BC03-0-060414*	2530	30
C0171056	DAL-SW-BC03-DB-060414	2660	30

*Laboratory Duplicate
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table II for re-extraction data

Table I. Summary of PFOA in Water Samples Continued

Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L) C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (%)
C0171059	DAL-SW-BC04-0-060414	NR	NR
C0171059 Rep	DAL-SW-BC04-0-060414*	NR	NR
C0171060	DAL-SW-BC04-DB-060414	NR	NR
C0171063	DAL-SW-BC05-0-060414	NR	NR
C0171063 Rep	DAL-SW-BC05-0-060414*	NR	NR
C0171064	DAL-SW-BC05-DB-060414	NR	NR
C0171067	DAL-SW-OSP01-0-060413	335	40
C0171067 Rep	DAL-SW-OSP01-0-060413*	307	40
C0171068	DAL-SW-OSP01-DB-060413	313	40
C0171071	DAL-SW-OSP02-0-060413	NR	NR
C0171071 Rep	DAL-SW-OSP02-0-060413*	NR	NR
C0171072	DAL-SW-OSP02-DB-060413	NR	NR
C0171075	DAL-SW-OSP03-0-060413	NR	NR
C0171075 Rep	DAL-SW-OSP03-0-060413*	NR	NR
C0171076	DAL-SW-OSP03-DB-060413	NR	NR
C0171079	DAL-GW-BPWELL-0-060413	NR	NR
C0171079 Rep	DAL-GW-BPWELL-0-060413*	NR	NR
C0171080	DAL-GW-BPWELL-DB-060413	NR	NR
C0171084	DPWS-WWI-DCTP01-0-060413	4200	50
C0171084 Rep	DPWS-WWI-DCTP01-0-060413*	4230	50
C0171085	DPWS-WWI-DCTP01-DB-060413	4390	50
C0171088	DPWS-WWE-DCTP01-0-060413^	7030	50
C0171088 Rep	DPWS-WWE-DCTP01-0-060413*^	7180	50
C0171089	DPWS-WWE-DCTP01-DB-060413	7020	50
C0171092	DAL-WW-EFF01-0-060413	4650	40
C0171092 Rep	DAL-WW-EFF01-0-060413*	4460	40
C0171093	DAL-WW-EFF01-DB-060413	4790	40
C0171096	DAL-LCH-MCLF01-0-060414	NR	NR
C0171096 Rep	DAL-LCH-MCLF01-0-060414*	NR	NR
C0171097	DAL-LCH-MCLF01-DB-060414	NR	NR
C0171100	DAL-GW-TRIP1-0-060412	ND	30
C0171103	DAL-WW-TRIP1-0-060413	ND	30

*Laboratory Duplicate
^ Sample was bottle was filled to 230 mL.
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table II for re-extraction data

Table II. Summary of PFOA in Re-extracted Water Samples

Exygen ID	Client Sample ID	Analyte Found (ppt, ng/L) C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0169341	DAL-GW-138R-0-060412	NR	NR
C0169347	DAL-GW-138S-DB-060412	NR	NR
C0170984	DAL-GW-601R-0-060413	107	30
C0170989	DAL-GW-601S-DB-060413	121	30
C0170997	DAL-GW-601L-DB-060413	19800	30
C0171021	DAL-SW-BPP01-DB-060413	526000	30
C0171035	DAL-SW-BCT01-0-060412	29000	30
C0171060	DAL-SW-BC04-DB-060414	27700	30
C0171063	DAL-SW-BC05-0-060414	782	30
C0171071	DAL-SW-OSP02-0-060413	2660	30
C0171076	DAL-SW-OSP03-DB-060413	2650	30
C0171079	DAL-GW-BPWELL-0-060413	641000	30
C0171097	DAL-LCH-MCLF01-DB-060414	48700	30

NR = Not reported due to quality control failures.

Table III. Summary of PFOA in Sludge Samples

Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0171106	DPWS-SL-DCTP01-0-0000	1860	40
C0171106 Rep	DPWS-SL-DCTP01-0-0000*	1890	40

*Laboratory Duplicate

Table IV. Summary of PFOA in Sediment Samples

Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0171107	DAL-SD-OSP01-0-0000	NR	NR
C0171107 Rep	DAL-SD-OSP01-0-0000*	NR	NR
C0171108	DAL-SD-OSP02-0-0000	NR	NR
C0171108 Rep	DAL-SD-OSP02-0-0000*	NR	NR

*Laboratory Duplicate
NR = Not reported due to quality control failures.

Table V. Matrix Spike Recovery of PFOA in Water Samples

	C8 Acid PFOA			
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-GW-TRIP1-LS-060411 (C0169334, 1.0 ppb Field Spike)	1000	ND	1040	104
DAL-GW-TRIP1-HS-060411 (C0169335, 10.0 ppb Field Spike)	10000	ND	10800	108
DAL-GW-138L-0-060411 (C0169336 Spk C, 10.0 ppb Lab Spike)	10000	219	7990	78
DAL-GW-138L-0-060411 (C0169336 Spk D, 1000 ppb Lab Spike)	1000000	219	896000	90
DAL-GW-138L-LS-060411 (C0169338, 10.0 ppb Field Spike)	10000	219	5760	55
DAL-GW-138L-MS-060411 (C0169339, 100 ppb Field Spike)	100000	219	38900	39
DAL-GW-138L-HS-060411 (C0169340, 1000 ppb Field Spike)	1000000	219	190000	19
DAL-GW-138R-0-060412 (C0169341 Spk E, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-GW-138R-0-060412 (C0169341 Spk F, 1000 ppb Lab Spike)	1000000	NR	NR	NR
DAL-GW-138R-LS-060412 (C0169343, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-GW-138R-MS-060412 (C0169344, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-GW-138R-HS-060412 (C0169345, 1000 ppb Field Spike)	1000000	NR	NR	NR
DAL-GW-138S-0-060412 (C0169346 Spk C, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-GW-138S-0-060412 (C0169346 Spk D, 1000 ppb Lab Spike)	1000000	NR	NR	NR
DAL-GW-138S-LS-060412 (C0169348, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-GW-138S-MS-060412 (C0169349, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-GW-138S-HS-060412 (C0169350, 1000 ppb Field Spike)	1000000	NR	NR	NR
DAL-GW-605R-0-060412 (C0169351 Spk E, 0.1 ppb Lab Spike)	100	92.1	175	83
DAL-GW-605R-0-060412 (C0169351 Spk F, 1.0 ppb Lab Spike)	1000	92.1	833	74
DAL-GW-605R-LS-060412 (C0169353, 0.1 ppb Field Spike)	100	92.1	138	46
DAL-GW-605R-HS-060412 (C0169354, 1.0 ppb Field Spike)^	800	92.1	566	59
DAL-GW-605L-0-060412 (C0169355 Spk G, 0.1 ppb Lab Spike)	100	ND	79.5	80
DAL-GW-605L-0-060412 (C0169355 Spk H, 1.0 ppb Lab Spike)	1000	ND	839	84
DAL-GW-605L-LS-060412 (C0169357, 0.1 ppb Field Spike)	100	ND	56.8	57
DAL-GW-605L-HS-060412 (C0169358, 1.0 ppb Field Spike)	1000	ND	487	49

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated

^ Sample was bottle was filled to 250 mL instead of 200 mL, causing the spiking level to be modified.

ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.

NR = Not reported due to quality control failures, see Table VI for re-extraction data.

Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

Sample Description	C8 Acid PFOA Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-GW-603S-0-060412 (C0169359 Spk C, 0.1 ppb Lab Spike)	100	317	418	*
DAL-GW-603S-0-060412 (C0169359 Spk D, 1.0 ppb Lab Spike)	1000	317	1130	81
DAL-GW-603S-LS-060412 (C0169361, 0.1 ppb Field Spike)	100	317	458	*
DAL-GW-603S-HS-060412 (C0169362, 1.0 ppb Field Spike)	1000	317	1040	72
DAL-GW-604L-0-060412 (C0169363 Spk E, 0.1 ppb Lab Spike)	100	1580	1680	*
DAL-GW-604L-0-060412 (C0169363 Spk F, 1.0 ppb Lab Spike)	1000	1580	2350	77
DAL-GW-604L-LS-060412 (C0169365, 0.1 ppb Field Spike)	100	1580	1670	*
DAL-GW-604L-HS-060412 (C0169366, 1.0 ppb Field Spike)	1000	1580	2210	63
DAL-GW-604S-0-060412 (C0169367 Spk G, 0.1 ppb Lab Spike)	100	913	987	*
DAL-GW-604S-0-060412 (C0169367 Spk H, 1.0 ppb Lab Spike)	1000	913	1800	89
DAL-GW-604S-LS-060412 (C0169369, 0.1 ppb Field Spike)	100	913	1300	*
DAL-GW-604S-HS-060412 (C0169370, 1.0 ppb Field Spike)	1000	913	2040	113
DAL-GW-601R-0-060413 (C0170984 Spk C, 0.1 ppb Lab Spike)	100	NR	NR	NR
DAL-GW-601R-0-060413 (C0170984 Spk D, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-GW-601R-LS-060413 (C0170986, 0.1 ppb Field Spike)	100	NR	NR	NR
DAL-GW-601R-HS-060413 (C0170987, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-GW-601S-0-060413 (C0170988 Spk E, 0.1 ppb Lab Spike)	100	NR	NR	NR
DAL-GW-601S-0-060413 (C0170988 Spk F, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-GW-601S-LS-060413 (C0170990, 0.1 ppb Field Spike)	100	NR	NR	NR
DAL-GW-601S-HS-060413 (C0170991, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-GW-602R-0-060413 (C0170992 Spk G, 0.1 ppb Lab Spike)	100	584	656	*
DAL-GW-602R-0-060413 (C0170992 Spk H, 1.0 ppb Lab Spike)	1000	584	2010	143
DAL-GW-602R-LS-060413 (C0170994, 0.1 ppb Field Spike)	100	584	762	*
DAL-GW-602R-HS-060413 (C0170995, 1.0 ppb Field Spike)	1000	584	1880	130

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

Sample Description	C8 Acid PFOA Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-GW-601L-0-060413 (C0170996 Spk C, 0.1 ppb Lab Spike)	100	NR	NR	NR
DAL-GW-601L-0-060413 (C0170996 Spk D, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-GW-601L-LS-060413 (C0170998, 0.1 ppb Field Spike)	100	NR	NR	NR
DAL-GW-601L-HS-060413 (C0170999, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-GW-602S-0-060413 (C0171000 Spk E, 0.1 ppb Lab Spike)	100	557	697	*
DAL-GW-602S-0-060413 (C0171000 Spk F, 1.0 ppb Lab Spike)	1000	557	1280	72
DAL-GW-602S-LS-060413 (C0171002, 0.1 ppb Field Spike)	100	557	573	*
DAL-GW-602S-HS-060413 (C0171003, 1.0 ppb Field Spike)	1000	557	1120	56
DAL-GW-602L-0-060413 (C0171004 Spk G, 0.1 ppb Lab Spike)	100	2820	3250	*
DAL-GW-602L-0-060413 (C0171004 Spk H, 1.0 ppb Lab Spike)	1000	2820	3720	90
DAL-GW-602L-LS-060413 (C0171006, 0.1 ppb Field Spike)	100	2820	2870	*
DAL-GW-602L-HS-060413 (C0171007, 1.0 ppb Field Spike)	1000	2820	4030	121
DAL-GW-604R-0-060413 (C0171008 Spk C, 0.1 ppb Lab Spike)	100	699	780	*
DAL-GW-604R-0-060413 (C0171008 Spk D, 1.0 ppb Lab Spike)	1000	699	1440	74
DAL-GW-604R-LS-060413 (C0171010, 0.1 ppb Field Spike)	100	699	789	*
DAL-GW-604R-HS-060413 (C0171011, 1.0 ppb Field Spike)	1000	699	1340	64
DAL-GW-603R-0-060413 (C0171012 Spk E, 0.1 ppb Lab Spike)	100	545	623	*
DAL-GW-603R-0-060413 (C0171012 Spk F, 1.0 ppb Lab Spike)	1000	545	1230	69
DAL-GW-603R-LS-060413 (C0171014, 0.1 ppb Field Spike)	100	545	682	*
DAL-GW-603R-HS-060413 (C0171015, 1.0 ppb Field Spike)	1000	545	1100	56
DAL-GW-603L-0-060413 (C0171016 Spk G, 0.1 ppb Lab Spike)	100	145	249	104
DAL-GW-603L-0-060413 (C0171016 Spk H, 1.0 ppb Lab Spike)	1000	145	1050	91
DAL-GW-603L-LS-060413 (C0171018, 0.1 ppb Field Spike)	100	145	214	69
DAL-GW-603L-HS-060413 (C0171019, 1.0 ppb Field Spike)	1000	145	882	74

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

	C8 Acid PFOA			
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-SW-BPP01-0-060413 (C0171020 Spk C, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-BPP01-0-060413 (C0171020 Spk D, 10 ppb Lab Spike)	100000	NR	NR	NR
DAL-SW-BPP01-LS-060413 (C0171022, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-SW-BPP01-MS-060413 (C0171023, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-SW-BPP01-HS-060413 (C0171024, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-SW-BPP02-0-060413 (C0171025 Spk E, 1.0 ppb Lab Spike)	1000	248000	224000	*
DAL-SW-BPP02-0-060413 (C0171025 Spk F, 100 ppb Lab Spike)	100000	248000	319000	71
DAL-SW-BPP02-LS-060413 (C0171027, 1.0 ppb Field Spike)	1000	248000	226000	*
DAL-SW-BPP02-MS-060413 (C0171028, 10 ppb Field Spike)	10000	248000	219000	*
DAL-SW-BPP02-HS-060413 (C0171029, 100 ppb Field Spike)	100000	248000	324000	76
DAL-SW-BPP03-0-060413 (C0171030 Spk G, 1.0 ppb Lab Spike)	1000	231000	229000	*
DAL-SW-BPP03-0-060413 (C0171030 Spk H, 100 ppb Lab Spike)	100000	231000	352000	121
DAL-SW-BPP03-LS-060413 (C0171032, 1.0 ppb Field Spike)	1000	231000	231000	*
DAL-SW-BPP03-MS-060413 (C0171033, 10 ppb Field Spike)	10000	231000	206000	*
DAL-SW-BPP03-HS-060413 (C0171034, 100 ppb Field Spike)	100000	231000	346000	115
DAL-SW-BCT01-0-060412 (C0171035 Spk C, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-BCT01-0-060412 (C0171035 Spk D, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-SW-BCT01-LS-060412 (C0171037, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-SW-BCT01-HS-060412 (C0171038, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-SW-BCT02-0-060412 (C0171039 Spk E, 1.0 ppb Lab Spike)	1000	132000	144000	*
DAL-SW-BCT02-0-060412 (C0171039 Spk F, 10 ppb Lab Spike)	10000	132000	138000	60
DAL-SW-BCT02-LS-060412 (C0171041, 1.0 ppb Field Spike)	1000	132000	119000	*
DAL-SW-BCT02-HS-060412 (C0171042, 10 ppb Field Spike)	10000	132000	138000	60

