

AR 226-3684

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

Jaisimha Kcsari P.E., DEE
Weston Solutions, Inc.
1400 Weston Way
West Chester, PA 19380
Phone: 610-701-3761

INTERIM REPORT COMPLETION DATE

May 09, 2006

PERFORMING LABORATORY

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

STUDY SPONSOR

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

PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

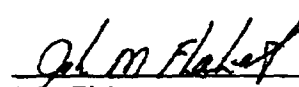
Total Pages: 132

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

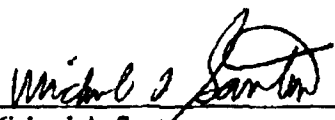
Date



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

5/10/06

Date



Michael A. Santoro
Sponsor Representative
3M Company

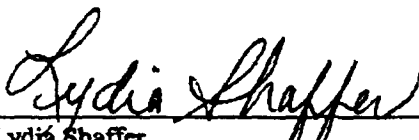
5/12/06

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 phases¹ 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.

<u>Phase</u>	<u>Date Inspected</u>	<u>Date Reported to Principal Investigator</u>	<u>Date Reported to Exygen Management</u>	<u>Date Reported to Study Director</u>
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

¹Note: 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.

Interim Report #10-Analysis of Fish and Clam Samples

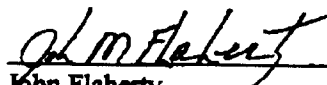
Exygen Study No.: P0001131

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

Principal Investigator, Exygen:



John Flaherty
Vice President
Exygen Research

5/19/06
Date

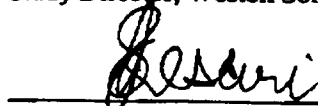
Exygen Research Facility Management:



Richard A. Grazzini
President
Exygen Research

9-11-2006
Date

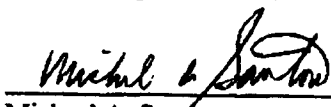
Study Director, Weston Solutions, Inc.



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

5/10/06
Date

Sponsor Representative, 3M Company:



Michael A. Santoro
Director of Regulatory Affairs

5/12/06
Date

Exygen Research

Page 4 of 132

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

STUDY MONITOR: Michael A. Santoro
3M Company
3M Building 0236-01-B-10
St. Paul, MN 55144

PERFORMING LABORATORY: Exygen Research
3058 Research Drive
State College, PA 16801

ANALYTICAL PHASE
TIMETABLE: Study Initiation Date: 11/05/04
Interim Analytical Start Date: 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:

<u>Name</u>	<u>Title</u>
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

TABLE OF CONTENTS

	<u>Page</u>
TITLE PAGE	1
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT	2
QUALITY ASSURANCE STATEMENT	3
CERTIFICATION OF AUTHENTICITY	4
STUDY IDENTIFICATION.....	5
PROJECT PERSONNEL.....	6
TABLE OF CONTENTS.....	7
LIST OF TABLES	8
LIST OF FIGURES.....	9
LIST OF APPENDICES	10
1.0 SUMMARY.....	11
2.0 OBJECTIVE	12
3.0 INTRODUCTION.....	13
4.0 ANALYTICAL TEST SAMPLES	13
5.0 REFERENCE MATERIAL	13
6.0 DESCRIPTION OF ANALYTICAL METHOD	15
6.1 Extraction Procedure	15
6.1.1 Sample Preparation	15
6.1.2 Glassware Preparation.....	15
6.1.3 Column Preparation	15
6.1.4 Sample Extraction.....	15
6.2 Preparation of Standards and Fortification Solutions.....	16
6.3 Chromatography	17
6.4 Instrument Sensitivity.....	17
6.5 Description of LC/MS/MS Instrument and Operating Conditions.....	17
6.6 Quantitation and Example Calculation.....	18
7.0 EXPERIMENTAL DESIGN	20
8.0 RESULTS	20
9.0 CONCLUSIONS.....	21
10.0 RETENTION OF DATA AND SAMPLES	21

