AR226_3689.

INTERIM REPORT # 10 - Analysis of Fish and Clam Samples

STUDY TITLE

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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

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INTERIM REPORT COMPLETION DATE

May 09, 2006

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PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 132

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number P0001131, entitled "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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.

John Flaherty
Principal Investigator
Exygen Research

5/9/06
Date

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

5/10/06
Date

Michael A. Santoro
Sponsor Representative
3M Company

5/12/06
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number P0001131, entitled, "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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
18. Raw Data Review and Interim Analytical Report Review	08/01-05/05	09/20/05	09/21/05	09/21/05
26. Raw Data Review and Interim Analytical Report Review	05/03/06	05/08/06	05/09/06	05/09/06

Lydia Shaffer
Technical Lead, Quality Assurance Unit

05/09/06
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 P0001131, is a true and complete representation of the raw data.

Submitted by: Exygen Research
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STUDY IDENTIFICATION

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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:	P0001131
EXYGEN STUDY NUMBER:	P0001131
TYPE OF STUDY:	Residue
SAMPLE MATRIX:	Fish and Clams
TEST SUBSTANCE:	Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS)
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
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PERFORMING LABORATORY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 11/05/04 Interim Analytical Start Date: 12/27/04 Interim Analytical Termination Date: 04/17/06 Interim Report Completion Date: 05/09/06

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
Paul Connolly	Technical Lead-LC/MS
Amy Sheehan	Associate Scientist
Christine Edwards	Technician
Brittany Kravets	Technician
Mindy Cressley	Technician
Krista Gallant	Technician
Scott Crain	Technician
Eric Hoover	Technician
Carlyle Horrell	Intern Technician
Shawn Robb	Technical Lead - Facilities
Mark Ammerman	Sample Custodian
Eric Edwards	Sample Custodian
Edward Carns	Sample Custodian

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1.0 SUMMARY

Exygen Research extracted and analyzed fish and clam samples for the determination of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) according to Exygen Method V0001783 (**Appendix A**).

The limit of quantitation (LOQ) for PFBS, PFHS, and PFOS in fish and clams was 0.2 ng/g. For one sample (C0048133) only one gram of fish was used for extraction, so the limit of quantitation for PFBS, PFHS, and PFOS in this fish sample was 1.0 ng/g.

The initial analytical results associated with fifty-one fish samples are not reported (NR) in Table I because the endogenous levels of PFOS were too high to calculate accurate matrix spike recoveries. These samples were re-extracted and the results of the re-analyses are summarized in **Tables II and V**.

Analytical results and assessed accuracies for the analysis of PFBS, PFHS and PFOS found in fish samples are summarized in **Table I.** Analytical results and assessed accuracies for the analysis of PFOS found in the re-extracted fish samples are summarized in **Table II.** Analytical results and assessed accuracies for the analysis of PFBS, PFHS and PFOS found in clam samples are summarized in **Table III.** Laboratory matrix spikes that were in the appropriate concentration range (primary concentration less than three times the spiking level) were used to calculate the assessed accuracies.

The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in fish samples were 79% ± 13%, 94% ± 12%, and 85% ± 20%, respectively. Fortification recoveries for PFBS, PFHS, and PFOS in fish samples are summarized in **Table IV.** The average percent recovery ± standard deviation for PFOS in the re-extracted fish samples was 109% ± 20%. Fortification recoveries for PFOS in the re-extracted fish samples are summarized in **Table V.** The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in clam samples were 84% ± 10%, 93% ± 13%, and 73% ± 16%, respectively. Fortification recoveries for PFBS, PFHS, and PFOS in clam samples are summarized in **Table VI.**

The sample IDs assigned by the client follow this formula: Dxx-x01-xxxxxx-x-04xxxx. The first string begins with D for Decatur, Alabama and the xx defines the sampling area within the Wheeler Reservoir of the Tennessee River where:
DL3 = Up river Letter of Intent (LOI) sampling location LOC-3
DL2 = Cross river LOI sampling location LOC-2
DL1 = Downstream Fox Creek mouth LOI sampling location LOC-1
DBC = Bakers Creek mouth
DOU = Cove at 3M WWTP outfall
DMC = Downstream Mallard Creek mouth

The second string defines the general category of the biota sample and the sampling round where:
F01 = Fish sample first round
I01 = Invertebrate sample first round

The third string defines the species (first two characters), the sample type (third character), and the sample number (last three characters) within that sampling area and type of sample where:
IP = channel catfish (*Ictalurus punctatus*)
MS = largemouth bass (*Micropterus salmoides*)
CF = Asiatic clam (*Corbicula fluminea*)

F = Filet tissue sample
W = Whole body sample

001 through 005 = sample number within the sampling area and sample type

The fourth string (single character) additionally defines the sample type where:
0 = Primary field sample
1 = Field duplicate sample
2 = Equipment rinseate blank sample

The fifth string defines the sample preparation date in YYMMDD format.

Examples of this sample ID system are:

Sample DBC-F01-IPF004-0-041021 is the filet tissue sample prepared on October 21, 2004 from the fourth channel catfish collected at the Bakers Creek mouth sampling location.

Sample DL1-F01-MSW002-0-041104 is the whole body sample prepared on November 4, 2004 from the second largemouth bass collected at the Fox Creek mouth sampling location.

2.0 OBJECTIVE

The objective of the analytical part of this study was to determine levels of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) in fish and clams according to Protocol P0001131 (**Appendix A**).

3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFBS, PFHS, and PFOS in fish and clams using the analytical methods entitled, "V0001783: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS."

The study was initiated on November 05, 2004, when the study director signed protocol number P0001131. The analytical start date for this interim report was December 27, 2004, and the analytical termination date for this interim report was April 17, 2006.

4.0 ANALYTICAL TEST SAMPLES

Forty fish samples (Exygen ID C0048126-C0048165) were received on dry ice on October 23, 2004 from Tim Frinak at Weston Solutions, Inc. Fifty-six fish samples (Exygen ID C0049450-C0049505) were received on dry ice on November 6, 2004 from Charles Young at Weston Solutions, Inc. Twenty-six fish samples (Exygen ID C0056354-C0056479) were received on dry ice on January 13, 2005 from Tim Frinak at Weston Solutions, Inc. Six clam samples, consisting of whole clams from each sample site (Exygen ID C0056480-C0056485) were received on dry ice on January 13, 2005 from Tim Frinak at Weston Solutions, Inc. A whole Whiting (Family: Gadidae) was purchased from a local grocer by Ed Carns on December 20, 2004 and was used as the control fish (Exygen ID C0054391). The samples were logged in by Exygen personnel and placed in frozen storage.

Sample login 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 standards, PFBS and PFHS, were supplied by 3M. PFBS was received from 3M at Exygen on July 6, 2000, and May, 13, 2005. PFHS was received from 3M at Exygen on January, 20, 2003. PFOS was purchased from Fluka Corporation and was received at Exygen on April, 23, 2003.

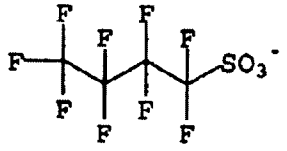
The available information for the reference materials is listed below. PFBS and PFHS were stored frozen and PFOS was stored refrigerated.

Compound	Exygen Inventory No.	Lot #	Purity (%)	Expiration Date
PFBS	SP0000252	101	96.7	12/04/06
PFBS	SP0005726	101	96.7	12/04/06
PFHS	SP0002401	SE036	98.6	10/18/06
PFOS	SP0002694	430180-1	101.2	10/31/07

The molecular structures of PFBS, PFHS and PFOS are given below:

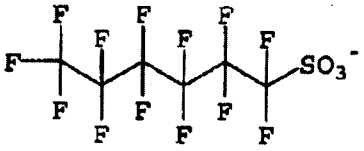
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Transitions Monitored: 299 → 99
Structure:



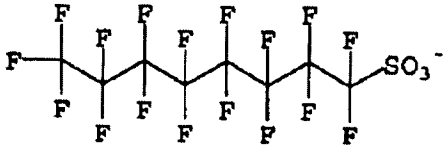
PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Transitions Monitored: 399 → 80
Structure:



PFOS

Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Transitions Monitored: 499 → 80
Structure:



6.0 DESCRIPTION OF ANALYTICAL METHOD

The analytical method "V0001783: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS" was used for the fish and clam samples in this study.

6.1 Extraction Procedure

6.1.1 Sample Preparation

Before the samples could be weighed for the extraction, they had to be processed. To process, the frozen samples were placed into a food processor and homogenized with dry ice. The samples were transferred to one-gallon Ziploc bags and placed in frozen storage with bag left open to allow the dry ice to sublime. After sublimation, the sample bags were sealed and remained in frozen storage until time of analysis. Sample processing records are located in the Sample Information section of the raw data package.

6.1.2 Glassware Preparation

The 125 mL pear-shaped flasks were silanized by first rinsing the flask with 30% dimethyldichlorosilane in toluene solution. The flasks were rinsed once with toluene followed by three rinses with methanol. The flasks were allowed to air dry before use.

6.1.3 Column Preparation

The 20 mL columns were packed at Exygen in sequence with 2 grams florisil, 2 grams silica gel, 2 grams of carbon, and 1 gram LC-NH_2. The columns were conditioned on the day of extraction with 20 mL of methanol followed by 20 mL of acetonitrile. All washes were discarded. These clean-up columns were used to remove matrix interference and not to retain PFBS, PFHS, and PFOS. Additional details about the column packing materials can be found in the raw data package associated with this report.

6.1.4 Sample Extraction

A 5-gram* portion of fish or clam sample was weighed into a fifty-milliliter centrifuge tube for the extraction. After fortification of appropriate samples, 30 mL of acetonitrile was added to the samples. The samples were allowed to shake on a wrist action shaker for ~15 minutes. The samples were placed into a freezer for ~1 hour. Freezing of the

samples allowed the fats and proteins to precipitate. The samples were centrifuged for ~10 minutes at ~2000 rpm. The supernatant was then loaded onto the conditioned clean-up column fitted inside the silanized pear shaped flask. The eluate was collected in the silanized pear-shaped flask. This process removed the fats and proteins from the extract. Ten milliliters of acetonitrile was added to the samples left in the centrifuge tube. The remaining sample in the tube was homogenized using a tissumizer for ~30 seconds. The tissumizer was rinsed with ten milliliters of acetonitrile. The rinse was added to the sample tube. The samples were allowed to shake on a wrist action shaker for another 10 minutes. The samples were placed into a freezer for ~1 hour. The samples were centrifuged again for ~10 minutes at ~2000 rpm. The supernatant was then loaded onto the same clean-up column. The eluate was collected into the same pear-shaped flask. The column was washed with 20 mL of acetonitrile, collecting the wash in the pear-shaped flask. Approximately 3 or 4 drops of 1-octanol was added to the extracts in the flasks. The samples were evaporated using a rotary evaporator at reduced pressure. The 1-octanol held the analytes in the pear-shaped flask while the solvent evaporated. Two milliliters of 2% ascorbic acid in methanol was added to the flasks to make final volume. The flask was swirled to dissolve and mix the sample. The sample was transferred to a HPLC vial using a disposable pipet. Each sample was analyzed by LC/MS/MS electrospray.

*Due to lack of sample, a one-gram portion of sample C0048133 (DMC-F01-MSW001-0-041216) was used. All other samples were weighed out in 5-gram portions.

6.2 Preparation of Standards and Fortification Solutions

Individual stock standard solutions of PFBS, PFHS and PFOS were prepared as specified in Exygen method V0001783. The stock standard solutions were prepared at a concentration of 100 µg/mL by dissolving 10 mg of each of the standards (corrected for purity and salt content) in methanol. From these solutions, mixed 1.0 µg/mL fortification standard solutions were prepared by taking 1 mL of each of the appropriate stock solutions and bringing the volume up to 100 mL with methanol. By taking 10 mL of the mixed 1.0 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a mixed 0.1 µg/mL fortification standard was prepared.

A mixed stock standard solution of PFBS, PFHS, and PFOS was also prepared. The stock standard solution was prepared at a concentration of 1000 µg/mL by dissolving 100 mg of each of the standards (corrected for purity and salt content, if necessary) in methanol. From this solution, a 100 µg/mL fortification standard solution was prepared by taking 10 mL of the stock and bringing the volume up to 100 mL with methanol. By taking 10 mL of the 100 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a 10 µg/mL fortification standard was prepared. By taking 10 mL of the 10 µg/mL fortification standard and bringing the volume up to 100 mL with

methanol, a 1.0 µg/mL fortification standard were prepared. By taking 10 mL of the 1.0 µg/mL fortification standard and bringing the volume up to 100 mL with methanol, a 0.1 µg/mL fortification standard was prepared.

A set of non-extracted standards containing PFBS, PFHS and PFOS was prepared in methanol. The following concentrations were prepared:

Conc. of Fort Solution (µg/mL)[1]	Fort Volume (mL)	Volume of Fortified Sample (mL)	Final Conc. of Calibration Std. (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
0.01	10	100	0.001
0.005	10	100	0.0005

[1] of PFBS, PFHS and PFOS

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

6.3 Chromatography

Quantification of PFBS, PFHS and PFOS was accomplished by LC/MS/MS electrospray. The retention times of PFBS, PFHS and PFOS were ~ 4.7 mins, ~ 10.8 mins, and ~ 12.9 mins, respectively.

6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.0005 µg/mL of PFBS, PFHS and PFOS.

6.5 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)
PFBS	negative	299 → 99	~4.7 min.
PFHS	negative	399 → 80	~10.8 min.
PFOS	negative	499 → 80	~12.9 min.

6.6 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 following equations.
Equation 1 calculated the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

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

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

Equation 2 was used to convert the amount of PFBS, PFHS and PFOS found in ng/mL to ng/g (ppb).

Equation 2:

$$\text{Analyte found (ppb, wet weight)} = \frac{[\text{Analyte found (ng/mL)} \times \text{final volume (2 mL)}]}{\text{sample weight (5 g)}}$$

For samples fortified with known amounts of PFBS, PFHS and PFOS prior to extraction, Equation 3 was used to calculate the percent recovery.

Equation 3:

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

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

An example of a calculation using an actual sample follows (calculation is for PFBS only):

Fish sample Exygen ID: C0049478 Spk E (Set: 061605E), fortified at 10 ng/g with where:

peak area	=	280750
intercept	=	3070
slope	=	13100
dilution factor	=	1
ng/g PFBS added (fort level)	=	10
amt in corresponding sample	=	NQ (not quantifiable)
final volume (mL)	=	2
sample weight (g)	=	5

From equation 1:

$$\text{Analyte found (ng/mL, wet weight)} = \frac{[280750 - 3070] \times 1}{13100}$$

$$= 21.2 \text{ ng/mL}$$

From equation 2:

$$\text{Analyte found ng/g (ppb)} = \frac{(21.2 \text{ ng/mL} \times 2 \text{ mL})}{5 \text{ g}}$$

$$= 8.48 \text{ ng/g (ppb)}$$

From equation 3:

$$\% \text{ Recovery} = \frac{(8.48 \text{ ng/g} - 0 \text{ ng/g})}{10 \text{ ng/g}} \times 100\%$$

$$= 85\ \%$$

7.0 EXPERIMENTAL DESIGN

For fish and clam samples designated as laboratory matrix spikes, PFBS, PFHS, and PFOS were added at a known concentration to the samples in the laboratory after the samples were weighed for extraction.

The fish samples were extracted in thirty sets, five that were re-extraction sets. The clam samples were extracted in one set. Each fish or clam set included one control fish matrix blank and two control fish matrix blanks fortified at known concentrations. Two sets of fish contained three samples, one set of fish contained four samples, one set of fish contained seven samples, and twenty-one sets of fish contained five samples. Three sets of re-extracted fish contained ten samples each. One set of re-extracted fish contained twelve samples. One set of re-extracted fish contained nine samples. One set of clams contained six samples.

Accuracies were assessed for each sample by reviewing the individual QC results obtained for each sample.

8.0 RESULTS

Analytical results and assessed accuracies for the analysis of PFBS, PFHS and PFOS found in fish samples are summarized in **Table I.** Please note that the chromatography for PFBS was poor for this matrix. Exygen recognizes this and great care has been taken to integrate each data set consistently. Analytical results and assessed accuracies for the analysis of PFOS found in the re-extracted fish samples are summarized in **Table II.** Analytical results and assessed accuracies for the analysis of PFBS, PFHS and PFOS found in clam samples are summarized in **Table III.**

The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in fish samples were 79% ± 13%, 94% ± 12%, and 85% ± 20%, respectively. Fortification recoveries for PFBS, PFHS, and PFOS in fish samples are summarized in **Table IV.** The average percent recovery ± standard deviation for PFOS in the re-extracted fish samples was 109% ± 20%. Fortification recoveries for PFOS in the re-extracted fish samples are summarized in **Table V.** The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in clam samples were 84% ± 10%, 93% ± 13%, and 73% ± 16%, respectively. Fortification recoveries for PFBS, PFHS, and PFOS in clam samples are summarized in **Table VI.**

9.0 CONCLUSIONS

The fish and clam samples were successfully extracted and analyzed for PFBS, PFHS and PFOS according to analytical method V0001783.