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

Sample Description	C8 Acid PFOA Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-SW-BCT03-0-060412 (C0171043 Spk G, 1.0 ppb Lab Spike)	1000	24700	26000	*
DAL-SW-BCT03-0-060412 (C0171043 Spk H, 10 ppb Lab Spike)	10000	24700	32100	74
DAL-SW-BCT03-LS-060412 (C0171045, 1.0 ppb Field Spike)	1000	24700	26500	*
DAL-SW-BCT03-HS-060412 (C0171046, 10 ppb Field Spike)	10000	24700	30400	57
DAL-SW-BC01-0-060412 (C0171047 Spk C, 1.0 ppb Lab Spike)	1000	103	863	76
DAL-SW-BC01-0-060412 (C0171047 Spk D, 10 ppb Lab Spike)	10000	103	7760	77
DAL-SW-BC01-LS-060412 (C0171049, 1.0 ppb Field Spike)	1000	103	647	54
DAL-SW-BC01-HS-060412 (C0171050, 10 ppb Field Spike)	10000	103	4780	47
DAL-SW-BC02-0-060412 (C0171051 Spk E, 1.0 ppb Lab Spike)	1000	126	850	72
DAL-SW-BC02-0-060412 (C0171051 Spk F, 10 ppb Lab Spike)	10000	126	8090	80
DAL-SW-BC02-LS-060412 (C0171053, 1.0 ppb Field Spike)	1000	126	654	53
DAL-SW-BC02-HS-060412 (C0171054, 10 ppb Field Spike)	10000	126	5500	54
DAL-SW-BC03-0-060414 (C0171055 Spk G, 1.0 ppb Lab Spike)	1000	2370	3360	99
DAL-SW-BC03-0-060414 (C0171055 Spk H, 10 ppb Lab Spike)	10000	2370	9900	75
DAL-SW-BC03-LS-060414 (C0171057, 1.0 ppb Field Spike)	1000	2370	3300	93
DAL-SW-BC03-HS-060414 (C0171058, 10 ppb Field Spike)	10000	2370	9200	68
DAL-SW-BC04-0-060414 (C0171059 Spk C, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-BC04-0-060414 (C0171059 Spk D, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-SW-BC04-LS-060414 (C0171061, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-SW-BC04-HS-060414 (C0171062, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-SW-BC05-0-060414 (C0171063 Spk E, 1.0 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-BC05-0-060414 (C0171063 Spk F, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-SW-BC05-LS-060414 (C0171065, 1.0 ppb Field Spike)	1000	NR	NR	NR
DAL-SW-BC05-HS-060414 (C0171066, 10 ppb Field Spike)	10000	NR	NR	NR

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

	C8 Acid PFOA			
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-SW-OSP01-0-060413 (C0171067 Spk G, 0.1 ppb Lab Spike)	100	335	375	*
DAL-SW-OSP01-0-060413 (C0171067 Spk H, 1.0 ppb Lab Spike)	1000	335	1070	74
DAL-SW-OSP01-LS-060413 (C0171069, 0.1 ppb Field Spike)	100	335	402	*
DAL-SW-OSP01-HS-060413 (C0171070, 1.0 ppb Field Spike)	1000	335	971	64
DAL-SW-OSP02-0-060413 (C0171071 Spk C, 1.0 ppb Lab Spike)	100	NR	NR	NR
DAL-SW-OSP02-0-060413 (C0171071 Spk D, 10 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-OSP02-LS-060413 (C0171073, 1.0 ppb Field Spike)	100	NR	NR	NR
DAL-SW-OSP02-HS-060413 (C0171074, 10 ppb Field Spike)	1000	NR	NR	NR
DAL-SW-OSP03-0-060413 (C0171075 Spk E, 1.0 ppb Lab Spike)	100	NR	NR	NR
DAL-SW-OSP03-0-060413 (C0171075 Spk F, 10 ppb Lab Spike)	1000	NR	NR	NR
DAL-SW-OSP03-LS-060413 (C0171077, 1.0 ppb Field Spike)	100	NR	NR	NR
DAL-SW-OSP03-HS-060413 (C0171078, 10 ppb Field Spike)	1000	NR	NR	NR
DAL-GW-BPWELL-0-060413 (C0171079 Spk G, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-GW-BPWELL-0-060413 (C0171079 Spk H, 1000 ppb Lab Spike)	1000000	NR	NR	NR
DAL-GW-BPWELL-LS-060413 (C0171081, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-GW-BPWELL-MS-060413 (C0171082, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-GW-BPWELL-HS-060413 (C0171083, 1000 ppb Field Spike)	1000000	NR	NR	NR
DPWS-WWI-DCTP01-0-060413 (C0171084 Spk C, 10 ppb Lab Spike)	10000	4200	13100	89
DPWS-WWI-DCTP01-0-060413 (C0171084 Spk D, 100 ppb Lab Spike)	100000	4200	96500	92
DPWS-WWI-DCTP01-LS-060413 (C0171086, 10 ppb Field Spike)	10000	4200	9220	50
DPWS-WWI-DCTP01-HS-060413 (C0171087, 100 ppb Field Spike)	100000	4200	46700	43
DPWS-WWE-DCTP01-0-060413 (C0171088 Spk E, 10 ppb Lab Spike)^	10000	7030	11700	47
DPWS-WWE-DCTP01-0-060413 (C0171088 Spk F, 100 ppb Lab Spike)^	100000	7030	95100	88
DPWS-WWE-DCTP01-LS-060413 (C0171090, 10 ppb Field Spike)	10000	7030	12600	56
DPWS-WWE-DCTP01-HS-060413 (C0171091, 100 ppb Field Spike)	100000	7030	64300	57

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
^ Sample was bottle was filled to 230 mL instead of 200 mL.
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table V. Matrix Spike Recovery of PFOA in Water Samples Continued

	C8 Acid PFOA			
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-WW-EFF01-0-060413 (C0171092 Spk G, 1.0 ppb Lab Spike)	1000	4650	5250	*
DAL-WW-EFF01-0-060413 (C0171092 Spk H, 10 ppb Lab Spike)	10000	4650	11700	71
DAL-WW-EFF01-LS-060413 (C0171094, 1.0 ppb Field Spike)	1000	4650	5890	*
DAL-WW-EFF01-HS-060413 (C0171095, 10 ppb Field Spike)	10000	4650	11200	66
DAL-LCH-MCLF01-0-060414 (C0171096 Spk C, 10 ppb Lab Spike)	10000	NR	NR	NR
DAL-LCH-MCLF01-0-060414 (C0171096 Spk D, 100 ppb Lab Spike)	100000	NR	NR	NR
DAL-LCH-MCLF01-LS-060414 (C0171098, 10 ppb Field Spike)	10000	NR	NR	NR
DAL-LCH-MCLF01-HS-060414 (C0171099, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-GW-TRIP1-LS-060412 (C0171101, 1.0 ppb Field Spike)	1000	ND	776	78
DAL-GW-TRIP1-HS-060412 (C0171102, 10 ppb Field Spike)	10000	ND	7950	80
DAL-WW-TRIP1-LS-060413 (C0171104, 1.0 ppb Field Spike)	1000	ND	857	86
DAL-WW-TRIP1-HS-060413 (C0171105, 10 ppb Field Spike)	10000	ND	9500	95
			Average:	**75**
			Standard Deviation:	**22**

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 25 ng/L.
NR = Not reported due to quality control failures, see Table VI for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table VI. Matrix Spike Recovery PFOA in Re-extracted Water Samples

		C8 Acid PFOA		
Sample Description	Amount Spiked (ng/L)	Amt Found in Sample (ng/L)	Amount Recovered (ng/L)	Recovery (%)
DAL-GW-138R-HS-060412 (C0169345, 1000 ppb Field Spike)	1000000	NR	NR	NR
DAL-GW-138S-DB-060412 (C0169347 Spk E, 100 ppb Lab Spike)	100000	NR	NR	NR
DAL-GW-138S-MS-060412 (C0169349, 100 ppb Field Spike)	100000	NR	NR	NR
DAL-GW-601R-LS-060413 (C0170986, 0.1 ppb Field Spike)	100	107	222	115
DAL-GW-601S-DB-060413 (C0170989 Spk G, 0.1 ppb Lab Spike)	100	121	223	102
DAL-GW-601S-LS-060413 (C0170990, 0.1 ppb Field Spike)	100	121	220	99
DAL-GW-601L-DB-060413 (C0170997 Spk E, 10 ppb Lab Spike)	10000	19800	30100	103
DAL-SW-BPP01-DB-060413 (C0171021 Spk E, 1000 ppb Lab Spike)	1000000	526000	1790000	126
DAL-SW-BCT01-HS-060412 (C0171038, 10 ppb Field Spike)	10000	29000	37300	83
DAL-SW-BC04-HS-060414 (C0171062, 10 ppb Field Spike)	10000	27700	39700	120
DAL-SW-BC05-LS-060414 (C0171065, 1.0 ppb Field Spike)	1000	782	1520	74
DAL-SW-OSP02-HS-060413 (C0171074, 10 ppb Field Spike)	1000	2660	3610	95
DAL-SW-OSP03-DB-060413 (C0171076 Spk G, 1.0 ppb Lab Spike)	1000	2650	3620	97
DAL-GW-BPWELL-HS-060413 (C0171083, 1000 ppb Field Spike)	1000000	641000	1540000	90
DAL-LCH-MCLF01-HS-060414 (C0171099, 100 ppb Field Spike)	100000	48700	132000	83
			Average:	**99**
			Standard Deviation:	**16**

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
NR = Not reported due to quality control failures.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table VII. Matrix Spike Recovery of PFOA in Sludge Samples

		C8 Acid PFOA		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	Amount Recovered (ng/g) wet weight	Recovery (%)
DPWS-SL-DCTP01-0-0000 **(C0171106 Spk C, 4 ppb Spike)**	4	424	439	*
DPWS-SL-DCTP01-0-0000 **(C0171106 Spk D, 400 ppb Spike)**	400	424	701	69
			Average:	**69**
			Standard Deviation:	**NA**

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table VIII. Matrix Spike Recovery PFOA in Sediment Samples

	C8 Acid PFOA			
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-OSP01-0-0000 **(C0171107 Spk C, 4 ppb Lab Spike)**	4	NR	NR	NR
DAL-SD-OSP01-0-0000 **(C0171107 Spk D, 400 ppb Lab Spike)**	400	NR	NR	NR
DAL-SD-OSP02-0-0000 **(C0171108 Spk E, 4 ppb Lab Spike)**	4	NR	NR	NR
DAL-SD-OSP02-0-0000 **(C0171108 Spk F, 400 ppb Lab Spike)**	400	NR	NR	NR

NR = Not reported due to quality control failures.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0169333	DAL-GW-TRIP1-0-060411	500	546	109
C0169334	DAL-GW-TRIP1-LS-060411	1000	1070	107
C0169335	DAL-GW-TRIP1-HS-060411	10000	10700	107
C0169336 Spk C	DAL-GW-138L-0-060411	10500	8240	78
C0169336 Spk D	DAL-GW-138L-0-060411	1000500	861000	86
C0169336	DAL-GW-138L-0-060411	500	413	83
C0169336 Rep	DAL-GW-138L-0-060411	500	450	90
C0169337	DAL-GW-138L-DB-060411	500	466	93
C0169338	DAL-GW-138L-LS-060411	10000	5020	50
C0169339	DAL-GW-138L-MS-060411	100000	37000	37
C0169340	DAL-GW-138L-HS-060411	1000000	166000	17
C0169341 Spk E	DAL-GW-138R-0-060412	10500	NR	NR
C0169341 Spk F	DAL-GW-138R-0-060412	1000500	NR	NR
C0169341	DAL-GW-138R-0-060412	500	NR	NR
C0169341 Rep	DAL-GW-138R-0-060412	500	NR	NR
C0169342	DAL-GW-138R-DB-060412	500	NR	NR
C0169343	DAL-GW-138R-LS-060412	10000	NR	NR
C0169344	DAL-GW-138R-MS-060412	100000	NR	NR
C0169345	DAL-GW-138R-HS-060412	1000000	NR	NR
C0169346 Spk C	DAL-GW-138S-0-060412	10500	NR	NR
C0169346 Spk D	DAL-GW-138S-0-060412	1000500	NR	NR
C0169346	DAL-GW-138S-0-060412	500	NR	NR
C0169346 Rep	DAL-GW-138S-0-060412	500	NR	NR
C0169347	DAL-GW-138S-DB-060412^	400	NR	NR
C0169348	DAL-GW-138S-LS-060412	10000	NR	NR
C0169349	DAL-GW-138S-MS-060412	100000	NR	NR
C0169350	DAL-GW-138S-HS-060412	1000000	NR	NR
C0169351 Spk E	DAL-GW-605R-0-060412	600	478	80
C0169351 Spk F	DAL-GW-605R-0-060412	1500	1090	73
C0169351	DAL-GW-605R-0-060412	500	371	74
C0169351 Rep	DAL-GW-605R-0-060412	500	401	80
C0169352	DAL-GW-605R-DB-060412	500	393	79
C0169353	DAL-GW-605R-LS-060412	100	53.8	54
C0169354	DAL-GW-605R-HS-060412^	800	440	55
C0169355 Spk G	DAL-GW-605L-0-060412	600	546	91
C0169355 Spk H	DAL-GW-605L-0-060412	1500	1230	82
C0169355	DAL-GW-605L-0-060412	500	403	81
C0169355 Rep	DAL-GW-605L-0-060412	500	438	88
C0169356	DAL-GW-605L-DB-060412	500	512	102
C0169357	DAL-GW-605L-LS-060412	100	54.2	54
C0169358	DAL-GW-605L-HS-060412	1000	449	45

^ Sample was bottle was filled to 250 mL, causing the 13C-PFOA spiking level to be modified.
NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0169359 Spk C	DAL-GW-603S-0-060412	600	520	87
C0169359 Spk D	DAL-GW-603S-0-060412	1500	1220	81
C0169359	DAL-GW-603S-0-060412	500	395	79
C0169359 Rep	DAL-GW-603S-0-060412	500	411	82
C0169360	DAL-GW-603S-DB-060412	500	511	102
C0169361	DAL-GW-603S-LS-060412	100	72.9	73
C0169362	DAL-GW-603S-HS-060412	1000	656	66
C0169363 Spk E	DAL-GW-604L-0-060412	600	547	91
C0169363 Spk F	DAL-GW-604L-0-060412	1500	1350	90
C0169363	DAL-GW-604L-0-060412	500	431	86
C0169363 Rep	DAL-GW-604L-0-060412	500	440	88
C0169364	DAL-GW-604L-DB-060412	500	446	89
C0169365	DAL-GW-604L-LS-060412	100	62.7	63
C0169366	DAL-GW-604L-HS-060412	1000	536	54
C0169367 Spk G	DAL-GW-604S-0-060412	600	491	82
C0169367 Spk H	DAL-GW-604S-0-060412	1500	1270	85
C0169367	DAL-GW-604S-0-060412	500	430	86
C0169367 Rep	DAL-GW-604S-0-060412	500	420	84
C0169368	DAL-GW-604S-DB-060412	500	472	94
C0169369	DAL-GW-604S-LS-060412	100	66.2	66
C0169370	DAL-GW-604S-HS-060412	1000	538	54
C0170984 Spk C	DAL-GW-601R-0-060413	600	NR	NR
C0170984 Spk D	DAL-GW-601R-0-060413	1500	NR	NR
C0170984	DAL-GW-601R-0-060413	500	NR	NR
C0170984 Rep	DAL-GW-601R-0-060413	500	NR	NR
C0170985	DAL-GW-601R-DB-060413	500	NR	NR
C0170986	DAL-GW-601R-LS-060413	100	NR	NR
C0170987	DAL-GW-601R-HS-060413	1000	NR	NR
C0170988 Spk E	DAL-GW-601S-0-060413	600	NR	NR
C0170988 Spk F	DAL-GW-601S-0-060413	1500	NR	NR
C0170988	DAL-GW-601S-0-060413	500	NR	NR
C0170988 Rep	DAL-GW-601S-0-060413	500	NR	NR
C0170989	DAL-GW-601S-DB-060413	500	NR	NR
C0170990	DAL-GW-601S-LS-060413	100	NR	NR
C0170991	DAL-GW-601S-HS-060413	1000	NR	NR
C0170992 Spk G	DAL-GW-602R-0-060413	600	530	88
C0170992 Spk H	DAL-GW-602R-0-060413	1500	2280	152
C0170992	DAL-GW-602R-0-060413	500	974	195
C0170992 Rep	DAL-GW-602R-0-060413	500	948	190
C0170993	DAL-GW-602R-DB-060413	500	941	188
C0170994	DAL-GW-602R-LS-060413	100	120	120
C0170995	DAL-GW-602R-HS-060413	1000	1050	105

NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0170996 Spk C	DAL-GW-601L-0-060413	600	NR	NR
C0170996 Spk D	DAL-GW-601L-0-060413	1500	NR	NR
C0170996	DAL-GW-601L-0-060413	500	NR	NR
C0170996 Rep	DAL-GW-601L-0-060413	500	NR	NR
C0170997	DAL-GW-601L-DB-060413	500	NR	NR
C0170998	DAL-GW-601L-LS-060413	100	NR	NR
C0170999	DAL-GW-601L-HS-060413	1000	NR	NR
C0171000 Spk E	DAL-GW-602S-0-060413	600	598	100
C0171000 Spk F	DAL-GW-602S-0-060413	1500	1210	81
C0171000	DAL-GW-602S-0-060413	500	473	95
C0171000 Rep	DAL-GW-602S-0-060413	500	449	90
C0171001	DAL-GW-602S-DB-060413	500	469	94
C0171002	DAL-GW-602S-LS-060413	100	53.9	54
C0171003	DAL-GW-602S-HS-060413	1000	581	58
C0171004 Spk G	DAL-GW-602L-0-060413	600	557	93
C0171004 Spk H	DAL-GW-602L-0-060413	1500	1330	89
C0171004	DAL-GW-602L-0-060413	500	423	85
C0171004 Rep	DAL-GW-602L-0-060413	500	437	87
C0171005	DAL-GW-602L-DB-060413	500	448	90
C0171006	DAL-GW-602L-LS-060413	100	67.6	68
C0171007	DAL-GW-602L-HS-060413	1000	710	71
C0171008 Spk C	DAL-GW-604R-0-060413	600	508	85
C0171008 Spk D	DAL-GW-604R-0-060413	1500	1160	77
C0171008	DAL-GW-604R-0-060413	500	433	87
C0171008 Rep	DAL-GW-604R-0-060413	500	428	86
C0171009	DAL-GW-604R-DB-060413	500	448	90
C0171010	DAL-GW-604R-LS-060413	100	68.3	68
C0171011	DAL-GW-604R-HS-060413	1000	575	58
C0171012 Spk E	DAL-GW-603R-0-060413	600	541	90
C0171012 Spk F	DAL-GW-603R-0-060413	1500	1140	76
C0171012	DAL-GW-603R-0-060413	500	436	87
C0171012 Rep	DAL-GW-603R-0-060413	500	433	87
C0171013	DAL-GW-603R-DB-060413	500	445	89
C0171014	DAL-GW-603R-LS-060413	100	68.6	69
C0171015	DAL-GW-603R-HS-060413	1000	486	49
C0171016 Spk G	DAL-GW-603L-0-060413	600	555	93
C0171016 Spk H	DAL-GW-603L-0-060413	1500	1350	90
C0171016	DAL-GW-603L-0-060413	500	434	87
C0171016 Rep	DAL-GW-603L-0-060413	500	438	88
C0171017	DAL-GW-603L-DB-060413	500	439	88
C0171018	DAL-GW-603L-LS-060413	100	76.6	77
C0171019	DAL-GW-603L-HS-060413	1000	676	68

NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0171020 Spk C	DAL-SW-BPP01-0-060413	1500	NR	NR
C0171020 Spk D	DAL-SW-BPP01-0-060413	100500	NR	NR
C0171020	DAL-SW-BPP01-0-060413	500	NR	NR
C0171020 Rep	DAL-SW-BPP01-0-060413	500	NR	NR
C0171021	DAL-SW-BPP01-DB-060413	500	NR	NR
C0171022	DAL-SW-BPP01-LS-060413	1500	NR	NR
C0171023	DAL-SW-BPP01-MS-060413	10500	NR	NR
C0171024	DAL-SW-BPP01-HS-060413	100500	NR	NR
C0171025 Spk E	DAL-SW-BPP02-0-060413	1500	770	51
C0171025 Spk F	DAL-SW-BPP02-0-060413	100500	84400	84
C0171025	DAL-SW-BPP02-0-060413	500	146	29
C0171025 Rep	DAL-SW-BPP02-0-060413	500	134	27
C0171026	DAL-SW-BPP02-DB-060413	500	134	27
C0171027	DAL-SW-BPP02-LS-060413	1500	366	24
C0171028	DAL-SW-BPP02-MS-060413	10500	3820	36
C0171029	DAL-SW-BPP02-HS-060413	100500	45000	45
C0171030 Spk G	DAL-SW-BPP03-0-060413	1500	811	54
C0171030 Spk H	DAL-SW-BPP03-0-060413	100500	86100	86
C0171030	DAL-SW-BPP03-0-060413	500	152	30
C0171030 Rep	DAL-SW-BPP03-0-060413	500	144	29
C0171031	DAL-SW-BPP03-DB-060413	500	138	28
C0171032	DAL-SW-BPP03-LS-060413	1500	404	27
C0171033	DAL-SW-BPP03-MS-060413	10500	4230	40
C0171034	DAL-SW-BPP03-HS-060413	100500	59800	60
C0171035 Spk C	DAL-SW-BCT01-0-060412	1500	NR	NR
C0171035 Spk D	DAL-SW-BCT01-0-060412	10500	NR	NR
C0171035	DAL-SW-BCT01-0-060412	500	NR	NR
C0171035 Rep	DAL-SW-BCT01-0-060412	500	NR	NR
C0171036	DAL-SW-BCT01-DB-060412	500	NR	NR
C0171037	DAL-SW-BCT01-LS-060412	1000	NR	NR
C0171038	DAL-SW-BCT01-HS-060412	10000	NR	NR
C0171039 Spk E	DAL-SW-BCT02-0-060412	1500	935	62
C0171039 Spk F	DAL-SW-BCT02-0-060412	10500	7620	73
C0171039	DAL-SW-BCT02-0-060412	500	152	30
C0171039 Rep	DAL-SW-BCT02-0-060412	500	147	29
C0171040	DAL-SW-BCT02-DB-060412	500	143	29
C0171041	DAL-SW-BCT02-LS-060412	1000	379	38
C0171042	DAL-SW-BCT02-HS-060412	10000	4980	50
C0171043 Spk G	DAL-SW-BCT03-0-060412	1500	1110	74
C0171043 Spk H	DAL-SW-BCT03-0-060412	10500	7610	72
C0171043	DAL-SW-BCT03-0-060412	500	276	55
C0171043 Rep	DAL-SW-BCT03-0-060412	500	266	53
C0171044	DAL-SW-BCT03-DB-060412	500	267	53
C0171045	DAL-SW-BCT03-LS-060412	1000	585	59
C0171046	DAL-SW-BCT03-HS-060412	10000	5130	51

NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0171047 Spk C	DAL-SW-BC01-0-060412	1500	1200	80
C0171047 Spk D	DAL-SW-BC01-0-060412	10500	8240	78
C0171047	DAL-SW-BC01-0-060412	500	389	78
C0171047 Rep	DAL-SW-BC01-0-060412	500	389	78
C0171048	DAL-SW-BC01-DB-060412	500	429	86
C0171049	DAL-SW-BC01-LS-060412	1000	509	51
C0171050	DAL-SW-BC01-HS-060412	10000	4680	47
C0171051 Spk E	DAL-SW-BC02-0-060412	1500	1240	83
C0171051 Spk F	DAL-SW-BC02-0-060412	10500	8430	80
C0171051	DAL-SW-BC02-0-060412	500	421	84
C0171051 Rep	DAL-SW-BC02-0-060412	500	450	90
C0171052	DAL-SW-BC02-DB-060412	500	433	87
C0171053	DAL-SW-BC02-LS-060412	1000	499	50
C0171054	DAL-SW-BC02-HS-060412	10000	5300	53
C0171055 Spk G	DAL-SW-BC03-0-060414	1500	1230	82
C0171055 Spk H	DAL-SW-BC03-0-060414	10500	8100	77
C0171055	DAL-SW-BC03-0-060414	500	419	84
C0171055 Rep	DAL-SW-BC03-0-060414	500	420	84
C0171056	DAL-SW-BC03-DB-060414	500	451	90
C0171057	DAL-SW-BC03-LS-060414	1000	589	59
C0171058	DAL-SW-BC03-HS-060414	10000	6300	63
C0171059 Spk C	DAL-SW-BC04-0-060414	1500	NR	NR
C0171059 Spk D	DAL-SW-BC04-0-060414	10500	NR	NR
C0171059	DAL-SW-BC04-0-060414	500	NR	NR
C0171059 Rep	DAL-SW-BC04-0-060414	500	NR	NR
C0171060	DAL-SW-BC04-DB-060414	500	NR	NR
C0171061	DAL-SW-BC04-LS-060414	1000	NR	NR
C0171062	DAL-SW-BC04-HS-060414	10000	NR	NR
C0171063 Spk E	DAL-SW-BC05-0-060414	1500	NR	NR
C0171063 Spk F	DAL-SW-BC05-0-060414	10500	NR	NR
C0171063	DAL-SW-BC05-0-060414	500	NR	NR
C0171063 Rep	DAL-SW-BC05-0-060414	500	NR	NR
C0171064	DAL-SW-BC05-DB-060414	500	NR	NR
C0171065	DAL-SW-BC05-LS-060414	1000	NR	NR
C0171066	DAL-SW-BC05-HS-060414	10000	NR	NR
C0171067 Spk G	DAL-SW-OSP01-0-060413	600	561	94
C0171067 Spk H	DAL-SW-OSP01-0-060413	1500	1250	83
C0171067	DAL-SW-OSP01-0-060413	500	479	96
C0171067 Rep	DAL-SW-OSP01-0-060413	500	477	95
C0171068	DAL-SW-OSP01-DB-060413	500	440	88
C0171069	DAL-SW-OSP01-LS-060413	100	78.3	78
C0171070	DAL-SW-OSP01-HS-060413	1000	650	65

NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0171071 Spk C	DAL-SW-OSP02-0-060413	600	NR	NR
C0171071 Spk D	DAL-SW-OSP02-0-060413	1500	NR	NR
C0171071	DAL-SW-OSP02-0-060413	500	NR	NR
C0171071 Rep	DAL-SW-OSP02-0-060413	500	NR	NR
C0171072	DAL-SW-OSP02-DB-060413	500	NR	NR
C0171073	DAL-SW-OSP02-LS-060413	100	NR	NR
C0171074	DAL-SW-OSP02-HS-060413	1000	NR	NR
C0171075 Spk E	DAL-SW-OSP03-0-060413	600	NR	NR
C0171075 Spk F	DAL-SW-OSP03-0-060413	1500	NR	NR
C0171075	DAL-SW-OSP03-0-060413	500	NR	NR
C0171075 Rep	DAL-SW-OSP03-0-060413	500	NR	NR
C0171076	DAL-SW-OSP03-DB-060413	500	NR	NR
C0171077	DAL-SW-OSP03-LS-060413	100	NR	NR
C0171078	DAL-SW-OSP03-HS-060413	1000	NR	NR
C0171079 Spk G	DAL-GW-BPWELL-0-060413	10500	NR	NR
C0171079 Spk H	DAL-GW-BPWELL-0-060413	1000500	NR	NR
C0171079	DAL-GW-BPWELL-0-060413	500	NR	NR
C0171079 Rep	DAL-GW-BPWELL-0-060413	500	NR	NR
C0171080	DAL-GW-BPWELL-DB-060413	500	NR	NR
C0171081	DAL-GW-BPWELL-LS-060413	10000	NR	NR
C0171082	DAL-GW-BPWELL-MS-060413	100000	NR	NR
C0171083	DAL-GW-BPWELL-HS-060413	1000000	NR	NR
C0171084 Spk C	DPWS-WWI-DCTP01-0-060413	10500	8680	83
C0171084 Spk D	DPWS-WWI-DCTP01-0-060413	100500	96200	96
C0171084	DPWS-WWI-DCTP01-0-060413	500	391	78
C0171084 Rep	DPWS-WWI-DCTP01-0-060413	500	393	79
C0171085	DPWS-WWI-DCTP01-DB-060413	500	426	85
C0171086	DPWS-WWI-DCTP01-LS-060413	10000	4600	46
C0171087	DPWS-WWI-DCTP01-HS-060413	100000	42500	43
C0171088 Spk E	DPWS-WWE-DCTP01-0-060413^	10435	8260	79
C0171088 Spk F	DPWS-WWE-DCTP01-0-060413^	100435	92400	92
C0171088	DPWS-WWE-DCTP01-0-060413^	435	343	79
C0171088 Rep	DPWS-WWE-DCTP01-0-060413^	435	350	80
C0171089	DPWS-WWE-DCTP01-DB-060413	500	391	78
C0171090	DPWS-WWE-DCTP01-LS-060413	10000	4870	49
C0171091	DPWS-WWE-DCTP01-HS-060413	100000	48800	49
C0171092 Spk G	DAL-WW-EFF01-0-060413	1500	1180	79
C0171092 Spk H	DAL-WW-EFF01-0-060413	10500	7880	75
C0171092	DAL-WW-EFF01-0-060413	500	360	72
C0171092 Rep	DAL-WW-EFF01-0-060413	500	335	67
C0171093	DAL-WW-EFF01-DB-060413	500	425	85
C0171094	DAL-WW-EFF01-LS-060413	1000	744	74
C0171095	DAL-WW-EFF01-HS-060413	10000	6690	67

NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in Water Samples Continued

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0171096 Spk C	DAL-LCH-MCLF01-0-060414	10500	NR	NR
C0171096 Spk D	DAL-LCH-MCLF01-0-060414	100500	NR	NR
C0171096	DAL-LCH-MCLF01-0-060414	500	NR	NR
C0171096 Rep	DAL-LCH-MCLF01-0-060414	500	NR	NR
C0171097	DAL-LCH-MCLF01-DB-060414	500	NR	NR
C0171098	DAL-LCH-MCLF01-LS-060414	10000	NR	NR
C0171099	DAL-LCH-MCLF01-HS-060414	100000	NR	NR
C0171100	DAL-GW-TRIP1-0-060412	500	412	82
C0171101	DAL-GW-TRIP1-LS-060412	1000	748	75
C0171102	DAL-GW-TRIP1-HS-060412	10000	8150	82
C0171103	DAL-WW-TRIP1-0-060413	500	501	100
C0171104	DAL-WW-TRIP1-LS-060413	1000	808	81
C0171105	DAL-WW-TRIP1-HS-060413	10000	8130	81
			Average:	**75**
			Standard Deviation:	**26**

^ Sample was bottle was filled to 230 mL, causing the 13C-PFOA spiking level to be modified.
NR = Not reported due to quality control failures, see Table X for re-extraction data.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table X. Surrogate Spike Recovery of ^{13}C PFOA in Re-extracted Water Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/L)	Amount Recovered (ng/L)	Recovery (%)
C0169341	DAL-GW-138R-0-060412	500	NR	NR
C0169345	DAL-GW-138R-HS-060412	1000000	NR	NR
C0169347 Spk E	DAL-GW-138S-DB-060412	100500	NR	NR
C0169347	DAL-GW-138S-DB-060412	500	NR	NR
C0169349	DAL-GW-138S-MS-060412	100000	NR	NR
C0170984	DAL-GW-601R-0-060413	500	639	128
C0170986	DAL-GW-601R-LS-060413	100	114	114
C0170989 Spk G	DAL-GW-601S-DB-060413	600	777	130
C0170989	DAL-GW-601S-DB-060413	500	772	154
C0170990	DAL-GW-601S-LS-060413	100	106	106
C0170997 Spk E	DAL-GW-601L-DB-060413	10500	14600	139
C0170997	DAL-GW-601L-DB-060413	500	540	108
C0171021 Spk E	DAL-SW-BPP01-DB-060413	1000500	1180000	118
C0171021	DAL-SW-BPP01-DB-060413	500	243	49
C0171035	DAL-SW-BCT01-0-060412	500	475	95
C0171038	DAL-SW-BCT01-HS-060412	10000	6850	69
C0171060	DAL-SW-BC04-DB-060414	500	432	86
C0171062	DAL-SW-BC04-HS-060414	10000	7270	73
C0171063	DAL-SW-BC05-0-060414	500	607	121
C0171065	DAL-SW-BC05-LS-060414	1000	761	76
C0171071	DAL-SW-OSP02-0-060413	500	669	134
C0171074	DAL-SW-OSP02-HS-060413	1000	641	64
C0171076 Spk G	DAL-SW-OSP03-DB-060413	1500	1590	106
C0171076	DAL-SW-OSP03-DB-060413	500	630	126
C0171079	DAL-GW-BPWELL-0-060413	500	341	68
C0171083	DAL-GW-BPWELL-HS-060413	1000000	230000	23
C0171097	DAL-LCH-MCLF01-DB-060414	500	398	80
C0171099	DAL-LCH-MCLF01-HS-060414	100000	53600	54
			Average:	**97**
			Standard Deviation:	**34**

NR = Not reported due to quality control failures.

Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table XI. Surrogate Spike Recovery of ^{13}C PFOA in Sludge Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/g)	Amount Recovered Wet Weight (ng/g)	Recovery (%)
C0171106	DPWS-SL-DCTP01-0-0000	4	1.82	46
C0171106 Rep	DPWS-SL-DCTP01-0-0000	4	1.67	42
C0171106 Spk C	DPWS-SL-DCTP01-0-0000	4	1.48	37
C0171106 Spk D	DPWS-SL-DCTP01-0-0000	400	291	73
			Average:	**49**
			Standard Deviation:	**16**

Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table XII. Surrogate Spike Recovery of ^{13}C PFOA in Sediment Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/g)	Amount Recovered (ng/g)	Recovery (%)
C0171107	DAL-SD-OSP01-0-0000	4	NR	NR
C0171107 Rep	DAL-SD-OSP01-0-0000	4	NR	NR
C0171107 Spk C	DAL-SD-OSP01-0-0000	4	NR	NR
C0171107 Spk D	DAL-SD-OSP01-0-0000	400	NR	NR
C0171108	DAL-SD-OSP02-0-0000	4	NR	NR
C0171108 Rep	DAL-SD-OSP02-0-0000	4	NR	NR
C0171108 Spk E	DAL-SD-OSP02-0-0000	4	NR	NR
C0171108 Spk F	DAL-SD-OSP02-0-0000	400	NR	NR

NR = Not reported due to quality control failures.

Table XIII. Total Percent Solids for Sludge and Sediment Samples

Exygen ID	Sample Description	Total Solids (%)
C0171106	DPWS-SL-DCTP01-0-0000	22.83
C0171107	DAL-SD-OSP01-0-0000	58.39
C0171108	DAL-SD-OSP02-0-0000	53.33

FIGURES

Figure 1. Typical Calibration Curve for PFOA in Reagent Water

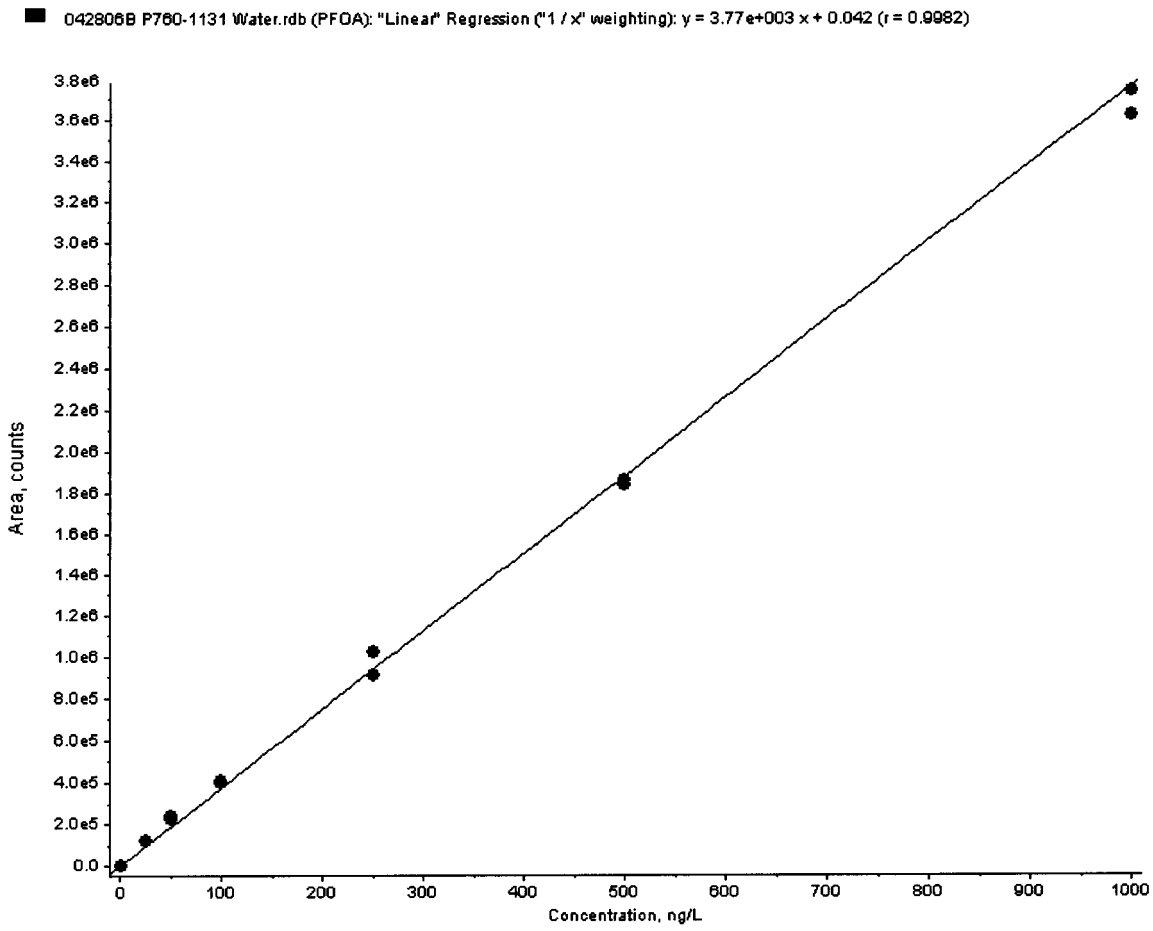


Figure 2. Extracted Standards of PFOA in Reagent Water, 25 ng/L and 50 ng/L, Respectively

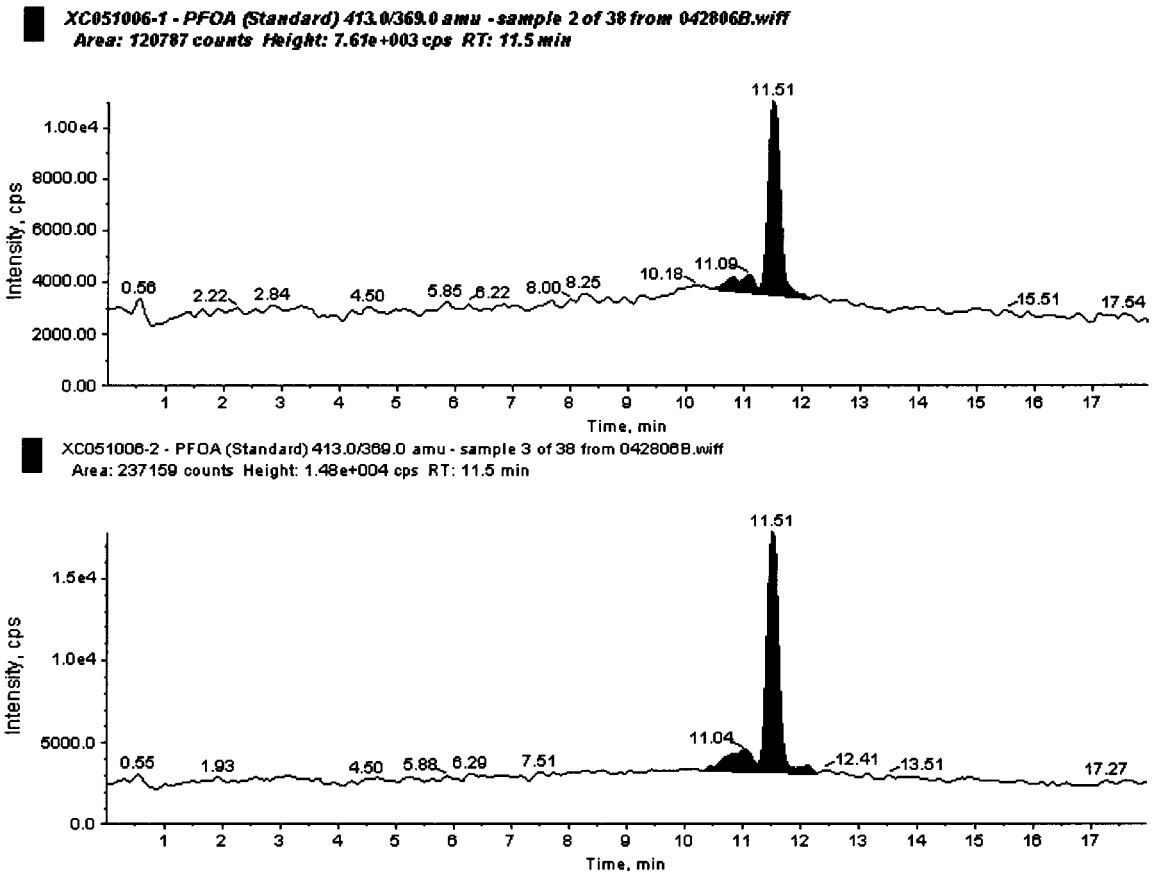


Figure 3. PFOA in Reagent Control, 50 ng/L Fortified Reagent Spike A, and 500 ng/L Fortified Reagent Spike B, Respectively

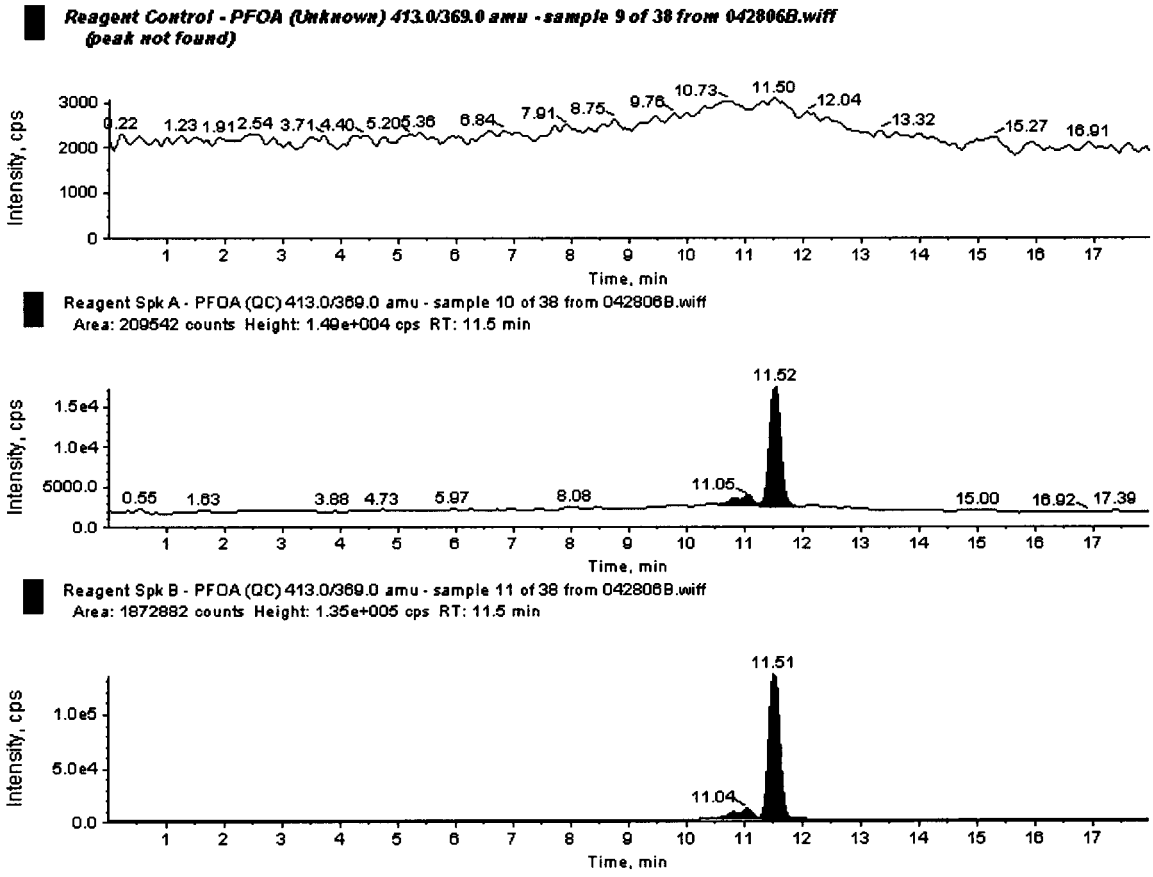


Figure 4. Chromatogram Representing a Water Sample Analyzed for PFOA (Exygen ID: C0169336, Data Set: 042806B)