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 final 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 PFBS, PFHS and PFOS in Fish Samples

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate Analyte Found (ng/g) wet wt.	C4 Sulfonate PFBS Assessed Accuracy (+/- %)	C6 Sulfonate PFHS Perfluorohexanesulfonate Analyte Found (ng/g) wet wt.	C6 Sulfonate PFHS Assessed Accuracy (+/- %)	C8 Sulfonate PFOS Perfluorooctanesulfonate Analyte Found (ng/g) wet wt.	C8 Sulfonate PFOS Assessed Accuracy (+/- %)
C0048126	DL3-F01-IPF001-0-041021	0.309	40	ND	30	5.16	30
C0048126 Rep	DL3-F01-IPF001-0-041021*	0.318	40	ND	30	5.64	30
C0048127	DL3-F01-IPF002-0-041021	0.266	40	ND	30	4.16	40
C0048127 Rep	DL3-F01-IPF002-0-041021*	0.382	40	ND	30	4.56	40
C0048128	DMC-F01-IPF001-0-041021	0.330	50	ND	50	7.40	50
C0048128 Rep	DMC-F01-IPF001-0-041021*	0.366	50	ND	50	8.36	50
C0048129	DMC-F01-IPF002-0-041021	0.712	30	ND	30	14.1	50
C0048129 Rep	DMC-F01-IPF002-0-041021*	0.792	30	ND	30	17.0	50
C0048130	DMC-F01-IPW001-0-041021	1.08	40	ND	30	70.0	40
C0048130 Rep	DMC-F01-IPW001-0-041021*	1.06	40	0.297	30	56.4	40
C0048131	DMC-F01-IPW002-0-041021	0.968	50	ND	30	34.4	30
C0048131 Rep	DMC-F01-IPW002-0-041021*	1.34	50	ND	30	34.2	30
C0048132	DMC-F01-MSW001-0-041021	0.218	30	0.524	30	270	50
C0048132 Rep	DMC-F01-MSW001-0-041021*	0.202	30	0.484	30	280	30
C0048133^	DMC-F01-MSW002-0-041021	ND^	30	ND^	30	530	30
C0048133 Rep^	DMC-F01-MSW002-0-041021*	ND^	30	ND^	30	546	30
C0048134	DL2-F01-IPF001-0-041021	0.660	50	ND	40	3.45	50
C0048134 Rep	DL2-F01-IPF001-0-041021*	1.00	50	ND	40	2.86	50
C0048135	DL2-F01-IPF002-0-041021	0.816	50	ND	40	4.72	50
C0048135 Rep	DL2-F01-IPF002-0-041021*	0.952	50	ND	40	5.04	50
C0048136	DL2-F01-IPF003-0-041021	0.247	30	ND	30	2.01	30
C0048136 Rep	DL2-F01-IPF003-0-041021*	0.216	30	ND	30	2.06	30
C0048137	DL2-F01-IPF004-0-041021	0.346	40	ND	30	4.52	40
C0048137 Rep	DL2-F01-IPF004-0-041021*	0.330	40	ND	30	4.12	40
C0048138	DL2-F01-IPF005-0-041021	0.338	40	ND	30	2.48	30
C0048138 Rep	DL2-F01-IPF005-0-041021*	0.318	40	ND	30	2.60	30
C0048139	DL2-F01-IPW001-0-041021	0.328	30	ND	30	5.86	30
C0048139 Rep	DL2-F01-IPW001-0-041021*	0.400	30	ND	30	6.12	30
C0048140	DL2-F01-MSF001-0-041021	0.333	30	ND	30	77.2	30
C0048140 Rep	DL2-F01-MSF001-0-041021*	0.348	30	ND	30	67.6	30
C0048141	DL2-F01-MSF002-0-041021	0.406	40	ND	30	94.8	40
C0048141 Rep	DL2-F01-MSF002-0-041021*	0.398	40	ND	30	98.4	40
C0048142	DL2-F01-MSF003-0-041021	0.416	40	ND	40	41.6	40
C0048142 Rep	DL2-F01-MSF003-0-041021*	0.376	40	ND	40	40.0	40
C0048143	DL2-F01-MSF004-0-041021	0.345	30	ND	30	83.6	30
C0048143 Rep	DL2-F01-MSF004-0-041021*	0.349	30	ND	30	83.2	30

*Laboratory Duplicate
^ 1.0 g of sample used for extraction instead of 5.0 g.
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
ND^ = Not detected at or above Limit of Quantitation which is 1.0 ng/g (wet weight).
NR = Not reported due to quality control result failures.

Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate Analyte Found (ng/g) wet wt.	C4 Sulfonate PFBS Assessed Accuracy (+/- %)	C6 Sulfonate PFHS Perfluorohexanesulfonate Analyte Found (ng/g) wet wt.	C6 Sulfonate PFHS Assessed Accuracy (+/- %)	C8 Sulfonate PFOS Perfluorooctanesulfonate Analyte Found (ng/g) wet wt.	C8 Sulfonate PFOS Assessed Accuracy (+/- %)
C0048144	DL1-F01-IPF001-0-041021	0.432	30	0.261	30	33.4	40
C0048144 Rep	DL1-F01-IPF001-0-041021*	0.428	30	0.252	30	32.6	40
C0048145	DL3-F01-IPF003-0-041021	0.448	30	ND	30	3.55	30
C0048145 Rep	DL3-F01-IPF003-0-041021*	0.440	30	0.229	30	3.35	30
C0048146	DL3-F01-IPF004-0-041021	0.784	40	ND	30	0.382	30
C0048146 Rep	DL3-F01-IPF004-0-041021*	0.900	40	ND	30	0.230	30
C0048147	DL3-F01-IPF005-0-041021	1.53	60	ND	30	5.48	40
C0048147 Rep	DL3-F01-IPF005-0-041021*	1.07	60	ND	30	5.64	40
C0048148	DBC-F01-IPF001-0-041021	1.34	50	0.692	30	NR	NR
C0048148 Rep	DBC-F01-IPF001-0-041021*	1.36	50	0.580	30	NR	NR
C0048149	DBC-F01-IPF002-0-041021	1.43	50	ND	30	110	30
C0048149 Rep	DBC-F01-IPF002-0-041021*	0.756	50	ND	30	109	30
C0048150	DBC-F01-IPF003-0-041021	0.996	50	0.844	30	NR	NR
C0048150 Rep	DBC-F01-IPF003-0-041021*	0.952	50	0.824	30	NR	NR
C0048151	DBC-F01-IPW001-0-041021	0.864	40	2.21	30	NR	NR
C0048151 Rep	DBC-F01-IPW001-0-041021*	0.508	40	2.20	30	NR	NR
C0048152	DBC-F01-IPW002-0-041021	0.700	40	9.12	30	NR	NR
C0048152 Rep	DBC-F01-IPW002-0-041021*	0.736	40	8.96	30	NR	NR
C0048153	DBC-F01-IPW003-0-041021	0.736	30	6.76	30	NR	NR
C0048153 Rep	DBC-F01-IPW003-0-041021*	0.760	30	7.44	30	NR	NR
C0048154	DOU-F01-IPF001-0-041021	4.36	40	1.02	30	NR	NR
C0048154 Rep	DOU-F01-IPF001-0-041021*	4.24	40	0.972	30	NR	NR
C0048156	DOU-F01-IPF002-0-041021	6.76	40	0.792	30	NR	NR
C0048156 Rep	DOU-F01-IPF002-0-041021*	8.28	40	0.720	30	NR	NR
C0048158	DOU-F01-IPF003-0-041021	5.88	40	1.02	30	NR	NR
C0048158 Rep	DOU-F01-IPF003-0-041021*	5.96	40	1.02	30	NR	NR
C0048157	DOU-F01-IPF004-0-041021	6.12	30	2.30	30	NR	NR
C0048157 Rep	DOU-F01-IPF004-0-041021*	6.28	30	2.38	30	NR	NR
C0048158	DOU-F01-IPF005-0-041021	4.56	30	0.956	30	NR	NR
C0048158 Rep	DOU-F01-IPF005-0-041021*	4.36	30	0.892	30	NR	NR
C0048159	DOU-F01-IPW001-0-041021	2.89	30	0.948	30	NR	NR
C0048159 Rep	DOU-F01-IPW001-0-041021*	2.81	30	0.964	30	NR	NR
C0048160	DOU-F01-IPW002-0-041021	18.4	50	3.69	30	NR	NR
C0048160 Rep	DOU-F01-IPW002-0-041021*	19.3	50	3.70	30	NR	NR
C0048161	DL2-F01-MSW001-0-041021	0.203	30	NO	30	120	50
C0048161 Rep	DL2-F01-MSW001-0-041021*	ND	30	NO	30	120	50
C0048162	DL2-F01-MSW002-0-041021	ND	30	ND	30	102	30
C0048162 Rep	DL2-F01-MSW002-0-041021*	ND	30	ND	30	100	30

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

Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048163	DL2-F01-MSW003-0-041021	ND	30	ND	30	92.4	30
C0048163 Rep	DL2-F01-MSW003-0-041021*	ND	30	ND	30	91.6	30
C0048164	DL2-F01-MSW004-0-041021	ND	30	ND	30	86.0	30
C0048164 Rep	DL2-F01-MSW004-0-041021*	ND	30	ND	30	94.8	30
C0048165	DL2-F01-MSW005-0-041021	ND	30	ND	30	168	30
C0048165 Rep	DL2-F01-MSW005-0-041021*	ND	30	ND	30	174	30
C0049450	DBC-F01-MSW001-0-041104	0.868	30	8.32	30	NR	NR
C0049450 Rep	DBC-F01-MSW001-0-041104*	0.852	30	8.32	30	NR	NR
C0049451	DBC-F01-MSW002-0-041104	0.868	30	3.43	30	NR	NR
C0049451 Rep	DBC-F01-MSW002-0-041104*	0.864	30	3.35	30	NR	NR
C0049452	DBC-F01-MSW003-0-041104	0.860	30	3.62	30	NR	NR
C0049452 Rep	DBC-F01-MSW003-0-041104*	0.764	30	3.64	30	NR	NR
C0049453	DBC-F01-MSW004-0-041104	0.884	30	9.72	30	NR	NR
C0049453 Rep	DBC-F01-MSW004-0-041104*	0.880	30	9.80	30	NR	NR
C0049454	DBC-F01-MSW005-0-041104	0.832	30	2.77	30	NR	NR
C0049454 Rep	DBC-F01-MSW005-0-041104*	0.832	30	2.66	30	NR	NR
C0049455	DBC-F01-MSF001-0-041104	0.820	30	3.58	30	NR	NR
C0049455 Rep	DBC-F01-MSF001-0-041104*	0.788	30	3.46	30	NR	NR
C0049456	DBC-F01-MSF002-0-041104	0.768	30	1.94	30	NR	NR
C0049456 Rep	DBC-F01-MSF002-0-041104*	0.780	30	1.90	30	NR	NR
C0049457	DBC-F01-IPF004-0-041104	0.504	30	0.584	30	142	30
C0049457 Rep	DBC-F01-IPF004-0-041104*	0.520	30	0.540	30	152	30
C0049458	DBC-F01-IPF005-0-041104	1.80	30	2.15	30	NR	NR
C0049458 Rep	DBC-F01-IPF005-0-041104*	1.84	30	2.18	30	NR	NR
C0049459	DBC-F01-IPW004-0-041104	0.484	30	3.28	30	NR	NR
C0049459 Rep	DBC-F01-IPW004-0-041104*	0.444	30	3.28	30	NR	NR
C0049460	DBC-F01-IPW005-0-041104	0.572	30	2.10	30	NR	NR
C0049460 Rep	DBC-F01-IPW005-0-041104*	0.532	30	2.10	30	NR	NR
C0049461	DOU-F01-IPW003-0-041104	3.12	30	6.00	30	NR	NR
C0049461 Rep	DOU-F01-IPW003-0-041104*	3.05	30	5.92	30	NR	NR
C0049462	DOU-F01-IPW004-0-041104	3.49	30	7.12	30	NR	NR
C0049462 Rep	DOU-F01-IPW004-0-041104*	3.11	30	6.84	30	NR	NR
C0049463	DOU-F01-IPW005-0-041104	2.97	30	5.12	30	NR	NR
C0049463 Rep	DOU-F01-IPW005-0-041104*	2.90	30	5.32	30	NR	NR
C0049464	DL2-F01-IPW002-0-041104	1.04	30	ND	30	8.20	40
C0049464 Rep	DL2-F01-IPW002-0-041104*	0.980	30	ND	30	6.60	40
C0049465	DL1-F01-MSW001-0-041104	1.20	30	0.898	30	NR	NR
C0049465 Rep	DL1-F01-MSW001-0-041104*	1.24	30	0.932	30	NR	NR

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Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049466	DL1-F01-MSW002-0-041104	1.30	30	1.72	30	NR	NR
C0049466 Rep	DL1-F01-MSW002-0-041104*	1.15	30	1.54	30	NR	NR
C0049467	DL1-F01-MSW003-0-041104	0.560	30	1.13	30	NR	NR
C0049467 Rep	DL1-F01-MSW003-0-041104*	0.528	30	1.14	30	NR	NR
C0049468	DL1-F01-MSW004-0-041104	1.22	30	0.908	30	161	30
C0049468 Rep	DL1-F01-MSW004-0-041104*	1.12	30	0.880	30	166	30
C0049469	DL1-F01-MSW005-0-041104	1.22	30	1.18	30	NR	NR
C0049469 Rep	DL1-F01-MSW005-0-041104*	1.20	30	1.18	30	NR	NR
C0049470	DL1-F01-MSF001-0-041104	ND	30	0.315	30	293	30
C0049470 Rep	DL1-F01-MSF001-0-041104*	ND	30	0.299	30	307	30
C0049471	DL1-F01-MSF002-0-041104	ND	30	0.624	30	NR	NR
C0049471 Rep	DL1-F01-MSF002-0-041104*	ND	30	0.604	30	NR	NR
C0049472	DL1-F01-MSF003-0-041104	0.203	30	0.652	30	NR	NR
C0049472 Rep	DL1-F01-MSF003-0-041104*	ND	30	0.676	30	NR	NR
C0049473	DL1-F01-MSF004-0-041104	ND	30	0.532	30	NR	NR
C0049473 Rep	DL1-F01-MSF004-0-041104*	ND	30	0.536	30	NR	NR
C0049474	DL1-F01-MSF005-0-041104	ND	30	0.684	30	NR	NR
C0049474 Rep	DL1-F01-MSF005-0-041104*	ND	30	0.644	30	NR	NR
C0049475	DL1-F01-IPW001-0-041104	0.772	30	0.566	30	17.4	30
C0049475 Rep	DL1-F01-IPW001-0-041104*	0.870	30	0.744	30	16.9	30
C0049476	DL1-F01-IPW002-0-041104	0.468	30	0.536	30	15.7	30
C0049476 Rep	DL1-F01-IPW002-0-041104*	0.472	30	0.616	30	15.6	30
C0049477	DL1-F01-IPW003-0-041104	ND	30	0.209	30	9.36	30
C0049477 Rep	DL1-F01-IPW003-0-041104*	ND	30	0.247	30	9.52	30
C0049478	DL1-F01-IPW004-0-041104	ND	30	0.238	30	59.6	30
C0049478 Rep	DL1-F01-IPW004-0-041104*	ND	30	0.246	30	57.2	30
C0049479	DL1-F01-IPF002-0-041104	ND	30	0.222	30	11.1	30
C0049479 Rep	DL1-F01-IPF002-0-041104*	ND	30	0.247	30	11.2	30
C0049480	DL-1-F01-IPF003-0-041104	ND	30	0.265	30	20.9	30
C0049480 Rep	DL-1-F01-IPF003-0-041104*	ND	30	0.262	30	21.4	30
C0049481	DL-1-F01-IPF004-0-041104	ND	30	ND	30	7.60	30
C0049481 Rep	DL-1-F01-IPF004-0-041104*	ND	30	ND	30	7.36	30
C0049482	DL-1-F01-IPF005-0-041104	ND	30	0.373	30	8.72	30
C0049482 Rep	DL-1-F01-IPF005-0-041104*	ND	30	0.366	30	8.56	30
C0049483	DL3-F01-MSW001-0-041105	ND	30	ND	30	139	30
C0049483 Rep	DL3-F01-MSW001-0-041105*	ND	30	ND	30	137	30
C0049484	DL3-F01-MSW002-0-041105	ND	30	ND	30	160	30
C0049484 Rep	DL3-F01-MSW002-0-041105*	ND	30	ND	30	162	30

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NR = Not reported due to quality control result failures.

Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049485	DL3-F01-MSW003-0-041105	ND	30	ND	30	146	30
C0049485 Rep	DL3-F01-MSW003-0-041105*	ND	30	ND	30	144	30
C0049486	DL3-F01-MSW004-0-041105	ND	30	0.285	30	198	30
C0049486 Rep	DL3-F01-MSW004-0-041105*	ND	30	0.282	30	199	30
C0049487	DL3-F01-MSW005-0-041105	ND	30	0.366	30	173	30
C0049487 Rep	DL3-F01-MSW005-0-041105*	ND	30	0.341	30	168	30
C0049488	DL3-F01-MSF001-0-041105	ND	30	ND	30	116	30
C0049488 Rep	DL3-F01-MSF001-0-041105*	ND	30	ND	30	115	30
C0049489	DL3-F01-MSF002-0-041105	ND	30	ND	30	116	30
C0049489 Rep	DL3-F01-MSF002-0-041105*	ND	30	ND	30	111	30
C0049490	DL3-F01-MSF003-0-041105	ND	30	ND	30	75.6	30
C0049490 Rep	DL3-F01-MSF003-0-041105*	ND	30	ND	30	72.0	30
C0049491	DL3-F01-MSF004-0-041105	ND	30	ND	30	130	30
C0049491 Rep	DL3-F01-MSF004-0-041105*	ND	30	ND	30	134	30
C0049492	DL3-F01-MSF005-0-041105	ND	30	ND	30	81.6	30
C0049492 Rep	DL3-F01-MSF005-0-041105*	ND	30	ND	30	83.6	30
C0049493	DMC-F01-IPW003-0-041105	ND	30	0.366	30	61.2	30
C0049493 Rep	DMC-F01-IPW003-0-041105*	ND	30	0.334	30	63.6	30
C0049494	DMC-F01-IPW004-0-041105	0.300	30	0.396	30	68.8	30
C0049494 Rep	DMC-F01-IPW004-0-041105*	0.345	30	0.464	30	69.6	30
C0049495	DMC-F01-IPW005-0-041105	ND	30	0.270	30	64.4	30
C0049495 Rep	DMC-F01-IPW005-0-041105*	ND	30	0.255	30	62.8	30
C0049496	DMC-F01-MSW003-0-041105	ND	30	0.592	30	354	30
C0049496 Rep	DMC-F01-MSW003-0-041105*	ND	30	0.560	30	330	30
C0049497	DMC-F01-MSW004-0-041105	0.318	30	1.90	30	259	30
C0049497 Rep	DMC-F01-MSW004-0-041105*	0.233	30	1.88	30	259	30
C0049498	DMC-F01-MSW005-0-041105	ND	30	0.960	30	NR	NR
C0049498 Rep	DMC-F01-MSW005-0-041105*	ND	30	0.936	30	NR	NR
C0049499	DMC-F01-MSF002-0-041105	ND	30	ND	30	NR	NR
C0049499 Rep	DMC-F01-MSF002-0-041105*	ND	30	0.367	30	NR	NR
C0049500	DMC-F01-MSF003-0-041105	ND	30	ND	30	56.8	40
C0049500 Rep	DMC-F01-MSF003-0-041105*	ND	30	ND	30	58.0	40
C0049501	DMC-F01-MSF004-0-041105	ND	30	0.254	30	116	30
C0049501 Rep	DMC-F01-MSF004-0-041105*	ND	30	0.228	30	119	30
C0049502	DMC-F01-MSF005-0-041105	ND	30	0.420	30	146	30
C0049502 Rep	DMC-F01-MSF005-0-041105*	ND	30	0.446	30	147	30
C0049503	DMC-F01-IPF003-0-041105	ND	30	ND	30	7.92	30
C0049503 Rep	DMC-F01-IPF003-0-041105*	ND	30	ND	30	7.92	30

*Laboratory Duplicate

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NR = Not reported due to quality control result failures.

Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049504	DMC-F01-IPF004-0-041105	ND	30	ND	30	44.0	30
C0049504 Rep	DMC-F01-IPF004-0-041105*	ND	30	ND	30	45.6	30
C0049505	DMC-F01-IPF005-0-041105	ND	30	ND	30	46.4	30
C0049505 Rep	DMC-F01-IPF005-0-041105*	ND	30	ND	30	46.0	30
C0056354	DL2-F01-MSF005-0-041216	ND	30	ND	30	49.2	30
C0056354 Rep	DL2-F01-MSF005-0-041216*	ND	30	ND	30	51.6	30
C0056455	DL2-F01-IPW006-0-041215	ND	30	ND	30	13.2	30
C0056455 Rep	DL2-F01-IPW005-0-041215*	ND	30	ND	30	13.3	30
C0056456	DOU-F01-MSW002-0-041216	0.206	40	1.72	30	NR	NR
C0056456 Rep	DOU-F01-MSW002-0-041216*	0.227	40	1.70	30	NR	NR
C0056457	DL2-F01-IPW004-0-041215	ND	30	ND	30	6.80	30
C0056457 Rep	DL2-F01-IPW004-0-041215*	ND	30	ND	30	6.80	30
C0056458	DOU-F01-MSW003-0-041216	ND	30	10.4	30	NR	NR
C0056458 Rep	DOU-F01-MSW003-0-041216*	ND	30	10.6	30	NR	NR
C0056459	DL3-F01-IPW004-0-041130	ND	30	ND	30	7.92	30
C0056459 Rep	DL3-F01-IPW004-0-041130*	ND	30	ND	30	7.52	30
C0056460	DOU-F01-MSW001-0-041216	7.08	30	15.4	30	NR	NR
C0056460 Rep	DOU-F01-MSW001-0-041216*	7.12	30	14.2	30	NR	NR
C0056461	DOU-F01-MSW004-0-041216	4.72	40	21.7	30	NR	NR
C0056461 Rep	DOU-F01-MSW004-0-041216*	4.68	40	22.5	30	NR	NR
C0056462	DOU-F01-MSW005-0-041216	0.364	30	1.34	30	NR	NR
C0056462 Rep	DOU-F01-MSW005-0-041216*	0.36	30	1.41	30	NR	NR
C0056463	DOU-F01-MSF005-0-041216	1.41	30	8.40	30	NR	NR
C0056463 Rep	DOU-F01-MSF005-0-041216*	1.43	30	8.44	30	NR	NR
C0056464	DL3-F01-IPW002-0-041130	ND	30	ND	30	12.0	50
C0056464 Rep	DL3-F01-IPW002-0-041130*	ND	30	ND	30	11.5	50
C0056465	DL3-F01-IPW003-0-041130	ND	30	ND	30	5.44	40
C0056465 Rep	DL3-F01-IPW003-0-041130*	ND	30	ND	30	5.28	40
C0056466	DOU-F01-MSF003-0-041216	1.72	30	8.56	30	NR	NR
C0056466 Rep	DOU-F01-MSF003-0-041216*	1.73	30	8.68	30	NR	NR
C0056467	DBC-F01-MSF005-1-041216	0.220	30	1.79	30	NR	NR
C0056467 Rep	DBC-F01-MSF005-1-041216*	ND	30	1.76	30	NR	NR
C0056468	DBC-F01-MSF005-0-041216	ND	30	1.68	30	NR	NR
C0056468 Rep	DBC-F01-MSF005-0-041216*	ND	30	1.59	30	NR	NR
C0056469	DOU-F01-MSF002-0-041216	0.888	30	4.56	30	NR	NR
C0056469 Rep	DOU-F01-MSF002-0-041216*	0.872	30	4.56	30	NR	NR
C0056470	DL3-F01-IPW001-0-041130	ND	30	0.246	30	9.04	30
C0056470 Rep	DL3-F01-IPW001-0-041130*	ND	30	0.219	30	8.60	30

*Laboratory Duplicate
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures.

Table I. Summary of PFBS, PFHS and PFOS in Fish Samples (continued)

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluoroctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0056471	DOU-F01-MSF002-1-041216	0.840	30	4.68	30	NR	NR
C0056471 Rep	DOU-F01-MSF002-1-041216*	0.860	30	4.72	30	NR	NR
C0056472	DOU-F01-MSF004-0-041216	0.792	30	2.35	40	NR	NR
C0056472 Rep	DOU-F01-MSF004-0-041216*	0.816	30	2.22	40	NR	NR
C0056473	DOU-F01-MSF001-0-041216	2.18	30	10.9	30	NR	NR
C0056473 Rep	DOU-F01-MSF001-0-041216*	2.26	30	10.7	30	NR	NR
C0056474	DBC-F01-MSF003-0-041216	ND	30	2.09	30	NR	NR
C0056474 Rep	DBC-F01-MSF003-0-041216*	ND	30	2.09	30	NR	NR
C0056475	DBC-F01-MSF004-0-041216	ND	30	3.87	30	NR	NR
C0056475 Rep	DBC-F01-MSF004-0-041216*	ND	30	3.77	30	NR	NR
C0056476	DMC-F01-MSF001-0-041216	ND	30	0.420	30	NR	NR
C0056476 Rep	DMC-F01-MSF001-0-041216*	ND	30	0.456	30	NR	NR
C0056477	DL1-F01-IPW005-0-041216	0.253	30	0.644	30	68.8	30
C0056477 Rep	DL1-F01-IPW005-0-041216*	0.249	30	0.644	30	67.6	30
C0056478	DL2-F01-IPW003-0-041201	ND	30	ND	30	7.48	40
C0056478 Rep	DL2-F01-IPW003-0-041201*	ND	30	ND	30	7.44	40
C0056479	DL3-F01-IPW005-0-041201	ND	30	ND	30	4.88	30
C0056479 Rep	DL3-F01-IPW005-0-041201*	ND	30	ND	30	4.72	30

*Laboratory Duplicate
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures.

Table II. Summary of PFOS in Re-extracted Fish Samples

Exygen ID	Client Sample ID	C8 Sulfonate PFOS Perfluorooctanesulfonate Analyte Found (ng/g) Wet Weight	Assessed Accuracy (+/- %)
C0048151	DBC-F01-IPW001-0-041021	2080	30
C0048152	DBC-F01-IPW002-0-041021	3330	30
C0048153	DBC-F01-IPW003-0-041021	1660	50
C0048154	DOU-F01-IPF001-0-041021	596	30
C0048155	DOU-F01-IPF002-0-041021	838	30
C0048158	DOU-F01-IPF005-0-041021	644	30
C0048160	DOU-F01-IPW002-0-041021	1210	40
C0049450	DBC-F01-MSW001-0-041104	9480	40
C0049451	DBC-F01-MSW002-0-041104	7520	30
C0049452	DBC-F01-MSW003-0-041104	12600	30
C0049453	DBC-F01-MSW004-0-041104	3440	30
C0049454	DBC-F01-MSW005-0-041104	5240	30
C0049455	DBC-F01-MSF001-0-041104	5240	30
C0049456	DBC-F01-MSF002-0-041104	4760	30
C0049458	DBC-F01-IPF005-0-041104	606	30
C0049459	DBC-F01-IPW004-0-041104	1230	40
C0049460	DBC-F01-IPW005-0-041104	2620	30
C0049461	DOU-F01-IPW003-0-041104	NR	NR
C0049462	DOU-F01-IPW004-0-041104	1740	30
C0049463	DOU-F01-IPW005-0-041104	2130	30
C0049465	DL1-F01-MSW001-0-041104	968	30
C0049466	DL1-F01-MSW002-0-041104	708	30
C0049467	DL1-F01-MSW003-0-041104	896	30
C0049469	DL1-F01-MSW005-0-041104	604	30
C0049471	DL1-F01-MSF002-0-041104	568	30
C0049472	DL1-F01-MSF003-0-041104	432	30

NR = Not reported due to quality control result failures.

Table II. Summary of PFOS in Re-extracted Fish Samples (continued)

Exygen ID	Client Sample ID	C8 Sulfonate PFOS Perfluorooctanesulfonate Analyte Found (ng/g) Wet Weight	Assessed Accuracy (+/- %)
C0049474	DL1-F01-MSF005-0-041104	740	30
C0049496	DMC-F01-MSW005-0-041105	560	30
C0049499	DMC-F01-MSF002-0-041105	620	30
C0056456	DOU-F01-MSW002-0-041216	28200	60
C0056458	DOU-F01-MSW003-0-041216	4040	30
C0056460	DOU-F01-MSW001-0-041216	NR	NR
C0056461	DOU-F01-MSW004-0-041216	5120	30
C0056462	DOU-F01-MSW005-0-041216	496	30
C0056463	DOU-F01-MSF005-0-041216	720	30
C0056466	DOU-F01-MSF003-0-041216	848	30
C0056467	DBC-F01-MSF005-1-041216	NR	NR
C0056468	DBC-F01-MSF006-0-041216	8960	50
C0056469	DOU-F01-MSF002-0-041216	640	30
C0056471	DOU-F01-MSF002-1-041216	892	30
C0056472	DOU-F01-MSF004-0-041216	1820	30
C0056473	DOU-F01-MSF001-0-041216	2070	30
C0056474	DBC-F01-MSF003-0-041216	4640	30
C0056475	DBC-F01-MSF004-0-041216	9280	50
C0056476	DMC-F01-MSF001-0-041216	840	30
C0048148	DBC-F01-IPF001-0-041021	384	30
C0048150	DBC-F01-IPF003-0-041021	293	30
C0048156	DOU-F01-IPF003-0-041021	359	30
C0048157	DOU-F01-IPF004-0-041021	424	30
C0048159	DOU-F01-IPW001-0-041021	387	30
C0049473	DL1-F01-MSF004-0-041104	348	30

NR = Not reported due to quality control result failures.

Table III. Summary of PFBS, PFHS and PFOS in Clam Samples

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0056480	DL3-I01-CFW001-0-041216	ND	40	ND	40	1.82	50
C0056480 Rep	DL3-I01-CFW001-0-041216*	ND	40	ND	40	1.82	50
C0056481	DMC-I01-CFW001-0-041216	ND	30	ND	30	6.16	50
C0056481 Rep	DMC-I01-CFW001-0-041216*	ND	30	ND	30	6.12	50
C0056482	DL2-I01-CFW001-0-041216	ND	30	ND	30	1.81	50
C0056482 Rep	DL2-I01-CFW001-0-041216*	ND	30	ND	30	1.77	50
C0056483	DBC-I01-CFW001-0-041215	ND	30	ND	30	4.48	30
C0056483 Rep	DBC-I01-CFW001-0-041215*	ND	30	ND	30	4.44	30
C0056484	DL1-I01-CFW001-0-041215	ND	30	ND	30	8.80	40
C0056484 Rep	DL1-I01-CFW001-0-041215*	ND	30	ND	30	8.84	40
C0056485	DOU-I01-CFW001-0-041216	ND	30	0.202	30	11.4	50
C0056485 Rep	DOU-I01-CFW001-0-041216*	ND	30	0.205	30	10.9	50

*Laboratory Duplicate
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPF001-0-041021 (C0048126 Spk C, 10 ppb)	10	0.309	8.56	83	ND	8.28	83	5.16	12.8	74
DL3-F01-IPF001-0-041021 (C0048126 Spk F, 100 ppb)	100	0.309	82.4	82	ND	73.2	73	5.16	78.4	73
DL3-F01-IPF002-0-041021 (C0048127 Spk D, 10 ppb)	10	0.265	8.52	83	ND	7.48	75	4.16	10.3	61
DL3-F01-IPF002-0-041021 (C0048127 Spk G, 100 ppb)	100	0.265	89.8	89	ND	79.8	80	4.16	80.8	77
DMC-F01-IPF001-0-041021 (C0048128 Spk E, 10 ppb)	10	0.330	4.48	42	ND	5.24	52	7.40	9.96	26
DMC-F01-IPF001-0-041021 (C0048128 Spk H, 100 ppb)	100	0.330	85.4	85	ND	74.8	75	7.40	81.8	74
DMC-F01-IPF002-0-041021 (C0048129 Spk C, 10 ppb)	10	0.712	7.68	70	ND	9.60	96	14.1	29.8	156
DMC-F01-IPF002-0-041021 (C0048129 Spk H, 100 ppb)	100	0.712	78.0	77	ND	96.0	96	14.1	104	90
DMC-F01-IPW001-0-041021 (C0048130 Spk D, 10 ppb)	10	1.08	7.60	65	ND	7.84	78	70.0	71.8	*
DMC-F01-IPW001-0-041021 (C0048130 Spk I, 100 ppb)	100	1.08	64.8	64	ND	80.0	80	70.0	131	61
DMC-F01-IPW002-0-041021 (C0048131 Spk E, 10 ppb)	10	0.866	5.76	58	ND	7.86	80	34.4	44.8	*
DMC-F01-IPW002-0-041021 (C0048131 Spk J, 100 ppb)	100	0.866	60.8	60	ND	74.8	75	34.4	101	67
DMC-F01-MSW001-0-041021 (C0048132 Spk E, 10 ppb Spike)	10	0.218	9.78	96	0.524	9.44	89	270	270	*
DMC-F01-MSW001-0-041021 (C0048132 Spk F, 100 ppb Spike)	100	0.218	91.2	91	0.524	93.2	93	270	375	105
DMC-F01-MSW002-0-041021^ (C0048133 Spk G, 50 ppb Spike)	50	ND^	45.2	90	ND^	52.0	104	530	470	*
DMC-F01-MSW002-0-041021^ (C0048133 Spk H, 500 ppb Spike)	500	ND^	432	86	ND^	482	96	530	924	79
DL2-F01-IPF001-0-041021 (C0048134 Spk F, 10 ppb Spike)	10	0.880	5.80	51	ND	8.88	88	3.45	8.36	49
DL2-F01-IPF001-0-041021 (C0048134 Spk K, 100 ppb Spike)	100	0.880	46.4	46	ND	58.8	59	3.45	56.4	53
DL2-F01-IPF002-0-041021 (C0048135 Spk G, 10 ppb Spike)	10	0.816	5.84	46	ND	6.40	64	4.72	8.56	38
DL2-F01-IPF002-0-041021 (C0048135 Spk L, 100 ppb Spike)	100	0.816	64.8	64	ND	78.8	79	4.72	72.4	68

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
^ 1.0 g of sample used for extraction instead of 5.0 g.
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
ND^ = Not detected at or above Limit of Quantitation which is 1.0 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DL2-F01-IPF003-0-041021 (C0048136 Spk C, 10 ppb Spike)	10	0.247	7.00	68	ND	7.96	80	2.01	9.20	72
DL2-F01-IPF003-0-041021 (C0048136 Spk H, 100 ppb Spike)	100	0.247	82.8	63	ND	73.2	73	2.01	74.0	72
DL2-F01-IPF004-0-041021 (C0048137 Spk D, 10 ppb Spike)	10	0.346	6.84	65	ND	7.60	76	4.52	11.3	68
DL2-F01-IPF004-0-041021 (C0048137 Spk I, 100 ppb Spike)	100	0.346	67.6	67	ND	76.9	77	4.52	80.4	76
DL2-F01-IPF005-0-041021 (C0048138 Spk E, 10 ppb Spike)	10	0.338	6.86	65	ND	7.68	77	2.46	9.68	72
DL2-F01-IPF005-0-041021 (C0048138 Spk J, 100 ppb Spike)	100	0.338	66.4	66	ND	80.6	81	2.46	82.0	80
DL2-F01-IPW001-0-041021 (C0048139 Spk F, 10 ppb Spike)	10	0.326	7.04	67	ND	7.60	76	5.66	13.9	80
DL2-F01-IPW001-0-041021 (C0048139 Spk K, 100 ppb Spike)	100	0.326	66.0	66	ND	77.2	77	5.66	85.2	79
DL2-F01-MSF001-0-041021 (C0048140 Spk G, 10 ppb Spike)	10	0.333	7.36	70	ND	8.20	82	77.2	78.0	*
DL2-F01-MSF001-0-041021 (C0048140 Spk L, 100 ppb Spike)	100	0.333	64.8	64	ND	77.8	78	77.2	158	81
DL2-F01-MSF002-0-041021 (C0048141 Spk C, 10 ppb Spike)	10	0.408	6.88	65	ND	7.48	75	94.8	101	*
DL2-F01-MSF002-0-041021 (C0048141 Spk H, 100 ppb Spike)	100	0.408	61.6	61	ND	66.0	66	94.8	156	61
DL2-F01-MSF003-0-041021 (C0048142 Spk D, 10 ppb Spike)	10	0.416	6.52	61	ND	6.76	68	41.6	41.2	*
DL2-F01-MSF003-0-041021 (C0048142 Spk I, 100 ppb Spike)	100	0.416	64.8	64	ND	70.6	71	41.6	104	62
DL2-F01-MSF004-0-041021 (C0048143 Spk E, 10 ppb Spike)	10	0.345	7.08	67	ND	7.56	76	83.6	91.2	*
DL2-F01-MSF004-0-041021 (C0048143 Spk J, 100 ppb Spike)	100	0.345	71.6	71	ND	81.2	81	83.6	167	83
DL1-F01-IPF001-0-041021 (C0048144 Spk F, 10 ppb Spike)	10	0.432	7.44	70	0.261	8.16	79	33.4	40.0	*
DL1-F01-IPF001-0-041021 (C0048144 Spk K, 100 ppb Spike)	100	0.432	67.2	67	0.261	76.4	76	33.4	106.0	73
DL3-F01-IPF003-0-041021 (C0048145 Spk G, 10 ppb Spike)	10	0.448	7.20	68	ND	8.08	81	3.55	10.8	73
DL3-F01-IPF003-0-041021 (C0048145 Spk L, 100 ppb Spike)	100	0.440	69.2	69	ND	81.8	82	3.55	90.0	78

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt.	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DL3-F01-IPF004-0-041021 (C0048146 Spk G, 10 ppb Spike)	10	0.784	8.84	81	ND	10.6	106	0.382	8.96	86
DL3-F01-IPF004-0-041021 (C0048146 Spk H, 100 ppb Spike)	100	0.784	88.6	88	ND	88.4	88	0.382	81.2	81
DL3-F01-IPF005-0-041021 (C0048147 Spk D, 10 ppb Spike)	10	1.53	5.44	39	ND	9.16	92	5.48	11.7	62
DL3-F01-IPF005-0-041021 (C0048147 Spk I, 100 ppb Spike)	100	1.53	60.4	59	ND	93.2	93	5.48	86.8	81
DBC-F01-IPF001-0-041021 (C0048148 Spk E, 10 ppb Spike)	10	1.34	6.24	49	0.892	10.8	101	NR	NR	NR
DBC-F01-IPF001-0-041021 (C0048148 Spk J, 100 ppb Spike)	100	1.34	56.4	55	0.892	86.8	86	NR	NR	NR
DBC-F01-IPF002-0-041021 (C0048149 Spk F, 10 ppb Spike)	10	1.43	5.84	44	ND	9.36	94	110	113	*
DBC-F01-IPF002-0-041021 (C0048149 Spk K, 100 ppb Spike)	100	1.43	59.2	58	ND	88.0	88	110	189	79
DBC-F01-IPF003-0-041021 (C0048150 Spk G, 10 ppb Spike)	10	0.896	6.72	57	0.644	10.4	98	NR	NR	NR
DBC-F01-IPF003-0-041021 (C0048150 Spk L, 100 ppb Spike)	100	0.896	60.8	60	0.644	86.8	86	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0048161 Spk C, 10 ppb Spike)	10	0.864	7.08	62	2.21	9.68	75	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0048161 Spk H, 100 ppb Spike)	100	0.864	70.0	69	2.21	86.0	96	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048162 Spk D, 10 ppb Spike)	10	0.700	7.56	86	6.12	16.2	91	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048162 Spk I, 100 ppb Spike)	100	0.700	69.6	69	9.12	96.0	87	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048163 Spk E, 10 ppb Spike)	10	0.736	8.32	76	6.76	16.5	97	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048163 Spk J, 100 ppb Spike)	100	0.736	70.8	70	6.76	90.8	84	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0048164 Spk F, 10 ppb Spike)	10	4.36	10.9	65	1.02	9.48	85	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0048164 Spk K, 100 ppb Spike)	100	4.36	76.8	72	1.02	86.0	85	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0048165 Spk G, 10 ppb Spike)	10	8.76	13.1	63	0.792	9.72	89	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0048165 Spk L, 100 ppb Spike)	100	8.76	76.0	69	0.792	80.0	79	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0048166 Spk C, 10 ppb Spike)	10	5.86	12.8	69	1.02	10.8	96	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0048166 Spk H, 100 ppb Spike)	100	5.86	81.2	75	1.02	88.4	87	NR	NR	NR

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt.	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DOU-F01-IPF004-0-041021 (C0048157 Spk D, 10 ppb Spike)	10	6.12	13.2	71	2.30	12.0	97	NR	NR	NR
DOU-F01-IPF004-0-041021 (C0048157 Spk I, 100 ppb Spike)	100	6.12	78.4	70	2.30	86.0	84	NR	NR	NR
DOU-F01-IPF005-0-041021 (C0048158 Spk E, 10 ppb Spike)	10	4.56	12.2	76	0.956	11.2	102	NR	NR	NR
DOU-F01-IPF005-0-041021 (C0048158 Spk J, 100 ppb Spike)	100	4.56	76.0	71	0.956	85.8	86	NR	NR	NR
DOU-F01-IPW001-0-041021 (C0048159 Spk F, 10 ppb Spike)	10	2.89	10.2	73	0.948	10.7	98	NR	NR	NR
DOU-F01-IPW001-0-041021 (C0048159 Spk K, 100 ppb Spike)	100	2.89	72.4	70	0.948	83.8	83	NR	NR	NR
DOU-F01-IPW002-0-041021 (C0048160 Spk G, 10 ppb Spike)	10	18.4	34.4	160	3.88	13.2	95	NR	NR	NR
DOU-F01-IPW002-0-041021 (C0048160 Spk L, 100 ppb Spike)	100	18.4	94.8	76	3.88	89.2	86	NR	NR	NR
DL2-F01-MSW001-0-041021 (C0048161 Spk I, 10 ppb Spike)	10	0.203	9.32	91	ND	8.88	87	120	126	*
DL2-F01-MSW001-0-041021 (C0048161 Spk J, 100 ppb Spike)	100	0.203	76.8	77	ND	85.2	85	120	177	57
DL2-F01-MSW002-0-041021 (C0048162 Spk K, 10 ppb Spike)	10	ND	9.66	97	ND	9.12	91	102	110	*
DL2-F01-MSW002-0-041021 (C0048162 Spk L, 100 ppb Spike)	100	ND	86.4	86	ND	93.6	94	102	185	83
DL2-F01-MSW003-0-041021 (C0048163 Spk C, 10 ppb Spike)	10	ND	9.24	92	ND	9.06	91	92.4	103	*
DL2-F01-MSW003-0-041021 (C0048163 Spk D, 100 ppb Spike)	100	ND	81.2	81	ND	95.2	95	92.4	182	90
DL2-F01-MSW004-0-041021 (C0048164 Spk E, 10 ppb Spike)	10	ND	7.56	76	ND	7.26	73	86.0	75.6	*
DL2-F01-MSW004-0-041021 (C0048164 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	93.6	94	86.0	166	78
DL2-F01-MSW005-0-041021 (C0048165 Spk G, 10 ppb Spike)	10	ND	7.96	80	ND	8.08	81	168	135	*
DL2-F01-MSW005-0-041021 (C0048165 Spk H, 100 ppb Spike)	100	ND	90.8	91	ND	100	100	168	234	66
DBC-F01-MSW001-0-041104 (C0048650 Spk C, 10 ppb Spike)	10	0.886	10.3	94	8.32	17.6	93	NR	NR	NR
DBC-F01-MSW001-0-041104 (C0048650 Spk D, 100 ppb Spike)	100	0.886	90.4	90	8.32	113	105	NR	NR	NR

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt.	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DBC-F01-MSW002-0-041104 (C0049451 Spk E, 10 ppb Spike)	10	0.868	9.96	91	3.43	12.8	94	NR	NR	NR
DBC-F01-MSW002-0-041104 (C0049451 Spk F, 100 ppb Spike)	100	0.868	94.8	94	3.43	113	110	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0049452 Spk G, 10 ppb Spike)	10	0.860	9.92	91	3.62	13.5	100	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0049452 Spk H, 100 ppb Spike)	100	0.860	86.0	85	3.62	104	100	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0049453 Spk I, 10 ppb Spike)	10	0.884	9.96	91	9.72	19.5	98	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0049453 Spk J, 100 ppb Spike)	100	0.884	97.2	96	9.72	124	114	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0049454 Spk K, 10 ppb Spike)	10	0.832	8.20	74	2.77	10.2	74	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0049454 Spk L, 100 ppb Spike)	100	0.832	91.6	91	2.77	108	105	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0049455 Spk M, 10 ppb Spike)	10	0.820	9.80	98	3.58	13.7	101	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0049455 Spk N, 100 ppb Spike)	100	0.820	88.4	88	3.58	108	102	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0049456 Spk O, 10 ppb Spike)	10	0.788	9.82	92	1.94	12.5	106	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0049456 Spk P, 100 ppb Spike)	100	0.788	90.8	90	1.94	102	100	NR	NR	NR
DBC-F01-IPF004-0-041104 (C0049457 Spk C, 10 ppb Spike)	10	0.504	10.5	100	0.584	11.8	110	142	152	*
DBC-F01-IPF004-0-041104 (C0049457 Spk D, 100 ppb Spike)	100	0.504	94.8	94	0.584	108	106	142	254	112
DBC-F01-IPF005-0-041104 (C0049458 Spk E, 10 ppb Spike)	10	1.60	10.4	88	2.15	12.2	101	NR	NR	NR
DBC-F01-IPF005-0-041104 (C0049458 Spk F, 100 ppb Spike)	100	1.60	90.8	89	2.15	105	103	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0049459 Spk G, 10 ppb Spike)	10	0.484	9.72	92	3.28	13.0	97	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0049459 Spk H, 100 ppb Spike)	100	0.484	87.6	87	3.28	104	101	NR	NR	NR
DBC-F01-IPW006-0-041104 (C0049460 Spk I, 10 ppb Spike)	10	0.572	10.4	98	2.10	13.2	111	NR	NR	NR
DBC-F01-IPW006-0-041104 (C0049460 Spk J, 100 ppb Spike)	100	0.572	92.8	92	2.10	102	100	NR	NR	NR

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
NO = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS Amount Spiked (ng/g)	C4 Sulfonate PFBS Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS Recovery (%)	C6 Sulfonate PFHS Amt Found in Sample (ng/g) wet wt.	C6 Sulfonate PFHS Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS Recovery (%)	C8 Sulfonate PFOS Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS Recovery (%)
DOU-F01-IPW003-0-041104 (C0049464 Spk K, 10 ppb Spike)	10	3.12	13.1	100	8.00	16.3	103	NR	NR	NR
DOU-F01-IPW003-0-041104 (C0049461 Spk L, 100 ppb Spike)	100	3.12	88.0	85	8.00	107	101	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk C, 10 ppb Spike)	10	3.49	12.8	93	7.12	17.4	103	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk D, 100 ppb Spike)	100	3.49	86.4	83	7.12	108	101	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk E, 10 ppb Spike)	10	2.97	10.5	75	5.12	13.5	84	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk F, 100 ppb Spike)	100	2.97	83.6	81	5.12	109	104	NR	NR	NR
DL2-F01-IPW002-0-041104 (C0049464 Spk G, 10 ppb Spike)	10	1.04	9.48	84	ND	10.4	104	6.20	14.3	81
DL2-F01-IPW002-0-041104 (C0049464 Spk H, 100 ppb Spike)	100	1.04	80.0	79	ND	97.8	98	6.20	124	116
DL1-F01-MSW001-0-041104 (C0049465 Spk I, 10 ppb Spike)	10	1.20	10.6	94	0.898	11.3	104	NR	NR	NR
DL1-F01-MSW001-0-041104 (C0049465 Spk J, 100 ppb Spike)	100	1.20	99.6	98	0.898	112	111	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk K, 10 ppb Spike)	10	1.30	9.76	85	1.72	12.4	107	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk L, 100 ppb Spike)	100	1.30	80.4	79	1.72	100	98	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk C, 10 ppb Spike)	10	0.580	8.82	84	1.13	10.2	91	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk D, 100 ppb Spike)	100	0.580	85.2	85	1.13	98.8	98	NR	NR	NR
DL1-F01-MSW004-0-041104 (C0049468 Spk E, 10 ppb Spike)	10	0.908	8.62	76	0.908	9.76	89	181	201	*
DL1-F01-MSW004-0-041104 (C0049468 Spk F, 100 ppb Spike)	100	0.908	84.8	84	0.908	99.6	99	181	289	108
DL1-F01-MSW005-0-041104 (C0049469 Spk G, 10 ppb Spike)	10	1.16	8.80	76	1.16	10.4	92	NR	NR	NR
DL1-F01-MSW005-0-041104 (C0049469 Spk H, 100 ppb Spike)	100	1.16	82.4	81	1.16	96.8	96	NR	NR	NR
DL1-F01-MSF001-0-041104 (C0049470 Spk I, 10 ppb Spike)	10	ND	7.76	78	0.315	8.52	92	293	303	*
DL1-F01-MSF001-0-041104 (C0049470 Spk J, 100 ppb Spike)	100	ND	78.4	78	0.315	90.8	90	293	394	101