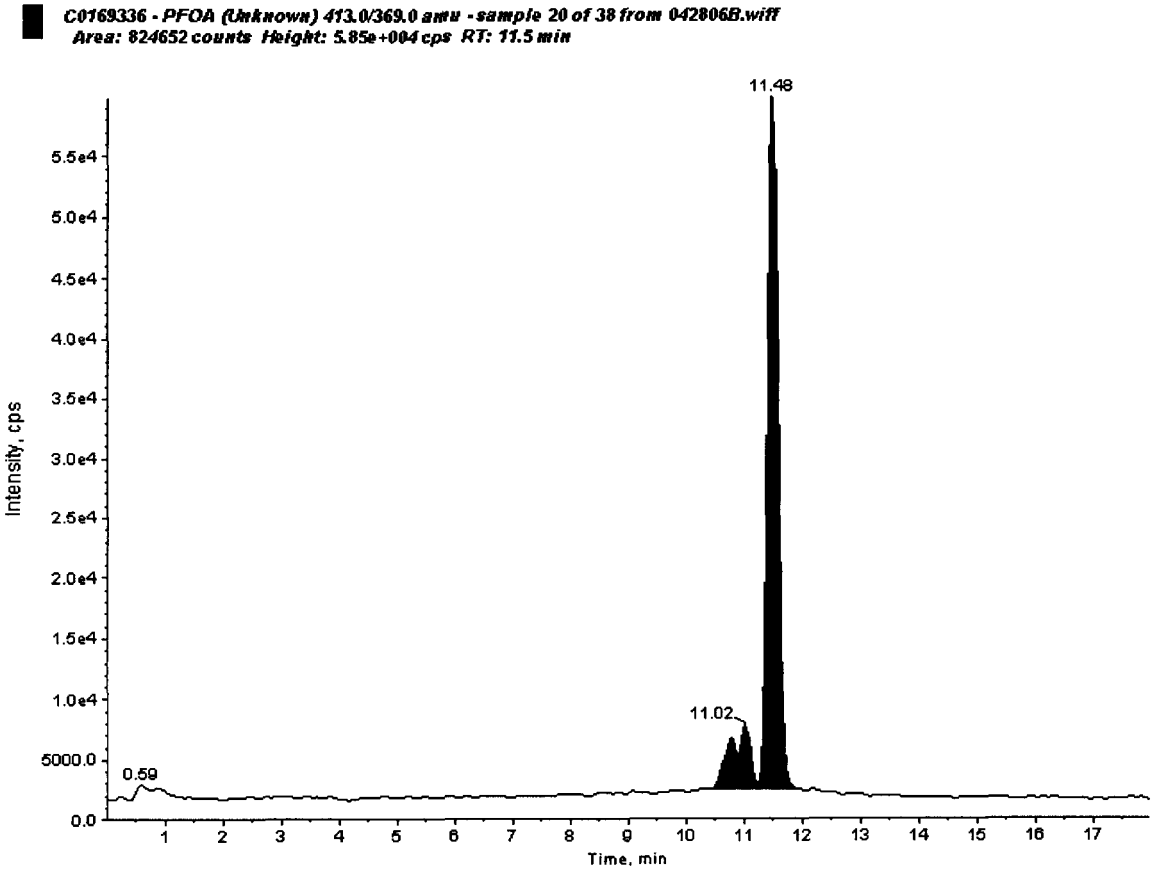


Figure 5. Chromatogram Representing a Sludge Sample Analyzed for PFOA (Exygen ID: C0171106, Data Set: 050906BR)

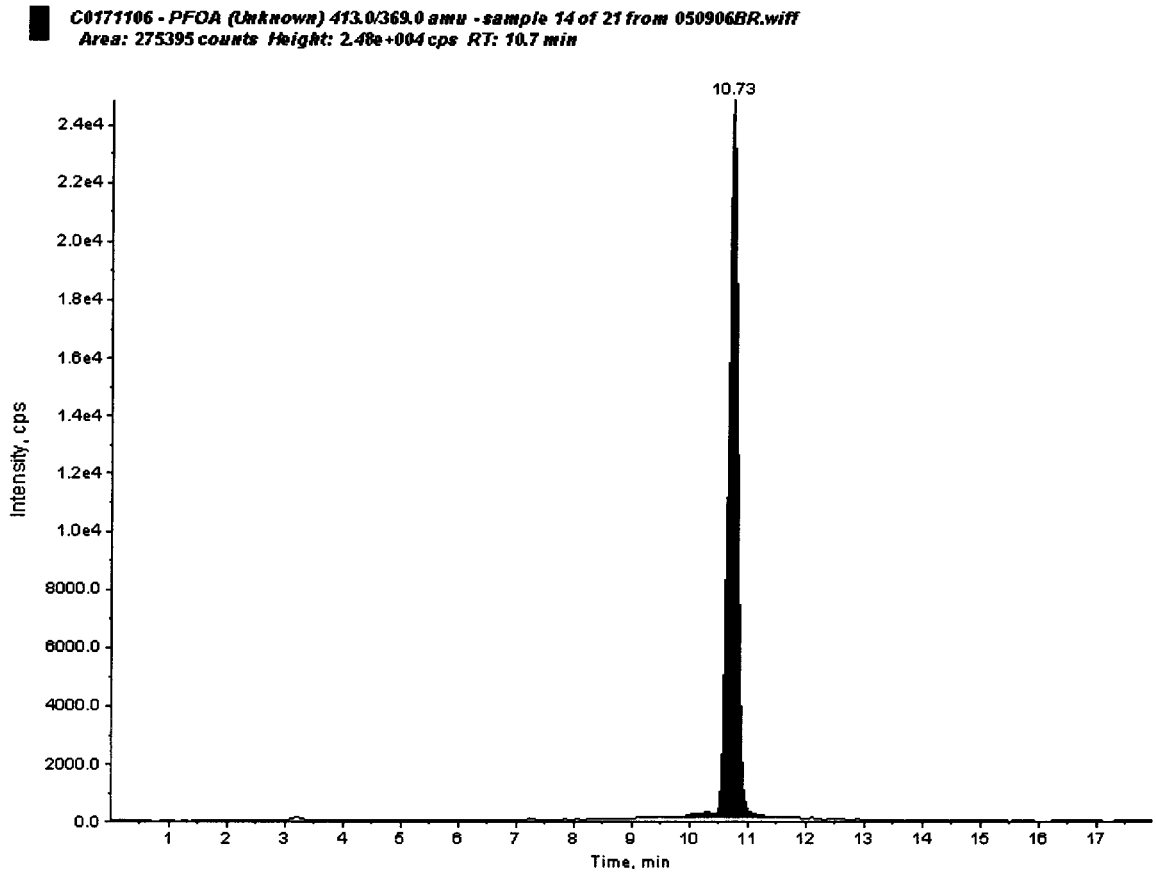


Figure 6. Chromatogram Representing a Sediment Sample Analyzed for PFOA (Exygen ID: C0171108, Data Set: 051106A)

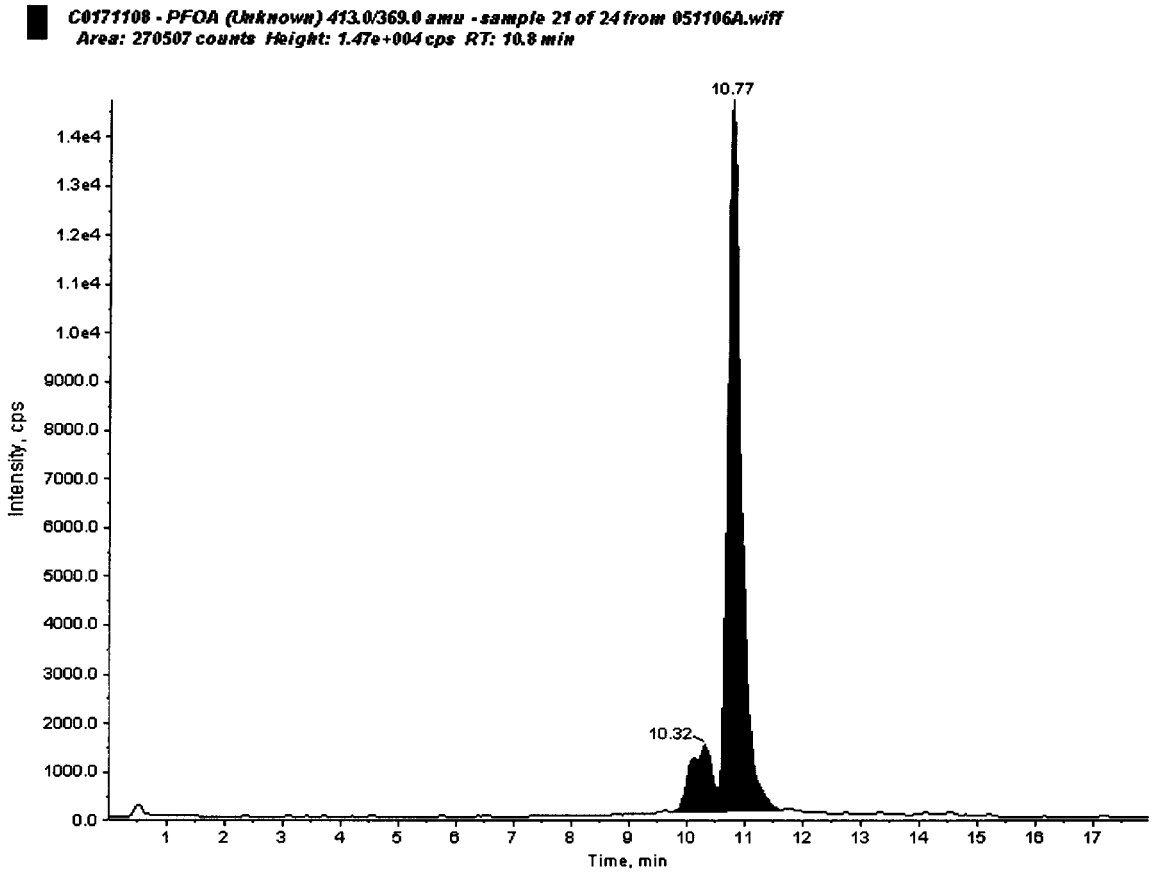


Figure 7. Typical Calibration Curve for ^{13}C PFOA in Reagent Water

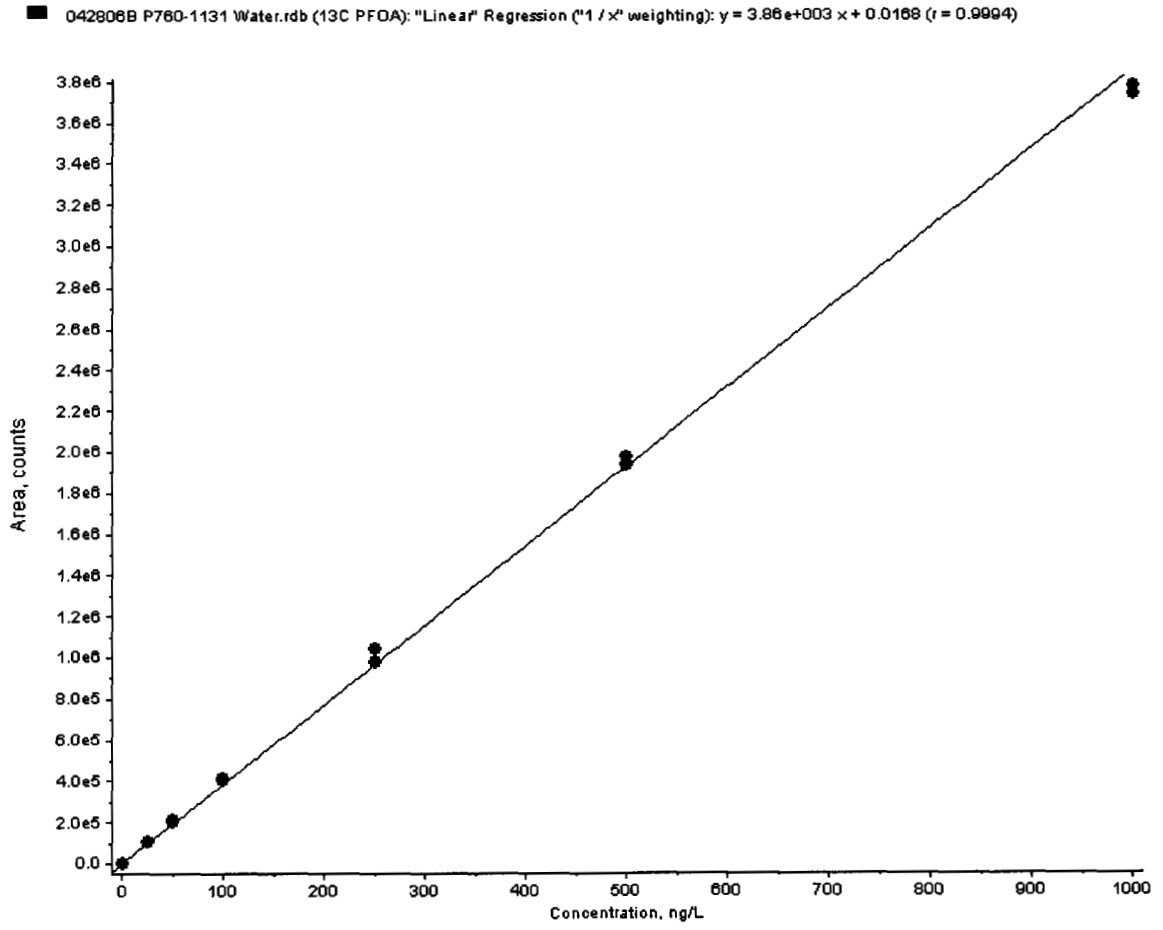


Figure 8. Extracted Standards of ^{13}C PFOA in Reagent Water, 25 ng/L and 50 ng/L, Respectively