*Sample residue exceeds the spiking level significantly, therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0048471 Spk K, 10 ppb Spike)	10	ND	8.60	86	0.624	10.5	99	NR	NR	NR
DL1-F01-MSF002-0-041104 (C0048471 Spk L, 100 ppb Spike)	100	ND	84.8	85	0.624	98.4	98	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0048472 Spk C, 10 ppb Spike)	10	0.200	8.78	86	0.652	10.2	95	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0048472 Spk D, 100 ppb Spike)	100	0.203	94.4	94	0.652	95.2	95	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0048473 Spk E, 10 ppb Spike)	10	ND	8.86	89	0.532	10.4	99	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0048473 Spk F, 100 ppb Spike)	100	ND	86.9	87	0.532	101	100	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0048474 Spk G, 10 ppb Spike)	10	ND	8.64	86	0.684	10.1	94	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0048474 Spk H, 100 ppb Spike)	100	ND	82.4	82	0.684	94.0	93	NR	NR	NR
DL1-F01-IPW001-0-041104 (C0048475 Spk I, 10 ppb Spike)	10	0.772	8.64	79	0.556	9.72	92	17.4	26.4	90
DL1-F01-IPW001-0-041104 (C0048475 Spk J, 100 ppb Spike)	100	0.772	84.0	83	0.556	97.8	97	17.4	113	96
DL1-F01-IPW002-0-041104 (C0048476 Spk K, 10 ppb Spike)	10	0.468	8.68	82	0.536	9.96	94	15.7	27.4	117
DL1-F01-IPW002-0-041104 (C0048476 Spk L, 100 ppb Spike)	100	0.468	76.4	76	0.536	88.0	87	15.7	100	84
DL1-F01-IPW003-0-041104 (C0048477 Spk C, 10 ppb Spike)	10	ND	9.06	91	0.209	9.44	92	9.36	15.7	73
DL1-F01-IPW003-0-041104 (C0048477 Spk D, 100 ppb Spike)	100	ND	90.4	90	0.209	105	105	9.36	109	100
DL1-F01-IPW004-0-041104 (C0048478 Spk E, 10 ppb Spike)	10	ND	8.48	85	0.238	9.80	96	59.6	72.0	*
DL1-F01-IPW004-0-041104 (C0048478 Spk F, 100 ppb Spike)	100	ND	71.8	72	0.238	84.8	85	59.6	140	80
DL1-F01-IPF002-0-041104 (C0048479 Spk G, 10 ppb Spike)	10	ND	8.00	80	0.222	9.28	91	11.1	18.9	78
DL1-F01-IPF002-0-041104 (C0048479 Spk H, 100 ppb Spike)	100	ND	88.4	88	0.222	98.4	98	11.1	108	97
DL-1-F01-IPF003-0-041104 (C0048480 Spk I, 10 ppb Spike)	10	ND	8.80	88	0.285	10.8	103	20.9	30.5	96
DL-1-F01-IPF003-0-041104 (C0048480 Spk J, 100 ppb Spike)	100	ND	78.8	79	0.285	90.4	90	20.9	109	88

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL-1-F01-IPF004-0-041104 (C0048461 Spk K, 10 ppb Spike)	10	ND	8.00	80	ND	9.46	95	7.80	15.8	82
DL-1-F01-IPF004-0-041104 (C0048461 Spk L, 100 ppb Spike)	100	ND	84.8	85	ND	98.8	99	7.80	102	94
DL-1-F01-IPF006-0-041104 (C0048462 Spk C, 10 ppb Spike)	10	ND	7.44	74	0.373	9.00	86	8.72	15.9	72
DL-1-F01-IPF006-0-041104 (C0048462 Spk D, 100 ppb Spike)	100	ND	84.6	85	0.373	98.4	98	8.72	102	93
DL3-F01-MSW001-0-041105 (C0048463 Spk E, 10 ppb Spike)	10	ND	8.36	84	ND	9.20	92	139	166	*
DL3-F01-MSW001-0-041105 (C0048463 Spk F, 100 ppb Spike)	100	ND	85.8	86	ND	99.6	100	139	256	119
DL3-F01-MSW002-0-041105 (C0048464 Spk G, 10 ppb Spike)	10	ND	8.44	84	ND	9.92	99	160	150	*
DL3-F01-MSW002-0-041105 (C0048464 Spk H, 100 ppb Spike)	100	ND	90.4	90	ND	106	106	160	263	103
DL3-F01-MSW003-0-041105 (C0048465 Spk I, 10 ppb Spike)	10	ND	8.60	86	ND	9.56	96	146	154	*
DL3-F01-MSW003-0-041105 (C0048465 Spk J, 100 ppb Spike)	100	ND	88.4	88	ND	102	102	146	254	108
DL3-F01-MSW004-0-041105 (C0048466 Spk K, 10 ppb Spike)	10	ND	8.48	85	0.286	9.88	94	198	225	*
DL3-F01-MSW004-0-041105 (C0048466 Spk L, 100 ppb Spike)	100	ND	86.4	86	0.286	102	102	198	301	103
DL3-F01-MSW005-0-041105 (C0048467 Spk C, 10 ppb Spike)	10	ND	9.56	96	0.366	11.1	107	173	183	*
DL3-F01-MSW005-0-041105 (C0048467 Spk D, 100 ppb Spike)	100	ND	100	100	0.366	105	105	173	246	73
DL3-F01-MSF001-0-041105 (C0048468 Spk E, 10 ppb Spike)	10	ND	9.48	95	ND	11.4	114	116	138	*
DL3-F01-MSF001-0-041105 (C0048468 Spk F, 100 ppb Spike)	100	ND	94.8	95	ND	109	109	116	234	118
DL3-F01-MSF002-0-041105 (C0048469 Spk G, 10 ppb Spike)	10	ND	9.86	99	ND	10.4	104	116	86.8	*
DL3-F01-MSF002-0-041105 (C0048469 Spk H, 100 ppb Spike)	100	ND	89.6	90	ND	103	103	116	216	100
DL3-F01-MSF003-0-041105 (C0048470 Spk I, 10 ppb Spike)	10	ND	9.32	93	ND	11.0	110	75.8	92.0	*
DL3-F01-MSF003-0-041105 (C0048470 Spk J, 100 ppb Spike)	100	ND	90.8	91	ND	107	107	75.8	190	114

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-MSF004-0-041105 (C0049481 Spk K, 10 ppb Spike)	10	ND	8.89	96	ND	11.0	110	130	144	*
DL3-F01-MSF004-0-041105 (C0049491 Spk L, 100 ppb Spike)	100	ND	90.4	90	ND	104	104	130	236	106
DL3-F01-MSF005-0-041105 (C0049482 Spk C, 10 ppb Spike)	10	ND	8.92	89	ND	10.3	103	81.8	87.2	*
DL3-F01-MSF005-0-041105 (C0049488 Spk G, 100 ppb Spike)	100	ND	85.5	86	ND	98.4	98	81.8	181	99
DMC-F01-IPW003-0-041105 (C0049489 Spk E, 10 ppb Spike)	10	ND	8.26	83	0.368	9.52	91	81.2	78.0	*
DMC-F01-IPW003-0-041105 (C0049493 Spk F, 100 ppb Spike)	100	ND	84.4	84	0.368	99.6	99	81.2	156	95
DMC-F01-IPW004-0-041105 (C0049494 Spk G, 10 ppb Spike)	10	0.300	8.04	77	0.306	9.28	89	88.8	107	*
DMC-F01-IPW004-0-041105 (C0049498 Spk H, 100 ppb Spike)	100	0.300	90.4	90	0.306	104	104	88.8	188	99
DMC-F01-IPW006-0-041105 (C0049499 Spk I, 10 ppb Spike)	10	ND	8.86	90	0.270	10.1	98	84.4	75.8	*
DMC-F01-IPW006-0-041105 (C0049499 Spk J, 100 ppb Spike)	100	ND	88.4	88	0.270	105	105	84.4	153	89
DMC-F01-MSW003-0-041105 (C0049488 Spk K, 10 ppb Spike)	10	ND	9.84	98	0.662	11.0	104	354	389	*
DMC-F01-MSW003-0-041105 (C0049496 Spk L, 100 ppb Spike)	100	ND	92.4	92	0.662	108	105	354	464	110
DMC-F01-MSW004-0-041105 (C0049497 Spk C, 10 ppb Spike)	10	0.318	9.52	92	1.90	12.1	102	259	259	*
DMC-F01-MSW004-0-041105 (C0049497 Spk G, 100 ppb Spike)	100	0.318	82.4	82	1.90	101	99	259	313	54
DMC-F01-MSW005-0-041105 (C0049485 Spk E, 10 ppb Spike)	10	ND	9.16	92	0.980	11.8	108	NR	NR	NR
DMC-F01-MSW005-0-041105 (C0049998 Spk F, 100 ppb Spike)	100	ND	93.8	94	0.980	118	117	NR	NR	NR
DMC-F01-MSF002-0-041105 (C0049499 Spk G, 10 ppb Spike)	10	ND	8.36	84	ND	10.4	104	NR	NR	NR
DMC-F01-MSF002-0-041105 (C0049499 Spk H, 100 ppb Spike)	100	ND	97.6	98	ND	104	104	NR	NR	NR
DMC-F01-MSF003-0-041105 (C0049500 Spk I, 10 ppb Spike)	10	ND	8.20	82	ND	10.4	104	88.8	108	*
DMC-F01-MSF003-0-041105 (C0049500 Spk J, 100 ppb Spike)	100	ND	87.2	87	ND	110	110	88.8	194	137

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DMC-F01-MSF004-0-041105 (C0046604 Spk K, 10 ppb Spike)	10	ND	8.76	88	0.254	11.1	108	116	123	*
DMC-F01-MSF004-0-041105 (C0046604 Spk L, 100 ppb Spike)	100	ND	88.0	88	0.254	107	107	116	226	110
DMC-F01-MSF005-0-041105 (C0046605 Spk C, 10 ppb Spike)	10	ND	8.52	85	0.420	12.2	118	146	193	*
DMC-F01-MSF005-0-041105 (C0046605 Spk D, 100 ppb Spike)	100	ND	83.6	84	0.420	110	110	146	280	114
DMC-F01-IPF003-0-041105 (C0046603 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	10.8	108	7.92	16.0	81
DMC-F01-IPF003-0-041105 (C0046603 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	108	108	7.92	101	93
DMC-F01-IPF004-0-041105 (C0046604 Spk G, 10 ppb Spike)	10	ND	7.92	79	ND	10.7	107	44.0	56.2	*
DMC-F01-IPF004-0-041105 (C0046604 Spk H, 100 ppb Spike)	100	ND	83.2	83	ND	107	107	44.0	134	90
DMC-F01-IPF005-0-041105 (C0046605b Spk I, 10 ppb Spike)	10	ND	7.88	79	ND	10.3	103	46.4	54.4	*
DMC-F01-IPF005-0-041105 (C0046605 Spk J, 100 ppb Spike)	100	ND	75.8	76	ND	96.4	96	46.4	121	75
DL2-F01-MSF006-0-041216 (C0064364 Spk E, 10 ppb Spike)	10	ND	8.44	84	ND	10.0	100	49.2	59.2	100
DL2-F01-IPW005-0-041215 (C0064364 Spk F, 100 ppb Spike)	100	ND	83.2	83	ND	102	102	49.2	142	93
DL2-F01-IPW005-0-041215 (C0064458 Spk C, 10 ppb Spike)	10	ND	8.00	80	ND	11.5	115	13.2	22.7	95
DL2-F01-IPW005-0-041215 (C0064458 Spk D, 100 ppb Spike)	100	ND	84.8	85	ND	107	107	13.2	121	108
DOU-F01-MSW002-0-041216 (C0064456 Spk I, 10 ppb Spike)	10	0.206	8.92	87	1.72	10.9	92	NR	NR	NR
DOU-F01-MSW002-0-041216 (C0064456 Spk J, 100 ppb Spike)	100	0.206	87.2	87	1.72	92.8	91	NR	NR	NR
DL2-F01-IPW004-0-041215 (C0064457 Spk C, 10 ppb Spike)	10	ND	8.08	81	ND	10.4	104	6.80	14.8	80
DL2-F01-IPW004-0-041215 (C0064457 Spk D, 100 ppb Spike)	100	ND	76.8	77	ND	96.2	96	6.80	97.2	90
DOU-F01-MSW003-0-041216 (C0064436 Spk K, 10 ppb Spike)	10	ND	8.12	81	10.4	19.4	90	NR	NR	NR
DOU-F01-MSW003-0-041216 (C0064436 Spk L, 100 ppb Spike)	100	ND	86.4	86	10.4	105	95	NR	NR	NR

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table 8.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DL3-F01-IPW004-0-041130 (C0056458 Spk E, 10 ppb Spike)	10	ND	7.52	75	ND	11.0	110	7.92	17.3	94
DL3-F01-IPW004-0-041130 (C0056459 Spk F, 100 ppb Spike)	100	ND	66.4	66	ND	118	118	7.92	114	106
DOU-F01-MSW001-0-041216 (C0056466 Spk G, 10 ppb Spike)	10	7.08	14.8	75	15.4	27.5	121	NR	NR	NR
DOU-F01-MSW001-0-041216 (C0056467 Spk H, 100 ppb Spike)	100	7.08	83.2	76	15.4	122.0	107	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0056461 Spk E, 10 ppb Spike)	10	4.72	10.8	61	21.7	29.3	76	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0056461 Spk F, 100 ppb Spike)	100	4.72	72.8	68	21.7	104	82	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0056462 Spk G, 10 ppb Spike)	10	0.364	7.08	67	1.34	8.52	72	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0056463 Spk H, 100 ppb Spike)	100	0.364	72.0	72	1.34	87.8	86	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0056463 Spk C, 10 ppb Spike)	10	1.41	8.60	72	8.40	17.8	92	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0056468 Spk D, 100 ppb Spike)	100	1.41	72.0	71	8.40	94.0	86	NR	NR	NR
DL3-F01-IPW002-0-041130 (C0056464 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	9.04	90	12.0	16.6	46
DL3-F01-IPW002-0-041130 (C0056464 Spk F, 100 ppb Spike)	100	ND	81.2	81	ND	110	110	12.0	105	93
DL3-F01-IPW003-0-041130 (C0056465 Spk G, 10 ppb Spike)	10	ND	7.96	80	ND	8.88	89	5.44	12.3	69
DL3-F01-IPW003-0-041130 (C0056466 Spk H, 100 ppb Spike)	100	ND	84.4	84	ND	102	102	5.44	92.0	87
DOU-F01-MSF003-0-041216 (C0056469 Spk K, 10 ppb Spike)	10	1.72	9.00	73	8.56	14.3	77	NR	NR	NR
DOU-F01-MSF003-0-041216 (C0056469 Spk L, 100 ppb Spike)	100	1.72	89.6	88	8.56	107	100	NR	NR	NR
DBC-F01-MSF006-1-041216 (C0056467 Spk C, 10 ppb Spike)	10	0.220	8.38	81	1.78	11.5	97	NR	NR	NR
DBC-F01-MSF006-1-041216 (C0056467 Spk D, 100 ppb Spike)	100	0.220	83.6	83	1.78	98.8	95	NR	NR	NR
DBC-F01-MSF005-0-041216 (C0056468 Spk K, 10 ppb Spike)	10	ND	8.36	84	1.88	11.4	97	NR	NR	NR
DBC-F01-MSF005-0-041216 (C0056468 Spk L, 100 ppb Spike)	100	ND	80.8	81	1.88	99.6	98	NR	NR	NR

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DOU-F01-MSF002-0-041216 (C0056469 Spk G, 10 ppb Spike)	10	0.886	9.24	84	4.66	14.5	98	NR	NR	NR
DOU-F01-MSF002-0-041216 (C0056469 Spk H, 100 ppb Spike)	100	0.886	83.6	83	4.66	104	99	NR	NR	NR
DL3-F01-IPW001-0-041130 (C0056470 Spk C, 10 ppb Spike)	10	ND	7.80	78	0.246	9.80	96	9.04	17.2	82
DL3-F01-IPW001-0-041130 (C0056470 Spk D, 100 ppb Spike)	100	ND	82.4	82	0.246	101	101	9.04	102	93
DOU-F01-MSF002-1-041216 (C0056471 Spk I, 10 ppb Spike)	10	0.840	9.20	84	4.66	14.5	98	NR	NR	NR
DOU-F01-MSF002-1-041216 (C0056471 Spk J, 100 ppb Spike)	100	0.840	82.0	81	4.66	103	98	NR	NR	NR
DOU-F01-MSF004-0-041216 (C0056472 Spk K, 10 ppb Spike)	10	0.792	7.76	70	2.35	8.80	65	NR	NR	NR
DOU-F01-MSF004-0-041216 (C0056472 Spk L, 100 ppb Spike)	100	0.792	81.2	80	2.35	86.6	83	NR	NR	NR
DOU-F01-MSF001-0-041216 (C0056473 Spk E, 10 ppb Spike)	10	2.18	11.1	89	10.9	20.8	99	NR	NR	NR
DOU-F01-MSF001-0-041216 (C0056473 Spk F, 100 ppb Spike)	100	2.18	82.8	81	10.9	104	93	NR	NR	NR
DBC-F01-MSF003-0-041216 (C0056474 Spk G, 10 ppb Spike)	10	ND	8.48	85	2.09	11.8	98	NR	NR	NR
DBC-F01-MSF003-0-041216 (C0056474 Spk H, 100 ppb Spike)	100	ND	84.0	84	2.09	106	104	NR	NR	NR
DBC-F01-MSF004-0-041216 (C0056475 Spk L, 10 ppb Spike)	10	ND	7.72	77	3.87	12.8	90	NR	NR	NR
DBC-F01-MSF004-0-041216 (C0056475 Spk J, 100 ppb Spike)	100	ND	75.8	76	3.87	98.8	95	NR	NR	NR
DMC-F01-MSF001-0-041216 (C0056476 Spk C, 10 ppb Spike)	10	ND	9.06	91	0.420	9.08	87	NR	NR	NR
DMC-F01-MSF001-0-041216 (C0056476 Spk D, 100 ppb Spike)	100	ND	86.0	86	0.420	102	102	NR	NR	NR
DL1-F01-IPW005-0-041216 (C0056477 Spk I, 10 ppb Spike)	10	0.253	7.40	71	0.644	9.86	93	68.8	83.2	*
DL1-F01-IPW005-0-041216 (C0056477 Spk J, 100 ppb Spike)	100	0.253	72.4	72	0.644	86.8	86	68.8	145	76
DL2-F01-IPW003-0-041201 (C0056478 Spk K, 10 ppb Spike)	10	ND	7.04	70	ND	9.04	90	7.48	13.7	62
DL2-F01-IPW003-0-041201 (C0056478 Spk L, 100 ppb Spike)	100	ND	71.8	72	ND	92.0	92	7.48	84.0	77