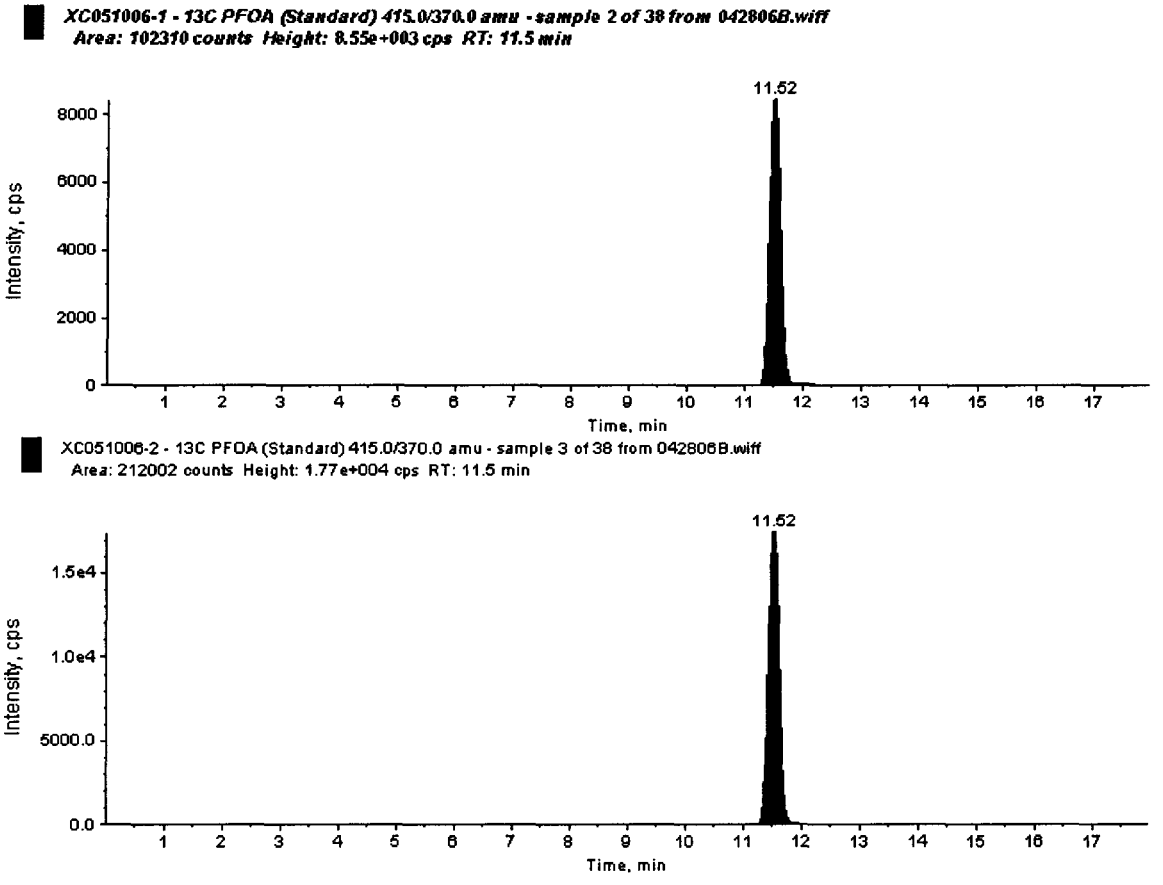


Figure 9. ^{13}C PFOA in Reagent Control, 50 ng/L Fortified Reagent Spike A, and 500 ng/L Fortified Reagent Spike B, Respectively

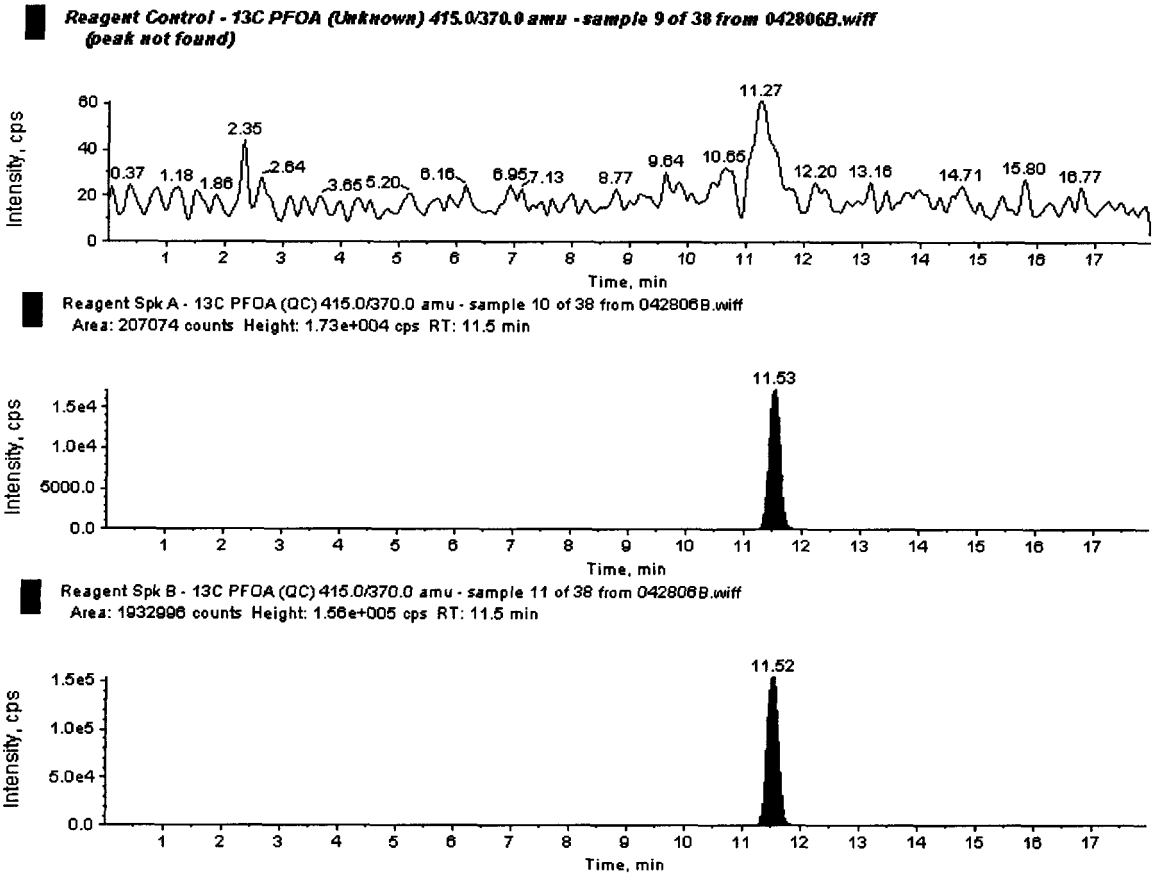


Figure 10. Chromatogram Representing a Water Sample Analyzed for ^{13}C PFOA (Exygen ID: C0169336, Data Set: 042806B)

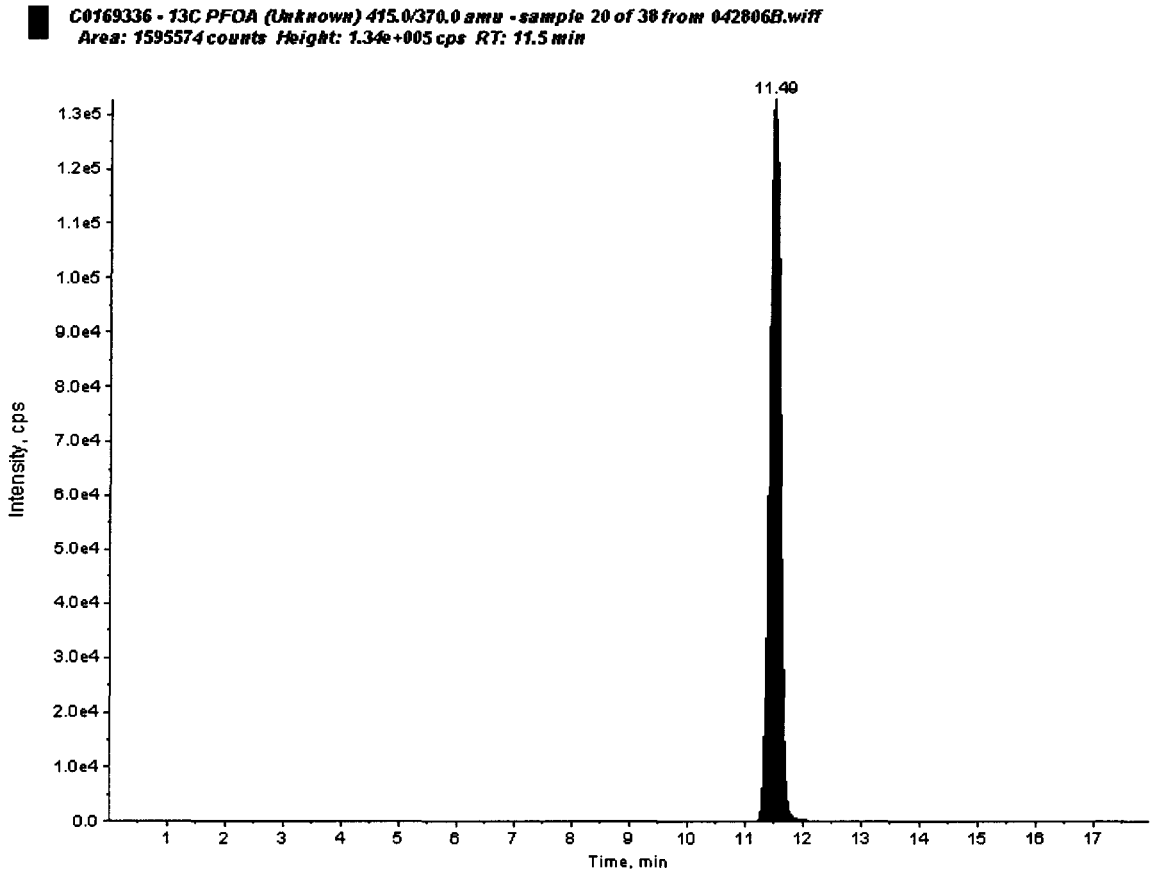


Figure 11. Chromatogram Representing a Sludge Sample Analyzed for ^{13}C PFOA (Exygen ID: C0171106, Data Set: 050906B)

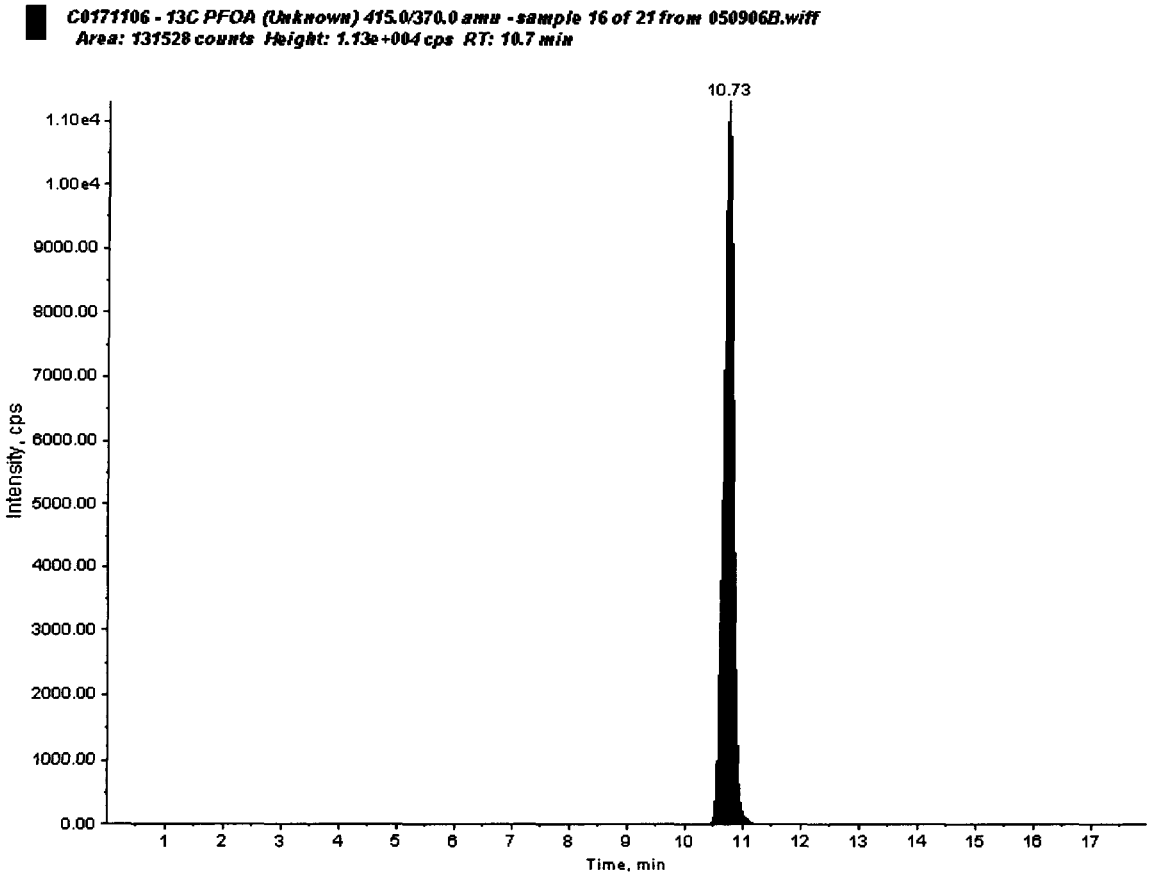
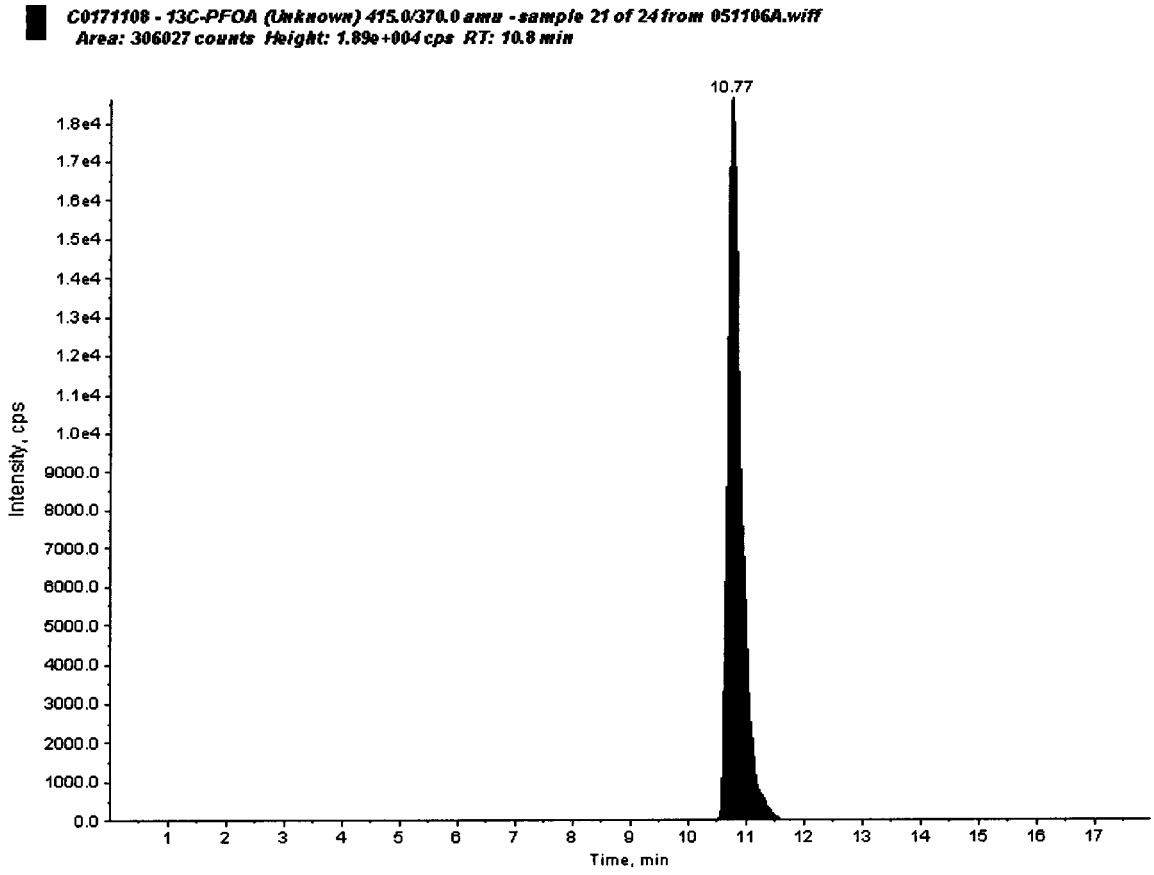