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples (continued)

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPW005-0-041201 (C8856479 Spk I, 10 ppb Spike)	10	ND	8.16	82	ND	8.96	90	4.88	14.0	91
DL3-F01-IPW005-0-041201 (C8856479 Spk J, 100 ppb Spike)	100	ND	84.8	85	ND	97.2	97	4.88	94.4	90
			Average:	79		Average:	94		Average:	86
			Standard Deviation:	13		Standard Deviation:	12		Standard Deviation:	29

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
NR = Not reported due to quality control result failure. Re-analysis results are shown in Table II.
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 PFOS in Re-extracted Fish Samples

Sample Description	C8 Sulfonate PFOS			
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DBC-F01-IPW001-0-041021 (C0048161 Spk C, 1000 ppb)	1000	2080	2860	78
DBC-F01-IPW002-0-041021 (C0048162 Spk D, 5000 ppb)	5000	3330	8680	107
DBC-F01-IPW003-0-041021 (C0048163 Spk E, 1000 ppb)	1000	1660	3160	150
DOU-F01-IPF001-0-041021 (C0048164 Spk F, 1000 ppb)	1000	898	1780	116
DOU-F01-IPF002-0-041021 (C0048165 Spk G, 1000 ppb)	1000	636	1740	110
DOU-F01-IPF005-0-041021 (C0048166 Spk H, 1000 ppb)	1000	544	1650	111
DOU-F01-IPW002-0-041021 (C0048169 Spk I, 1000 ppb)	1000	1210	2810	140
DBC-F01-MSW001-0-041104 (C0049460 Spk J, 10,000 ppb)	10000	9460	23200	137
DBC-F01-MSW002-0-041104 (C0049461 Spk K, 10,000 ppb)	10000	7520	16800	91
DBC-F01-MSW003-0-041104 (C0049462 Spk L, 10,000 ppb)	10000	12600	23200	106
DBC-F01-MSW004-0-041104 (C0049463 Spk M, 5000 ppb)	5000	3440	8520	102
DBC-F01-MSW005-0-041104 (C0049464 Spk N, 10,000 ppb)	10000	5240	15200	100
DBC-F01-MSF001-0-041104 (C0049465 Spk C, 10,000 ppb)	10000	5240	14400	92
DBC-F01-MSF002-0-041104 (C0049466 Spk D, 10,000 ppb)	10000	4760	15200	104
DBC-F01-IPF005-0-041104 (C0049468 Spk E, 1000 ppb)	1000	808	1600	99
DBC-F01-IPW004-0-041104 (C0049469 Spk F, 1000 ppb)	1000	1230	2590	136
DBC-F01-IPW005-0-041104 (C0049470 Spk G, 5000 ppb)	5000	2820	6520	78
DOU-F01-IPW003-0-041104 (C0049471 Spk H, 1000 ppb)	1000	2270	2640	37*
DOU-F01-IPW004-0-041104 (C0049472 Spk I, 1000 ppb)	1000	1740	2740	100
DOU-F01-IPW005-0-041104 (C0049473 Spk J, 5000 ppb)	5000	2130	7600	109
DL1-F01-MSW001-0-041104 (C0049475 Spk K, 1000 ppb)	1000	968	1860	89
DL1-F01-MSW002-0-041104 (C0049476 Spk C, 1000 ppb)	1000	706	1780	107
DL1-F01-MSW003-0-041104 (C0049477 Spk D, 1000 ppb)	1000	896	1930	123
DL1-F01-MSW005-0-041104 (C0049479 Spk E, 1000 ppb)	1000	804	1680	108

*Not reported due to quality control result failures. Values are not included in the summary statistics.

Table V. Matrix Spike Recovery of PFOS in Re-extracted Fish Samples (continued)

		C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0048471 Spk F, 1000 ppb)	1000	668	1660	112
DL1-F01-MSF003-0-041104 (C0048472 Spk G, 1000 ppb)	1000	432	1670	124
DL1-F01-MSF005-0-041104 (C0048474 Spk H, 1000 ppb)	1000	740	1830	109
DMC-F01-MSW005-0-041105 (C0048469 Spk I, 1000 ppb)	1000	580	1780	122
DMC-F01-MSF002-0-041105 (C0048469 Spk J, 1000 ppb)	1000	620	1500	88
DOU-F01-MSW002-0-041216 (C0056466 Spk K, 10,000 ppb)	10000	28200	32700	45
DOU-F01-MSW003-0-041216 (C0056468 Spk L, 5000 ppb)	5000	4040	10200	123
DOU-F01-MSW001-0-041216 (C0056460 Spk C, 10000 ppb)	10000	7600	25000	174*
DOU-F01-MSW004-0-041216 (C0056461 Spk D, 5000 ppb)	5000	5120	9860	95
DOU-F01-MSW005-0-041216 (C0056462 Spk E, 1000 ppb)	1000	486	1780	126
DOU-F01-MSF005-0-041216 (C0056463 Spk F, 1000 ppb)	1000	720	1820	110
DOU-F01-MSF003-0-041216 (C0056469 Spk G, 1000 ppb)	1000	846	1720	87
DBC-F01-MSF005-1-041216 (C0056467 Spk H, 10000 ppb)	10000	9320	32400	230*
DBC-F01-MSF005-0-041216 (C0056466 Spk I, 10000 ppb)	10000	8960	24100	151
DOU-F01-MSF002-0-041216 (C0056469 Spk J, 1000 ppb)	1000	640	1720	108
DOU-F01-MSF002-1-041216 (C0056471 Spk K, 1000 ppb)	1000	692	1950	126
DOU-F01-MSF004-0-041216 (C0056472 Spk L, 1000 ppb)	1000	1820	2700	88
DOU-F01-MSF001-0-041216 (C0056473 Spk C, 5000 ppb)	5000	2070	7960	118
DBC-F01-MSF003-0-041216 (C0056474 Spk D, 10000 ppb)	10000	4640	15500	109
DBC-F01-MSF004-0-041216 (C0056475 Spk E, 10000 ppb)	10000	9280	24500	152
DMC-F01-MSF001-0-041216 (C0056476 Spk F, 1000 ppb)	1000	840	2040	120
DBC-F01-IPF001-0-041021 (C0048148 Spk G, 1000 ppb)	1000	384	1350	97
DBC-F01-IPF003-0-041021 (C0048150 Spk H, 1000 ppb)	1000	298	1340	105
DOU-F01-IPF003-0-041021 (C0048158 Spk I, 1000 ppb)	1000	359	1400	104

*Not reported due to quality control result failures. Values are not included in the summary statistics.

Table VI. Matrix Spike Recovery PFBS, PFHS and PFOS in Clam Samples

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS: Amt Found in Sample (ng/g) wet wt.	C4 Sulfonate PFBS: Amount Recovered (ng/g) wet wt.	C4 Sulfonate PFBS: Recovery (%)	C6 Sulfonate PFHS: Amt Found in Sample (ng/g) wet wt	C6 Sulfonate PFHS: Amount Recovered (ng/g) wet wt.	C6 Sulfonate PFHS: Recovery (%)	C8 Sulfonate PFOS: Amt Found in Sample (ng/g) wet wt.	C8 Sulfonate PFOS: Amount Recovered (ng/g) wet wt.	C8 Sulfonate PFOS: Recovery (%)
DL3-I01-CFW001-0-041216 (C0089460 Spk C, 10 ppb)	10	ND	8.98	90	ND	6.18	62	1.82	6.32	46
DL3-I01-CFW001-0-041216 (C0089460 Spk G, 100 ppb)	100	ND	82.4	82	ND	95.8	96	1.82	85.2	83
DMC-I01-CFW001-0-041216 (C0089461 Spk E, 10 ppb)	10	ND	9.04	90	ND	9.44	94	6.16	11.8	51
DMC-I01-CFW001-0-041216 (C0089461 Spk F, 100 ppb)	100	ND	92.9	93	ND	98.8	98	5.16	92.6	87
DL2-I01-CFW001-0-041216 (C0089462 Spk G, 10 ppb)	10	ND	7.29	73	ND	7.64	76	1.81	7.52	57
DL2-I01-CFW001-0-041216 (C0089462 Spk H, 100 ppb)	100	ND	83.6	84	ND	101	101	1.81	88.4	87
DSC-I01-CFW001-0-041216 (C0089463 Spk I, 10 ppb)	10	ND	10.3	103	ND	10.8	108	4.48	13.0	86
DSC-I01-CFW001-0-041215 (C0089463 Spk J, 100 ppb)	100	ND	84.4	84	ND	97.2	97	4.48	86.4	82
DL1-I01-CFW001-0-041216 (C0089464 Spk K, 10 ppb)	10	ND	8.96	90	ND	9.32	93	8.80	16.6	82
DL1-I01-CFW001-0-041216 (C0089464 Spk L, 100 ppb)	100	ND	86.2	86	ND	103	103	8.80	96.8	88
DOU-I01-CFW001-0-041216 (C0089465 Spk M, 10 ppb)	10	ND	8.48	85	0.202	8.32	81	11.4	17.2	58
DOU-I01-CFW001-0-041216 (C0089465 Spk N, 100 ppb)	100	ND	84.4	84	0.202	98.0	96	11.4	96.0	87
			Average:	84		Average:	93		Average:	73
			Standard Deviation:	10		Standard Deviation:	13		Standard Deviation:	16

ND = Not detected at or above Limit of Quantitation which is 0.2 ng/g (wet weight).
Note: Since this summary table shows rounded results, recovery values may vary slightly from the values in the raw data.

FIGURES

Figure 1. Typical Calibration Curve for PFBS in Methanol

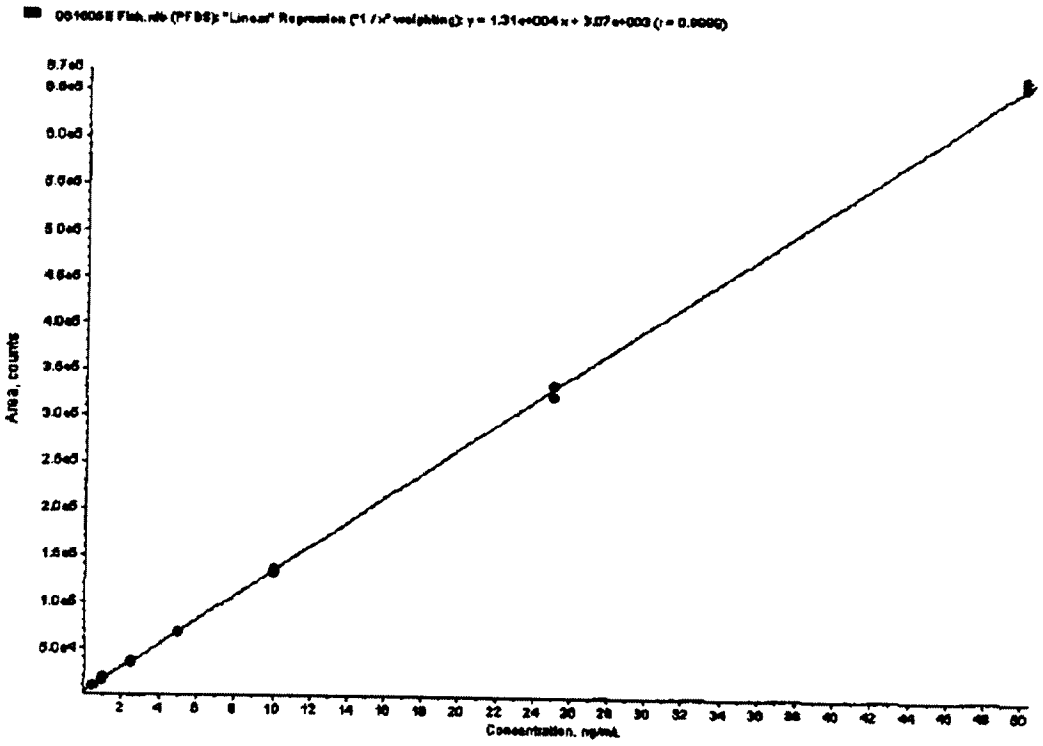


Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

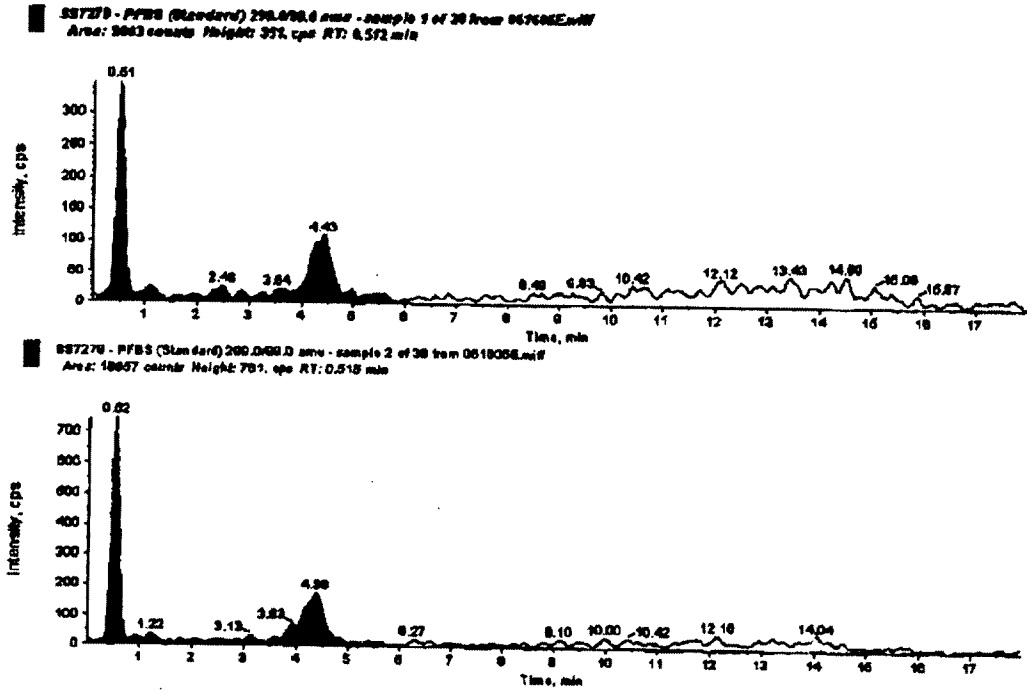


Figure 3. PFBS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

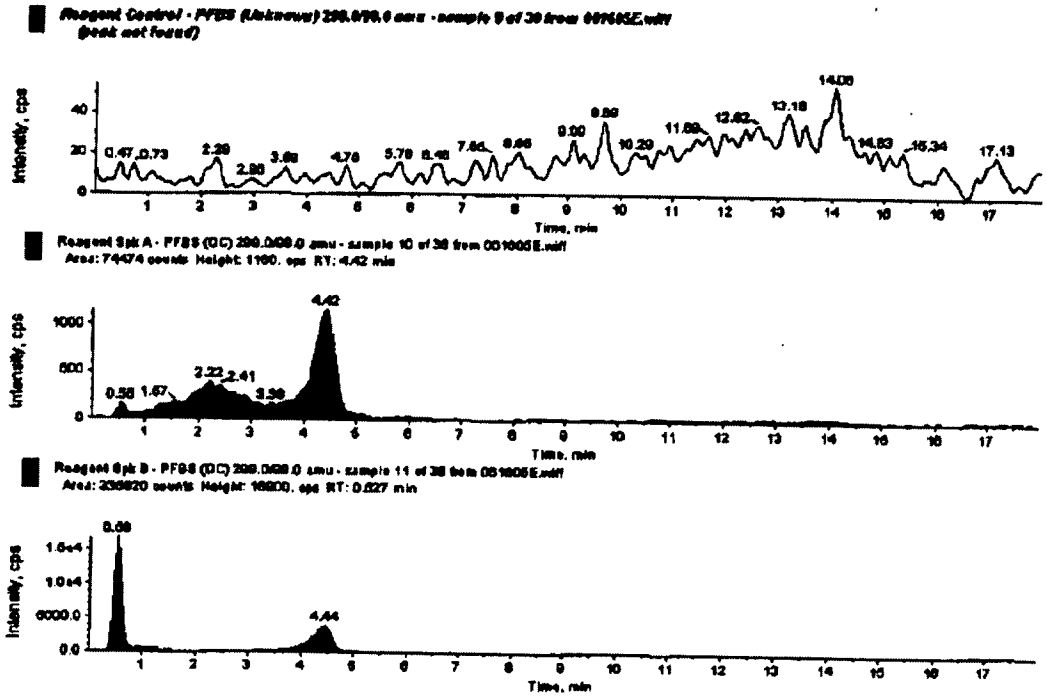


Figure 4. Chromatogram Representing a Fish Sample Analyzed for PFBS (Exygen ID: C0049478, Data Set:061605E)