Figure 12. Chromatogram Representing a Sediment Sample Analyzed for ^{13}C PFOA (Exygen ID: C0171108, Data Set: 051106A)



APPENDIX A

Study Protocol P0000760 (Exygen Study No. P0000760) with Analytical Methods and Protocol Amendments

STUDY PROTOCOL

Study Title:

Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0000760

Performing Laboratory:
Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039

Sponsor Representative:
Michael A. Santoro
Director of Regulatory Affairs
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: (651) 733-6374

DISTRIBUTION:

1) Jaisimha Kesari, Study Director, Weston Solutions

2) John M. Flaherty, Principal Investigator, Exygen Research

3) Michael A. Santoro, Sponsor Representative, 3M Company

4) Exygen Research Quality Assurance Unit

PROTOCOL APPROVAL

Study Title: Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Livers and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0000760

APPROVALS

Jaisimha Kesari, Study Director
Weston Solutions — 11/5/04 Date

Michael A. Santoro, Sponsor Representative
3M Company — 11/11/04 Date

John M. Flaherty, Principal Investigator
Exygen Research — 10/29/04 Date

Richard A. Grazzini, President, Facility Management
Exygen Research — 29-OCT-2004 Date

Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research — 10/29/04 Date

TABLE OF CONTENTS

INTRODUCTION

The purpose of this study is to perform analysis for perfluorooctanoic acid (PFOA) in water, soil, sediment, fish, clams, vegetation, small mammal livers and small mammal serum using LC/MS/MS for the 3M Decatur Monitoring Program.

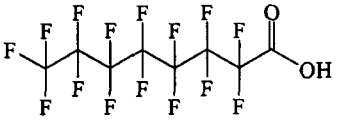
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIAL

The test material is perfluorooctanoic acid (PFOA) and was purchased from Sigma-Aldrich.

PFOA

Chemical Name: Perfluorooctanoic acid
Molecular Weight: 414
Lot Number: 23116HB
Purity: 97.64%
Transitions Monitored: 413 → 369 (for quantification) and
413 → 219 (for confirmation)
Structure:



SURROGATE FIELD SPIKE COMPOUND

The surrogate field spiking compound is ^{13}C labeled perfluorooctanoic acid (^{13}C PFOA) and was purchased by 3M from Perkin-Elmer Life and Analytical Sciences.

^{13}C PFOA

Chemical Name: 1,2-13C perfluorooctanoic acid
Molecular Weight: 416
Lot Number: 3507-195
Purity: 97%
Transition Monitored: 415 → 370

OBJECTIVE

The purpose of this study is to perform analysis for perfluorooctanoic acid (PFOA) in water, soil, sediment, fish, clams, vegetation, small mammal livers and small mammal serum for the 3M Decatur Monitoring Program using the current versions of the following Exygen analytical methods:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"
V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"
V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"
V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"
V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"
V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"
V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

TESTING FACILITY

Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039

STUDY DIRECTOR

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PRINCIPAL INVESTIGATOR

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john.flaherty@exygen.com

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

It is proposed that the analytical portion of this study be conducted from October 01, 2004 to December 31, 2005. The actual experimental start and termination dates will be included in the final report.

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

- Water (groundwater and surface water)
- Soil
- Sediment
- Fish
- Clams
- Vegetation
- Small Mammal Liver
- Small Mammal Serum

The samples will be collected by Weston Solutions. The control samples will be purchased and prepared by the testing facility. Purchase and processing

details for the control samples will be included in the final report associated with this study.

The test systems were chosen to access the environmental impact of PFOA in the Decatur, Alabama area.

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

Water, soil, sediment, fish, clam, vegetation, small mammal liver and small mammal serum samples will be received at Exygen directly from Weston Solutions. The details of sample procurement for this study are outlined in the 3M work plan entitled "Phase 2 Work Plan for Sampling Environmental Media." The number and types of samples collected will vary depending availability in the field. The total number of samples received and analyzed for each matrix will be documented in the final report associated with this study.

Water, soil, and sediment samples will be used as received without further processing at Exygen. These samples will be stored refrigerated at 2°C-8°C. Fish, clam, vegetation and small mammal liver samples will be processed according to the appropriate analytical method (see Appendix I). These samples will be stored frozen at ≤ -10°C. Small mammal whole blood samples will be centrifuged in the field at the time of collection and the serum fraction will be used for the study. Small mammal serum will be stored frozen at ≤ -10°C.

The receipt and processing of the samples will be documented in the final report and raw data associated with the study.

SAMPLE IDENTIFICATION

Prior to analysis, each sample will be assigned a laboratory sample reference number. The reference number will be unique and will distinguish each laboratory sample that is processed throughout the analytical procedure. Chromatographic data will be identified by the laboratory sample reference number.

Sample storage conditions and locations will be documented throughout the study.

ANALYTICAL PROCEDURE SUMMARY

References:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"

V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"

V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"

V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"

V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"

V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"

V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

The above methods use analytical conditions capable of separating the isomers of PFOA. The final report will include the isomers summed into total PFOA found.

VERIFICATION OF ANALYTICAL PROCEDURE

A laboratory control sample will be used for the preparation of fortified control samples. The test substance will be made into solutions as per the method, and added to the matrices via a micropipette.

For water sampling, Exygen will supply one bottle per sample collected. The bottles will be 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottles. These bottles have been routinely used for PFOA sample collection at the testing facility and have been shown to be free of PFOA. All containers used for water sample collection will be shipped to the sample location containing 100 ng of ^{13}C PFOA (equivalent to 500 ng/L in the final sample). Samples will be added to each container to a volumetric fill line at 200 mL. A field duplicate, a low field spike and a high field spike of each sample will be collected. The low and high field spikes will contain PFOA as well as perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS). These compounds are included in the solutions used to spike the samples. The results for PFBS, PFHS and PFOS will not be reported in this study. Exygen will supply one field blank (control water) and two field blank spikes (control water fortified with PFOA at a low

and high level) for every twenty samples collected. At the testing facility, each water sample (excluding field duplicates and field spikes) will be extracted in duplicate and will also be fortified at a low and high concentration with PFOA and processed through the described procedure to determine method accuracy and to check for bias.

For soil, sediment, clams, and vegetation, Exygen will supply one 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottle per sample collected or a zip-seal bag. All containers/bags used for sample collection will be shipped to the sample location. Samples will be added to each container or bag in the field. At the testing facility, each sample will be extracted in duplicate and will also be fortified at a known concentration with PFOA at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias. ^{13}C-PFOA will also be added to each sample in the laboratory prior to extraction at the level specified below.

For small mammal liver, Exygen will supply a 50 mL polypropylene centrifuge tube. For small mammal serum, Exygen will supply a collection kit for each sample containing serum separator tubes (red top), vacutainers, needle holders and needles, transfer pipettes, and polypropylene tubes. At the testing facility, each liver and serum sample will be extracted in duplicate and will also be fortified at a known concentration with PFOA at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias. ^{13}C-PFOA will also be added to each sample in the laboratory prior to extraction at the level specified below.

Low and high spiking levels for each matrix are defined below:

Matrix	Low PFOA Spiking Level	High PFOA Spiking Level	^{13}C-PFOA Spiking Level
Water	500 ng/L	5000 ng/L	500 ng/L
Soil	4 ng/g	40 ng/g	40 ng/g
Sediment	4 ng/g	40 ng/g	40 ng/g
Fish	10 ng/g	100 ng/g	100 ng/g
Clams	10 ng/g	100 ng/g	100 ng/g
Vegetation	10 ng/g	100 ng/g	100 ng/g
Small Mammal Liver	10 ng/g	100 ng/g	100 ng/g
Small Mammal Serum	10 ng/g	100 ng/mL	100 ng/mL

Recoveries are anticipated to be between 70% and 130% of the fortified levels; however, the exact precision and accuracy will be determined by the analysis of the quality control samples described above. A statement of accuracy will be included in the final report.

Prior to sample analysis, a laboratory control spike study will be conducted in water to justify the use of ^{13}C PFOA results to establish precision and accuracy of PFOA samples. The following samples are to be prepared:

- Two control water blanks
- Two control water samples fortified at 250 ng/L with ^{13}C PFOA
- Two control water samples fortified at 250 ng/L with ^{13}C PFOA and at 250 ng/L with PFOA.
- Two control water samples fortified at 5000 ng/L with ^{13}C PFOA and at 5000 ng/L with PFOA.

The above samples are to be extracted and analyzed according to method V0001780 entitled "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS."

METHOD FOR CONTROL OF BIAS

Control of bias will be addressed by taking representative sub-samples from a homogeneous mixture of each matrix from untreated control samples, and by analyzing at least two levels of fortifications.

STATISTICAL METHODS

Statistics will be limited to those specified in the subject methods and to the calculation of average recoveries, as applicable.

GLP STATEMENT

All aspects of this study shall be performed and reported in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792. The final report or data package (supplied to the Sponsor) shall contain a statement that the study was conducted in compliance with current and applicable GLP standards and will outline any deviations in the study from those standards. This statement will be signed by the Study Director and Sponsor Representative.

REPORT

A final report will be prepared by the principal investigator or their designee at the conclusion of the study. The report will include, but will not be limited to, the following:

- The name and address of the Study Director, Sponsor Representative, and of the testing facility.
- A statement of GLP compliance (any related documentation, such as chain-of-custody records, must be in the study records).
- The signed and dated statement by the Exygen Research Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and Management.
- A description of the exact analytical conditions employed in the study. If the subject method was followed exactly, it is necessary to include only a copy of the analytical method. Any modifications to this method will be incorporated into the report. If the method is photo-reduced, the project number and page number must be included on each page.
- Description of the instrumentation used and operating conditions.
- All results from all sets analyzed. Control and fortified samples will be identified and the data table will include sample number and fortification level.
- Representative chromatograms for each analyte in each matrix, including chromatograms of a standard and a control sample, and a chromatogram at a fortification level. The location of the analyte peaks will be clearly identified in all chromatograms.
- All circumstances that may have affected the quality or integrity of the data will be documented in the report.
- Locations where raw data and the final report are to be archived.
- Additions or corrections to the final report shall be in the form of an amendment signed by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and the Sponsor Representative.

SAFETY AND HEALTH

- Laboratory personnel will practice good sanitation and health habits.
- Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test or reference substance(s).

AMENDMENTS TO PROTOCOL

All significant changes to the analytical protocol outlined here will be expressed in writing, signed and dated by the Study Director and Sponsor Representative. Amendments usually will be issued prior to initiation of study plan change. However, when a change is required without sufficient time for the issue of a written amendment, that change may be effected verbally with supporting documentation signed and dated by the Study Director and followed with a written amendment as soon as possible. In this case, the effective date of the written amendment will be the date of the documented change. Copies of the signed amendments will be appended to all distributed study plan copies. The original amendment will be maintained with the original study plan. Any deviations from the study plan or from the analytical method as provided will be documented and reported promptly to the Sponsor Representative.

DATA RECORD KEEPING

Records to be maintained include the following (as appropriate):

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

Chromatograms- All chromatograms will contain the following:

- Sample identification, injection date, arrow or other indication of the area of interest, and injection number corresponding to the run.
- Additionally, fortifications will include the amount of analyte added and the sample number of the sample that was fortified.
- Analytical standard chromatograms will additionally include the concentration (e.g., µg/mL).

- As part of the documentation the following sheets will be included in each analytical set: a run sheet listing the samples to be run in the set, and an instrument conditions sheet describing the instrument type and operating conditions.

- All applicable requirements for reporting of study results as per 40 CFR 792.185.

QUALITY ASSURANCE

The QA Unit of Exygen Research will inspect the study at intervals adequate to assure compliance with GLP's, and will report the findings of audits to the Study Director, Exygen Management, and the Sponsor Representative.

RETENTION OF DATA AND ARCHIVING

All hard copy raw data, including, but not limited to, the original chromatograms, worksheets, correspondence, and results shall be included with the data package submitted to the Study Director. These will be archived with the original study plan, amendments, final report, and all pertinent information from the Sponsor.

The testing facility shall keep all electronic raw data and any instrument, equipment, and storage logs for the period of time specified in 40 CFR 792.195. An exact copy of the materials submitted to the study director will also be kept at Exygen Research.

Exygen will obtain permission from the study director before discarding or returning samples.

APPENDIX I

ANALYTICAL METHODS

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"

V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"

V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"

V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"

V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"

V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"

V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001780

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

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Exygen Protocol Number: P0000760

Exygen Research Method Number V0001780

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in water.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 40 mL of test sample for extraction.
3.2 No sample processing is needed for water samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Any samples containing particles should be centrifuged at ~3000 rpm for ~5 minutes and the supernatant used for the extraction.
3.6 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Ammonium Acetate – A.C.S. Reagent Grade
4.4 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

5.12 SPE vacuum manifold.
5.13 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001780

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure, identical to samples.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ppb)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
10	200	40	50	XCmmddyy-2
10	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
100	200	40	500	XCmmddyy-5
100	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 8× its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

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9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards extracted.

9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using HPLC water) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Measure 40 mL of sample or a portion of sample diluted to 40 mL with water into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.3 Load sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.4 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001781

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001781

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in soil.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 15 g of test sample for extraction.
3.2 No sample processing is needed for soil samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Ammonium Acetate – A.C.S. Reagent Grade
4.4 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Ultrasonic bath.

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5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure, identical to samples.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ppb)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
10	200	40	50	XCmmddyy-2
10	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
100	200	40	500	XCmmddyy-5
100	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 8× its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards extracted.
9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.
9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.
10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes.
11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.
11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.
11.5 Centrifuge for ~10 minutes at ~3000 rpm.
11.6 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry
11.7 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.
11.8 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
11.9 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of

extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) *software system.*

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/L to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/L)} \times \text{volume extracted (0.04L)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation to calculate the amount of PFOA found in ppb based on dry weight.

PFOA found (ppb) dry weight = PFOA found (ppb) x [100% / total solids(%)]

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001782

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
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10/26/04
Date

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Vice President, Operations, Exygen Research

10/26/04
Date

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Exygen Protocol Number: P0000760

Exygen Research Method Number V0001782

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in sediment.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 30 g of test sample for extraction.
3.2 No sample processing is needed for sediment samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetic Acid – Reagent grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.