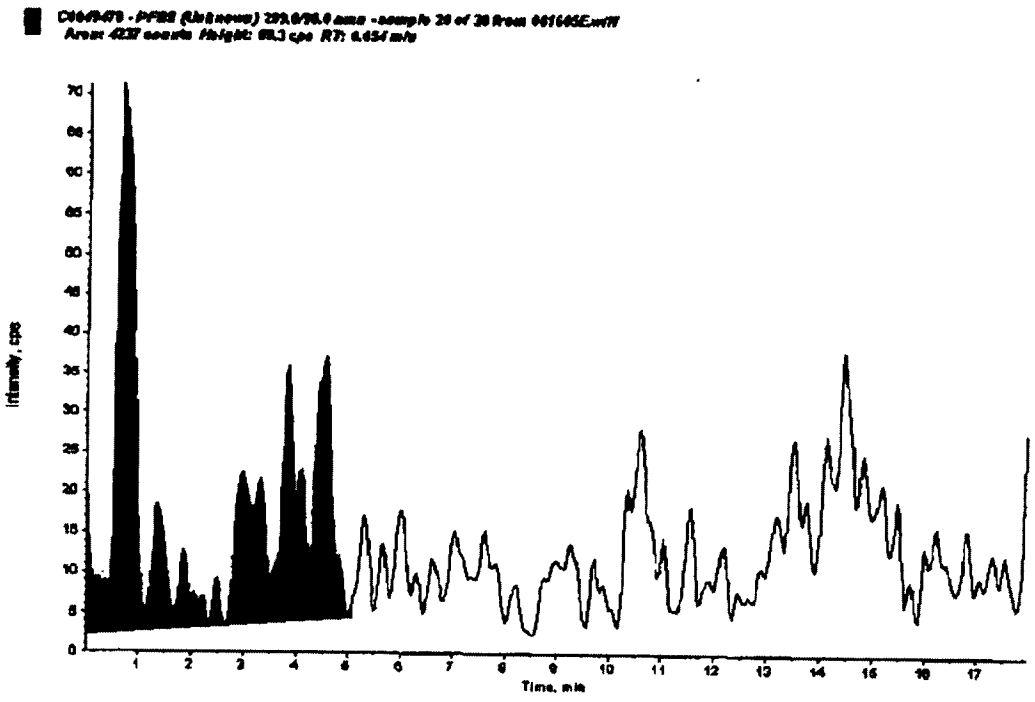


Figure 5. Typical Calibration Curve for PFHS in Methanol

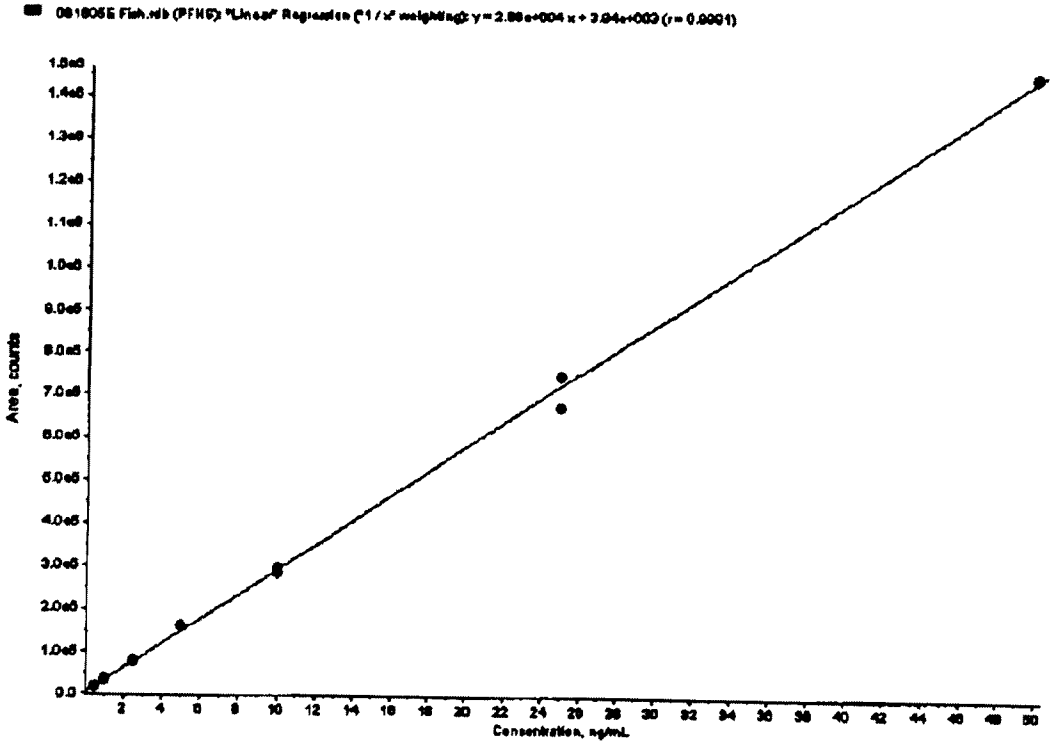


Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

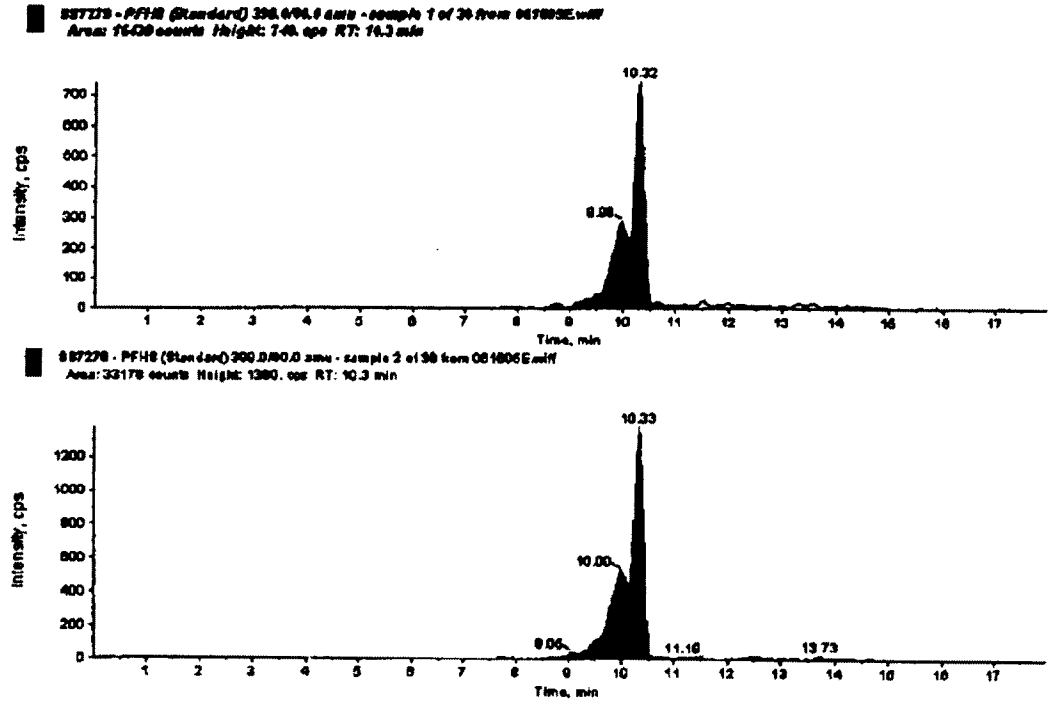


Figure 7. PFHS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

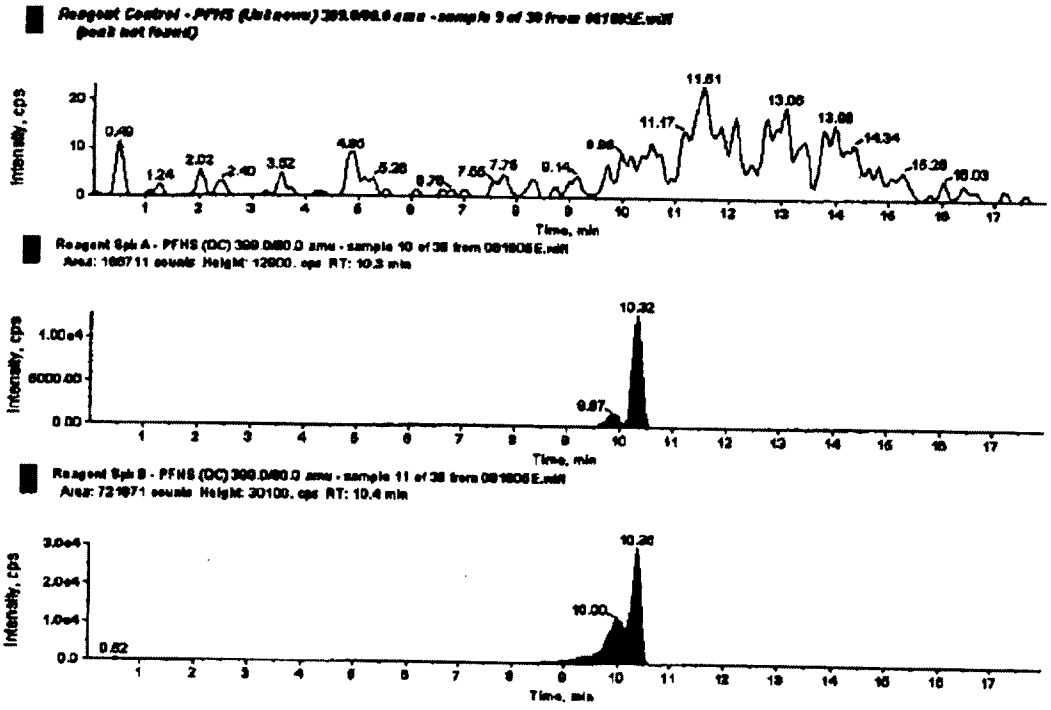


Figure 8. Chromatogram Representing a Fish Sample Analyzed for PFHS (Exygen ID: C0049478, Data Set: 061605E)

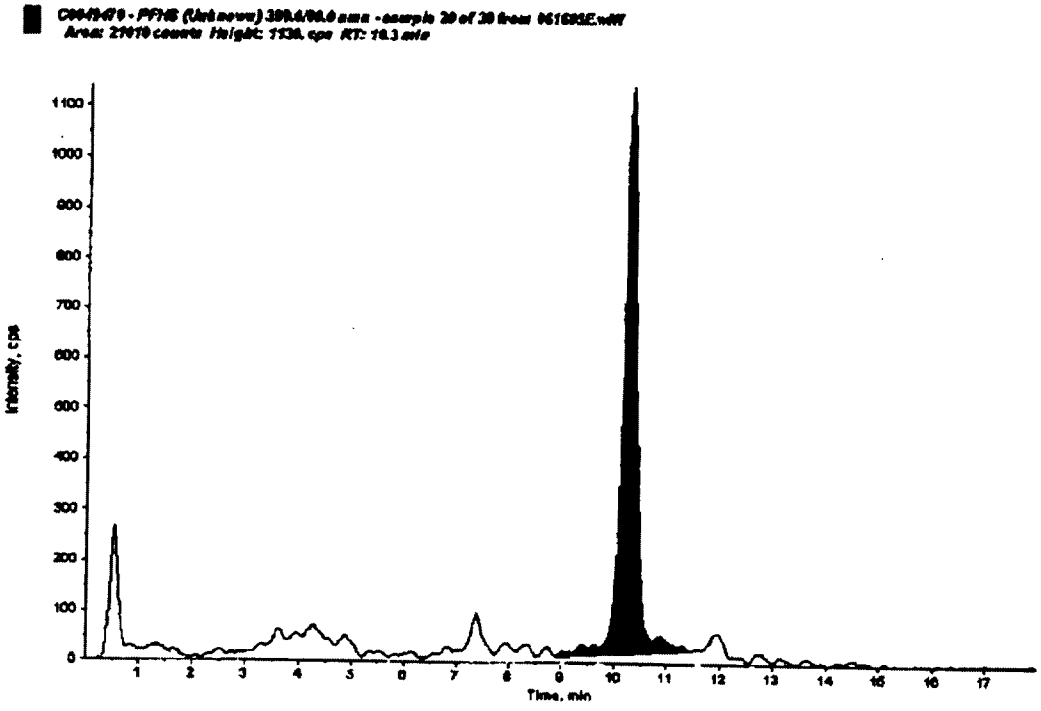


Figure 9. Typical Calibration Curve for PFOS in Methanol

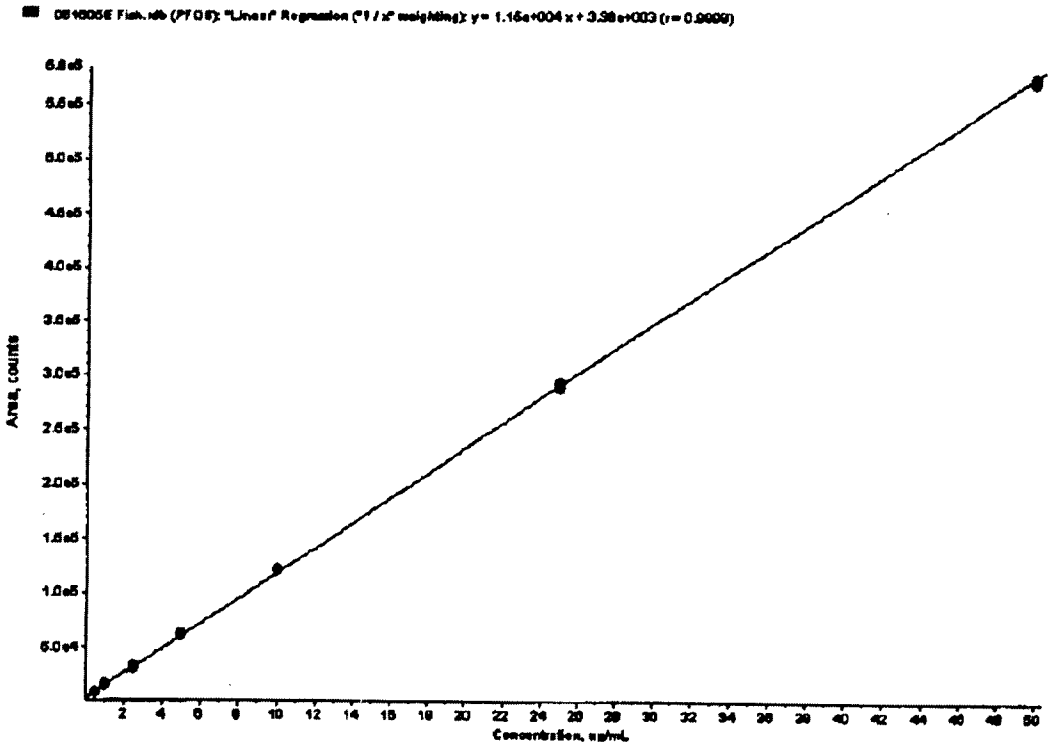


Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

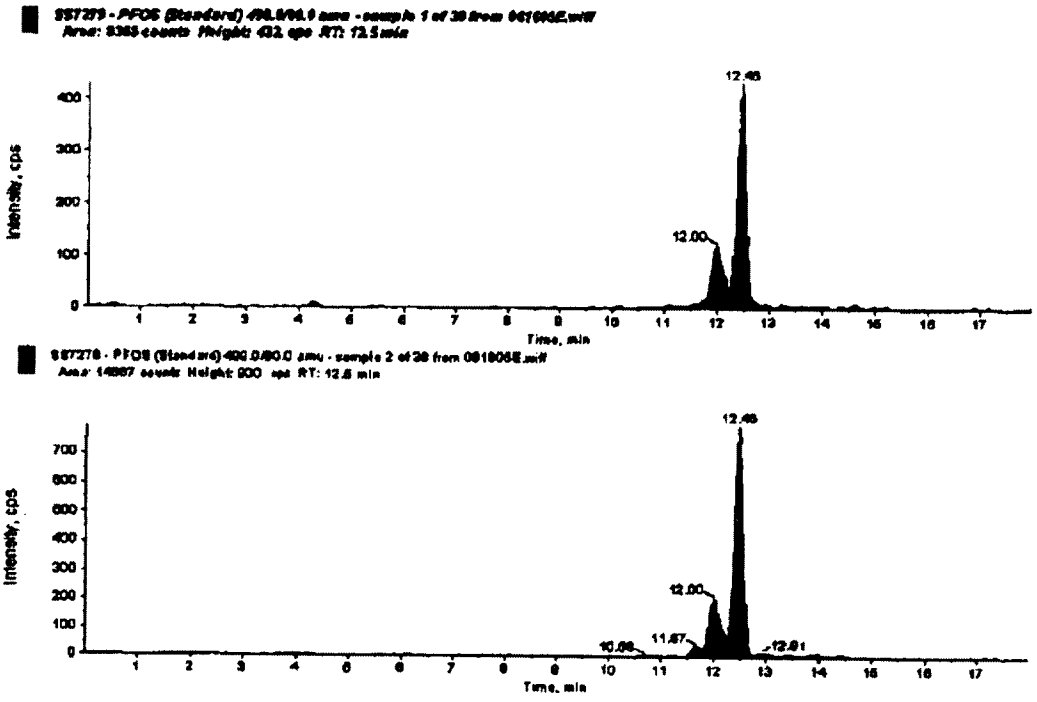


Figure 11. PFOS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

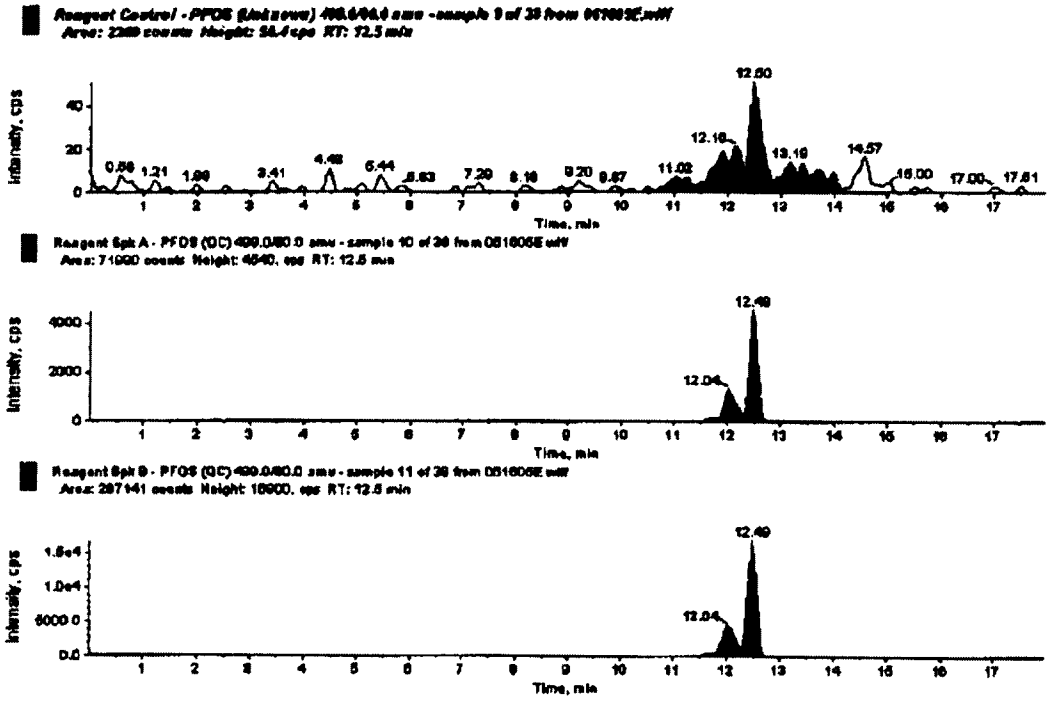
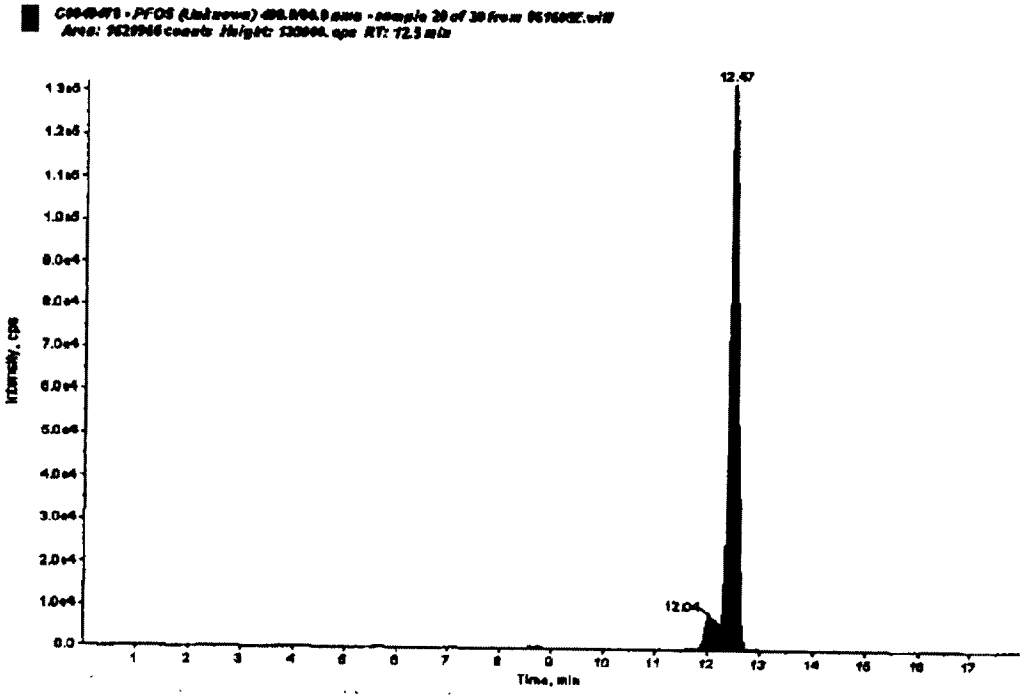


Figure 12. Chromatogram Representing a Fish Sample Analyzed for PFOS (Exygen ID: C0049478, Data Set:061605E)



APPENDIX A

Study Protocol P0001131 (Exygen Study No. P0001131) with Analytical Method, Protocol Amendments 1 and 2 and Deviations 2 and 3

STUDY PROTOCOL

Study Title:

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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: P0001131

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 Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) 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: P0001131

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 — Date

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

Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research — 11/29/04 Date

TABLE OF CONTENTS

Exygen Protocol Number: P0001131

INTRODUCTION

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

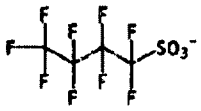
This study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

The test materials are perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) and are all supplied by 3M.

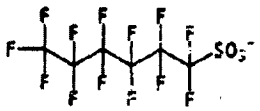
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Lot Number: 101
Purity: 96.7%
Transitions Monitored: 299 → 99
Structure:



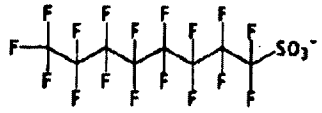
PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SB036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



Exygen Protocol Number: P0001131

PFOS
Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Lot Number: 217
Purity: 86.9%
Transitions Monitored: 499 → 99
Structure:



OBJECTIVE

The purpose of this study is to perform analysis for perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) 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
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Phone: (814) 272-1039

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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 PFBS, PFHS and PFOS 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 PFBS, PFHS and PFOS. The final report will include the isomers summed into total PFBS, total PFHS, and total PFOS 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 fluorochemical sample

collection at the testing facility and have been shown to be free of PFBS, PFHS and PFOS. 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 spike bottles will contain PFBS, PFHS and PFOS as well as perfluorooctanoic acid (PFOA) and 1,2-13C perfluorooctanoic acid (^{13}C PFOA). PFOA and ^{13}C PFOA are included in the solutions used to spike the samples. The results for PFOA and ^{13}C PFOA will not be reported in this study. Exygen will supply one field blank (control water) and two field blank spikes (control water fortified with PFBS, PFHS and PFOS 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 PFBS, PFHS and PFOS 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 PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

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 PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

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

Matrix	Low Spiking Level	High Spiking Level
Water	500 ng/L	5000 ng/L
Soil	4 ng/g	40 ng/g
Sediment	4 ng/g	40 ng/g
Fish	10 ng/g	100 ng/g
Clams	10 ng/g	100 ng/g
Vegetation	10 ng/g	100 ng/g
Small Mammal Liver	10 ng/g	100 ng/g
Small Mammal Serum	10 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.

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

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.

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.

Exygen Protocol Number: P0001131

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: P0001131

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
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/24/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001720

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 (20-100uL, 100-300uL).
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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V3001700

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 MS/MS 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0801706

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 (ppt)	Fortification Volume (µL)	Volume of Purified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (in sample)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
20	200	40	50	XCmmddyy-2
40	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
200	200	40	500	XCmmddyy-5
400	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 0.4 its initial concentration, due to the concentration of the standard during the extraction (SPE).
XC = extracted calibration standard.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001180

ANALYTICAL METHOD

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

9.2.3 A two 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 droplets). 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

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001700

ANALYTICAL METHOD

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

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

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

13.0 Acceptance Criteria

13.1 Chromatograms 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 Hunt Test, may be excluded from the calculation of the calibration curve. However, the total number of estimated 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 MassLynx software program:

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

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0907720

ANALYTICAL METHOD

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0001131

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
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/24/04
Date

John Fisher
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V000.701

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 Requirements

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: Phenyhexyl RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82905-052 150)

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: ~ 25 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 MS/MS 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: P0001131

Exygen Research Method Number V0001781

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 (assessed 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 (ng/mL)	Fortification Volume (µL)	Volume of Purified Cleaned Extracts (mL)	Final Concentration of Calibration Standard (ng/g)*	Calibration Standard ID (example)
0	0	40	0	XCmatdyy-0
10	100	40	25	XCmatdyy-1
10	200	40	50	XCmatdyy-2
10	400	40	100	XCmatdyy-3
100	100	40	250	XCmatdyy-4
100	200	40	500	XCmatdyy-5
100	400	40	1000	XCmatdyy-6

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

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Exygen Research Method Number: V0001701

ANALYTICAL METHOD

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

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 8°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 (hortify 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 ~2000 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 drops/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 chromatography 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

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ANALYTICAL METHOD

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

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 3.5 (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 Rexer 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001701

ANALYTICAL METHOD

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

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:

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

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.

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

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

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

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(%)]

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001702

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

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001703

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 wet 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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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001702

ANALYTICAL METHOD

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

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: Phenomenex RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82586-052130)
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 MS/MS 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001787

ANALYTICAL METHOD

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

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0004.702

ANALYTICAL METHOD

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

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 extracted 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 drops/sec). Do not let columns 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 drops/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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001752

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 3.5 (or equivalent) software system.

12.5 Sample responses 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 Hugt Erver 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001702

ANALYTICAL METHOD

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

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:

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

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.

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

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

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

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

$$\text{PFOA found (ppb) dry weight} = \text{PFOA found (ppb)} \times [100\% / \text{total solids(\%)}]$$

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: Y0001783

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

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 8

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001783

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 freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer 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 Supelclean 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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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001703

ANALYTICAL METHOD

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

5.4 Rotary evaporator.
5.5 Titrator/stir.
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 (50-100mL, 100-200mL).
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. 505717).
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: Phosphate 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.

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Exygen Research Method Number V0000 783

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 MS/MS 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% acetic acid in methanol is prepared by dissolving 2 g of acetic acid in 100 mL of methanol.

8.2.2 30% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane 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 (accounted 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.

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Exygen Research Method Number: VM001R3

ANALYTICAL METHOD

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

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 (freshly 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 Label 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 columns 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001703

ANALYTICAL METHOD

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

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 eliminated pear-shape flask.

11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat plugs 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 30 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.

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Exygen Research Method Number V0001FD

ANALYTICAL METHOD

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

12.5 Sample responses 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 remain 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0004.R3

ANALYTICAL METHOD

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0001131

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
3668 Rassmuk Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

Ken Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: VB081704

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 freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer 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 Supelclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethylbichlorosilane – 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 solvent 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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Exygen Protocol Number: P0001131

Exygen Research Method Number V9001704

ANALYTICAL METHOD

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

5.4 Rotary evaporator.
5.5 125 mL pour-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. 57177).
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: Phosphate RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82305-052130)
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.

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ANALYTICAL METHOD

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

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

Exygen Research Method Number: M0001704

ANALYTICAL METHOD

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

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)
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
0.005	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 3°C to 8°C, up to six months.

9.2.4 Aliquots 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 tissue 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 $LC\text{-}NH_2$. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the columns 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001784

ANALYTICAL METHOD

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

11.9 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 eluate 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 unextracted 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V080176

ANALYTICAL METHOD

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

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 Rorer 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.

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001705

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

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/24/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

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 freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer 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.

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Exygen Protocol Number. P0001131

Exygen Research Method Number V0001783

ANALYTICAL METHOD

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

5.9 Disposable pipettes.
5.10 Micropipettes (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: Phenomenex RP (Keystone Scientific), 2.1 mm x 50 mm, 3µ (P/N: 82305-052130)
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: ~ 25 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 MS/MS system.

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Exygen Protocol Number: P0001131

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

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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001781

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 unspiked 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 homogenizer 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 ~30 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 droplets). 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

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

13.5 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 Baver 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:

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V020178.1

ANALYTICAL METHOD

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

14.2 Per 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).

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

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Exygen Protocol Number: P0001131

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
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

Date

Mike Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001784

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 be allowed to completely thaw to room temperature before use.
3.3 Sample collection procedure 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.

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Exygen Research Method Number V0001706

ANALYTICAL METHOD

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

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: Phenyhexyl RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82305-052130)

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
21.5	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 MS/MS 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0801706

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	2.5	100	2.5
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 8°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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001704

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 (larify 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 Loosen 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 droptons). 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 run 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.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001706

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:

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

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.

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001706

ANALYTICAL METHOD

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

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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State College, PA 16801
Phone: 814-272-1039
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PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 01/19/05
Exygen Study Number: P0001131 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 3/2/05
Safety Director/Management Signature N/A — Date N/A
Sponsor Signature (if required) Mary Ellenstein for Michael A. Kenitzer — Date 3/10/05

Exygen QAU Review 1 of 1 pages

LIBRARY ID: VV0001228-6 ADMINISTRATIVE FORM

Exygen RESEARCH
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PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 03/07/06
Exygen Study Number P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

1) Report, page 11 of 65
2) Test Materials, page 6 of 65: PFOS transition monitored 499 -> 99.

AMENDED TO

1) Instead of one final report, interim reports will be issued.
2) PFOS transition monitored may also be 499 -> 80.

RATIONALE

1) Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timelier manner. ~~more either out~~
2) The API 4000 LC/MS/MS systems detect the 499 -> 80 PFOS transition with greater sensitivity than the 499 -> 99 transition.

IMPACT ON STUDY

1) The client will be able to receive and review the data more quickly.
2) The 499 -> 80 transition can be detected with greater sensitivity; therefore, giving better chromatography.

Study Director Signature	Date 3/9/06
Principal Investigator Signature	Date 3/9/06
Study Director Management Signature MA	Date
Exygen Management Signature	Date 8-MAR-06
Sponsor Signature (if required)	Date 3/16/06

Exygen QAU Review LJS 03/08/06

LIBRARY ID: V0001228-8 ADMINISTRATIVE FORM

09-20-2005 02:35pm From-MESTON SOLUTIONS T-384 P.003/004 F-437

Exygen RESEARCH
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DEVIATION FORM

Page 1 of 1

General:
X Project Specific Deviation ___ Facility Deviation Date of Occurrence: 08/29/05

Exygen Project # : P780/P1131 Deviation # : 2/2

Client Project # : NA Reference # : 05-133 9/20/05

Regulatory Driver:
___ GMP
X GLP
___ Other
___ None

Deviation Type: *(Include V# for methods and SOPs)*
X Protocol ___ Method ___ SOP
V#: NA
Notebook reference: NA

Sample Description:
Login # : L4254/L4255 Container # : C0056480-86 Lot # : NA

Summary of Deviation:
The protocol states that control and fortified control samples of each matrix will be analyzed; however, control clam was not obtained. Fish was used as the control for the analysis of these six clam samples.

Cause: ___ Preparation ___ Analysis ___ Instrument ___ Client Request X Other

Impact:
No negative impact on this study.

Corrective Actions:
Deviation issued.

Signatures:

Principal Investigator	Date	Exygen Management	Date
Study Director	9/20/05 Date	NA Sponsor Representative	Date
Quality Assurance	9/20/05 Date	NA Sponsor Management	Date

08-20-2005 02:35pm From-WESTON SOLUTIONS T-384 P.004/004 F-437

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DEVIATION FORM

Page 1 of 1

General:
X Project Specific Deviation ___ Facility Deviation Date of Occurrence: 12/27/04

Exygen Project #: P760/P1131 Deviation #: 3/3

Client Project #: NA Reference #: 05-134 8/24/05

Regulatory Driver:
___ GMP
X GLP
___ Other
___ None

Deviation Type: *(Include VR for methods and SOPs)*
___ Protocol ___ Method X SOP
VR: 202-20 Section 5.2.3
Notebook reference: NA

Sample Description:
Login #: NA Container #: NA Lot #: NA

Summary of Deviation:
SL2114 (30% Dimethyldichlorosilane in Toluene) was given the expiration date of 02/13/05, but RE544 (Toluene) used to make SL2114 expired on 03/28/04. SL2114 was used to silanized glassware prepared for the fish extraction from 12/27/04 through 01/07/05.

Cause: ___ Preparation ___ Analysis ___ Instrument ___ Client Request X Other

Impact:
No negative impact on the study. Toluene was used only as a solvent for the glassware preparation. Dimethyldichlorosilane, which is the coating agent, was not expired.

Corrective Actions:
Deviation issued.

Signatures:

Principal Investigator	Date 9/20/05	Exygen Management	Date 30-SEP-05
Study Director	Date 9/20/05	Sponsor Representative NA	Date
Quality Assurance	Date 9/20/05	Sponsor Management NA	Date