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5.13 Vortexer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 1% acetic acid in water is prepared by adding 10 mL of acetic acid to 1000 mL of water.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	10	100	10.0
100	5	100	5.0
100	2	100	2.0
10	10	100	1.0
5	10	100	0.5
2	10	100	0.2

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one *untreated control and two untreated controls fortified at known* concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 35 mL of 1% acetic acid, cap, vortex and shake on a wrist action shaker for ~60 minutes.

11.3 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.4 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.5 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.

11.6 Add 20 mL of methanol to the sediment left in the bottom of the 50 mL centrifuge tube. Cap, vortex and shake on a wrist action shaker for ~30 minutes.

11.7 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.8 Decant the methanol onto the same SPE cartridge. Collect the eluate.

11.9 Wash the column with 4 mL of methanol. Collect the eluate and add it to the eluate collected in step 11.8.

11.10 Condition a second C_{18} SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.11 Add the methanol to ~200 mL of water and load on the second conditioned SPE cartridge.

11.12 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.13 Analyze samples using electrospray LC/MS/MS.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001782

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

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

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation (if necessary) to calculate the amount of PFOA found in ppb based on dry weight.

PFOA found (ppb) dry weight = PFOA found (ppb) x [100% / total solids(%)] .

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001783

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

Analytical Testing Facility: Exygen Research
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Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 8

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in fish and clams.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 *Reagents and Standards*

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Superclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (*LC/MS/MS*).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

5.4 Rotary evaporator.
5.5 Tissumizer.
5.6 125 mL pear-shaped flasks.
5.7 50 mL disposable polypropylene centrifuge tubes.
5.8 15 mL disposable polypropylene centrifuge tubes.
5.9 Disposable micropipets (50-100uL, 100-200uL).
5.10 125-mL LDPE narrow-mouth bottles.
5.11 2 mL clear HPLC vial kit.
5.12 Disposable pipettes.
5.13 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.14 SPE tubes (20mL) (Supelco cat. no. N057177).
5.15 Wrist action shaker.
5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by *LC/MS/MS*

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Place the tubes in a freezer for ~1 hour.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.8 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat phase using a tissumizer for ~30 seconds and rinse the tissumizer with ~10 mL of acetonitrile into the tube.

11.10 Shake the sample again for ~10 minutes on a wrist-action shaker.

11.11 Place the tubes in a freezer for ~ 1 hour more.

11.12 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.13 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.

11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.

11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).

11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.

11.17 Transfer the extracts to HPLC vials using disposable pipets.

11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

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

14.2 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{\text{[PFOA found (ng/mL) x final volume (mL) x DF]}}{\text{sample weight (g)}}$$

DF = factor by which the final volume was diluted, if necessary.

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}]}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001784

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research
Date: 10/26/04

John Flaherty
Vice President, Operations, Exygen Research
Date: 10/26/04

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001784

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in vegetation.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Superclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

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5.4 Rotary evaporator.
5.5 125 mL pear-shaped flasks.
5.6 50 mL disposable polypropylene centrifuge tubes.
5.7 15 mL disposable polypropylene centrifuge tubes.
5.8 Disposable micropipets (50-100uL, 100-200uL).
5.9 125-mL LDPE narrow-mouth bottles.
5.10 2 mL clear HPLC vial kit.
5.11 Disposable pipettes.
5.12 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.13 SPE tubes (20mL) (Supelco cat. no. N057177).
5.14 Wrist action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

11.8 Add 20 mL of acetonitrile to the sample in the 50 mL centrifuge tube.
11.9 Shake the sample again for ~10 minutes on a wrist-action shaker.
11.10 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~5 minutes.
11.11 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.
11.12 Repeat steps 11.8 through 11.11 again.
11.13 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).
11.14 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.
11.15 Transfer the extracts to HPLC vials using disposable pipets.
11.16 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

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

14.2 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{\text{[PFOA found (ng/mL) x final volume (mL) x DF]}}{\text{sample weight (g)}}$$

DF = factor by which the final volume was diluted, if necessary.

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{\text{[total analyte found (ng/g) - analyte found in control (ng/g)]}}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001785

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research
Date: 10/26/04

John Flaherty
Vice President, Operations, Exygen Research
Date: 10/26/04

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal liver.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 5 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternately, if there is an insufficient amount of sample (~less than 5 g), then no processing is necessary and the sample can be used as supplied.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.

5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Tissuemizer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 1 g of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate weights of liver may be measured depending on the sample size available for use.

11.2 Add water to the sample for a final volume of 10 mL.

11.3 Homogenize sample using a tissuemizer for ~1 minute.

11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.

11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.

11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.

11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry

11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

Page 5 of 7

versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/g, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 10

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample weight (g)}}$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001786

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

Analytical Testing Facility: Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001786

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal serum.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 1 mL of test sample for extraction.
3.2 No sample processing is needed for serum samples. However, frozen serum samples must to allowed to completely thaw to room temperature before use.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Vortexer.

Page 2 of 7

5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 *Chromatographic System*

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 *MS/MS System*

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001786

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

11.0 Sample Extraction

11.1 Measure 1 mL of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate volumes of serum may be measured depending on the sample size available for use.
11.2 Add water to the sample for a final volume of 20 mL. Cap tightly
11.3 Vortex for ~1 minute.
11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.
11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.
11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry
11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.
11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).
11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001786

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 20

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

Page 6 of 7

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ppb.

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample volume (mL)}}$$

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PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 01/19/05
Exygen Study Number: P0000760 Client Study Number: None
Page 1 of 1

DESCRIPTION OF AMENDED SECTION

1) Analytical Procedure Summary V0001780:Section 9.1
2) Verification of Analytical Procedure

AMENDED TO

1) Add to Section 9.1: Section 9.1.6, Alternate weights of standards may be used to prepare alternate concentrations of stock solutions as necessary. Alternate levels of fortification solutions may also be prepared.
2) Low and high spiking levels of the analytes for each matrix may be altered depending on sample size available for extraction and/or to cover analyte concentrations expected in the samples.

RATIONALE

1) Higher concentrations of standards need to be prepared in order to spike the sample bottles at higher levels.
2) The sample size available for small mammal liver and serum was smaller than expected. Spiking at the pre-determined levels in the protocol puts the spiked concentration lower than the detection limit. Also, the analyte levels in the ground water samples are expected to greatly exceed the pre-determined spiking levels listed in the protocol. When the levels in the samples greatly exceed the spiking levels, an accurate recovery value cannot be calculated for the QC sample. Higher spiking levels in the bottles will cover the analyte concentrations expected in the water samples.

IMPACT ON STUDY

The LOQ is 100 ng/g for a 0.1 g sample of small mammal liver and is 1000 ng/mL for a 0.01 mL sample of small mammal serum.
Higher levels of spiking for the water samples will ensure that more QC recovery data can be used.

Study Director Signature	Date 3/9/05
Principal Investigator Signature	Date 2/2/05
Study Director Management Signature	Date NA
Sponsor Signature (if required)	Date 3/10/05

Exygen QAU Review LJS 02/01/05

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PROTOCOL AMENDMENT

Amendment Number: 2 Page 1 of 1
Effective Date: 03/07/05
Exygen Study Number P0000760 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

Report, page 11 of 65

AMENDED TO

Instead of one final report, interim reports will be issued.

RATIONALE

Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timelier ~~manor~~ manner. 03/09/05 mcc

IMPACT ON STUDY

The client will be able to receive and review the data more quickly.

Study Director Signature	Date 3/9/05
Principal Investigator Signature	Date 3/8/05
Study Director Management Signature N/A	Date —
Exygen Management Signature	Date 8-MAR-05
Sponsor Signature (if required)	Date 3/10/05

Exygen QAU Review LJS 03/08/05

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PROTOCOL AMENDMENT

Page 1 of 1

Amendment Number: 3
Effective Date: 04/13/05
Exygen Study Number P760
Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

Analytical Procedure Summary: V0001780:"Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS." Sections 9.2 and 11.0 of the method.

AMENDED TO

Section 9.2. Non-extracted calibration standards may be prepared in order to quantitate samples directly injected onto the LCMSMS system.
Section 11.0. Samples may be analyzed without going through the extraction procedure. Instead, they may be diluted, if appropriate, and directly injected onto the LCMSMS system for analysis.

RATIONALE

Some extracted results between laboratory and field spikes were not in agreement and/or were unusable. Directly injecting the extracts may add some insight into the matrix effects on the data results.

IMPACT ON STUDY

More usable data results may be obtained.

Study Director Signature	Date 6/02/05
Principal Investigator Signature	Date 6/2/05
Study Director Management Signature N/A	Date N/A
Exygen Management Signature	Date 2-JUN-05
Sponsor Signature (if required)	Date 06/02/05

Exygen QAU Review 4/21/05

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PROTOCOL AMENDMENT

Page 1 of 1

Amendment Number: 4
Effective Date: 07/18/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

Verification of Analytical Procedure, page 10 of protocol.

AMENDED TO

The field duplicate can be used for the laboratory spikes and replicate when the primary sample volume is limited.

RATIONALE

The sample size for a water sample is 200 mL. If a sample site requires re-extraction for any reason, there would not be enough of the primary sample to repeat two laboratory spikes and a replicate. The field duplicate is technically the same sample as the primary sample and therefore, can be used for laboratory spikes and replicates as needed.

IMPACT ON STUDY

No negative impact on the study. Using the duplicate sample allows for the full QC of the sample site to be completed.

Study Director Signature — Date 7/25/05

Principal Investigator Signature — Date 7/26/05

NA
Study Director Management Signature — Date

Exygen Management Signature — Date 26 JUL 05

Sponsor Signature (if required) — Date 7/25/05

Exygen QAU Review 7/22/05

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PROTOCOL AMENDMENT

Amendment Number: 5
Effective Date: 09/13/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

Analytical Procedure Summary: V0001780:"Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS."

AMENDED TO

The reanalysis of two water samples, DF09-GW-131R-0-040126 and DFB-GW-135L-0-040121 will be performed using the following procedure:
An aliquot of the water sample is spiked with the surrogate standard and/or other matrix spike compounds as appropriate. Add methanol to the fortified aqueous sample. Mix and aliquot for LC/MS/MS analysis. The LC conditions include the use of a Betasil C18, 2.1 x 50 mm column, a gradient of 30% B to 100% B in 10 minutes, where A=2mM ammonium acetate in water and B=Methanol (6 min equilibration). Use a 5 µL injection volume and a 0.3 mL/min flow rate. LC conditions may be further modified as appropriate for the system being used. An interim report will be issued that will include the exact details of the reanalysis.

RATIONALE

DF09-GW-131R-0-040126 and DF8b-GW-135L-0-040121 could not be reported quantitatively in interim report #3 due to quality control sample failures. The study director and sponsor have requested reanalysis using the above conditions.

IMPACT ON STUDY

No negative impact on the study. More usable data may be obtained.

Study Director Signature	Date
Principal Investigator Signature	Date 9/14/05
N/A Study Director Management Signature	Date –
Exygen Management Signature	Date 14-Sep-05
Sponsor Signature (if required)	Date

Exygen QAU Review m.w 9/13/05

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PROTOCOL AMENDMENT

Amendment Number: 5
Effective Date: 09/13/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

Analytical Procedure Summary: V0001760: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS."

AMENDED TO

The reanalysis of two water samples, DF09-GW-131R-0-040126 and DF8-GW-135L-0-040121 will be performed using the following procedure:
An aliquot of the water sample is spiked with the surrogate standard and/or other matrix spike compounds as appropriate. Add methanol to the fortified aqueous sample. Mix and aliquot for LC/MS/MS analysis. The LC conditions include the use of a Betasil C18, 2.1 x 50 mm column, a gradient of 30% B to 100% B in 10 minutes, where A=2mM ammonium acetate in water and B=Methanol (6 min equilibration). Use a 5 µL injection volume and a 0.3 mL/min flow rate. LC conditions may be further modified as appropriate for the system being used. An interim report will be issued that will include the exact details of the reanalysis.

RATIONALE

DF09-GW-131R-0-040126 and DF8b-GW-135L-0-040121 could not be reported quantitatively in interim report #3 due to quality control sample failures. The study director and sponsor have requested reanalysis using the above conditions.

IMPACT ON STUDY

No negative impact on the study. More usable data may be obtained.

Study Director Signature _____ Date _____

Principal Investigator Signature _____ Date _____

Study Director Management Signature _____ Date _____

Exygen Management Signature _____ Date _____

Sponsor Signature (if required) _____ Date 9/14/05

Exygen QAU Review _____ 9/13/05

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PROTOCOL AMENDMENT

Amendment Number: 5 Page 1 of 1
Effective Date: 09/13/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

Analytical Procedure Summary: V0001780:"Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS."

AMENDED TO

The reanalysis of two water samples, DF09-GW-131R-0-040126 and DFB-GW-135L-0-040121 will be performed using the following procedure:
An aliquot of the water sample is spiked with the surrogate standard and/or other matrix spike compounds as appropriate. Add methanol to the fortified aqueous sample. Mix and aliquot for LC/MS/MS analysis. The LC conditions include the use of a Betasil C18, 2.1 x 50 mm column, a gradient of 30% B to 100% B in 10 minutes, where A=2mM ammonium acetate in water and B=Methanol (6 min equilibration). Use a 5 µL injection volume and a 0.3 mL/min flow rate. LC conditions may be further modified as appropriate for the system being used. An interim report will be issued that will include the exact details of the reanalysis.

RATIONALE

DF09-GW-131R-0-040126 and DF8b-GW-135L-0-040121 could not be reported quantitatively in interim report #3 due to quality control sample failures. The study director and sponsor have requested reanalysis using the above conditions.

IMPACT ON STUDY

No negative impact on the study. More usable data may be obtained.

Study Director Signature — Date 9/14/05

Principal Investigator Signature — Date

Study Director Management Signature — Date

Exygen Management Signature — Date

Sponsor Signature (if required) — Date

Exygen QAU Review mw 9/13/05

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PROTOCOL AMENDMENT

Amendment Number: 6 Page 1 of 1
Effective Date: 09/20/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

The identification of the two water samples listed in protocol amendment No 5 are incorrect.

AMENDED TO

The two water samples for reanalysis should be DF09-GW-131R-0-040120 and DF8b-GW-135R-0-040121.

RATIONALE

The correct samples are identified for reanalysis.

IMPACT ON STUDY

No negative impact on the study.

Study Director Signature — Date

Principal Investigator Signature — Date 9/21/05

NA
Study Director Management Signature — Date —

Exygen Management Signature — Date 23-SEP-05

Sponsor Signature (if required) — Date

Exygen QAU Review 9/20/05

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PROTOCOL AMENDMENT

Amendment Number: 6
Effective Date: 09/20/05
Exygen Study Number P760 Client Study Number: NA

DESCRIPTION OF AMENDED SECTION

The identification of the two water samples listed in protocol amendment No 5 are incorrect.

AMENDED TO

The two water samples for reanalysis should be DF09-GW-131R-0-040120 and DF8b-GW-135R-0-040121.

RATIONALE

The correct samples are identified for reanalysis.

IMPACT ON STUDY

No negative impact on the study.

Study Director Signature — Date

Principal Investigator Signature — Date

NA

Study Director Management Signature — Date —

Exygen Management Signature — Date

Sponsor Signature (if required) — Date 9/20/05

Exygen QAU Review 9/20/05

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