LIST OF TABLES

	<u>Page</u>
Table I. Summary of PFBS, PFHS and PFOS in Fish Samples.....	23
Table II. Summary of PFOS in Re-extracted Fish Samples.....	30
Table III. Summary of PFBS, PFHS and PFOS in Clam Samples	32
Table IV. Matrix Spike Recovery PFBS, PFHS and PFOS in Fish Samples	33
Table V. Matrix Spike Recovery of PFOS in Re-extracted Fish Samples.....	46
Table VI. Matrix Spike Recovery PFBS, PFHS and PFOS in Clam Samples.....	49

LIST OF FIGURES

	<u>Page</u>
Figure 1. Typical Calibration Curve for PFBS in Methanol	51
Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively	52
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	53
Figure 4. Chromatogram Representing a Fish Sample Analyzed for PFBS (Exygen ID: C0049478, Data Set:061605E)	54
Figure 5. Typical Calibration Curve for PFHS in Methanol	55
Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively	56
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	57
Figure 8. Chromatogram Representing a Fish Sample Analyzed for PFHS (Exygen ID: C0049478, Data Set: 061605E)	58
Figure 9. Typical Calibration Curve for PFOS in Methanol	59
Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively	60
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	61
Figure 12. Chromatogram Representing a Fish Sample Analyzed for PFOS (Exygen ID: C0049478, Data Set:061605E)	62

LIST OF APPENDICES**Page**

Appendix A	Study Protocol P0001131 (Exygen Study No. P0001131) with Analytical Method V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS", Protocol Amendments 1 and 2, and Deviations 2 and 3.....	63
-------------------	---	-----------

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

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

<u>Compound</u>	<u>Exygen Inventory No.</u>	<u>Lot #</u>	<u>Purity (%)</u>	<u>Expiration Date</u>
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:

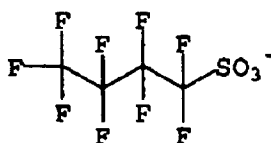
PFBS

Chemical Name: Perfluorobutanesulfonate

Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3K^+$)

Transitions Monitored: 299 → 99

Structure:

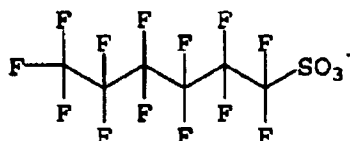
**PFHS**

Chemical Name: Perfluorohexanesulfonate

Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3K^+$)

Transitions Monitored: 399 → 80

Structure:

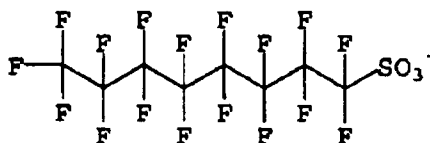
**PFOS**

Chemical Name: Perfluorooctanesulfonate

Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3K^+$)

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-NH₂. 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

<u>Time (min)</u>	<u>% A</u>	<u>% B</u>
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:

<u>Analyte</u>	<u>Mode</u>	<u>Transition Monitored</u>	<u>Approximate Retention Time (min)</u>
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.

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Equation 2:

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:

$$\begin{aligned} \text{Analyte found (ng/mL, wet weight)} &= \frac{[280750 - 3070] \times 1}{13100} \\ &= 21.2 \text{ ng/mL} \end{aligned}$$

From equation 2:

$$\begin{aligned} \text{Analyte found ng/g (ppb)} &= \frac{(21.2 \text{ ng/mL} \times 2 \text{ mL})}{5 \text{ g}} \\ &= 8.48 \text{ ng/g (ppb)} \end{aligned}$$

From equation 3:

$$\begin{aligned}\% \text{ Recovery} &= \frac{(8.48 \text{ ng/g} - 0 \text{ ng/g})}{10 \text{ ng/g}} \times 100\% \\ &= 85 \%\end{aligned}$$

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

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

TABLES

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	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	5.84	30
C0048127	DL3-F01-IPF002-0-041021	0.266	40	ND	30	4.16	40
C0048127 Rep	DL3-F01-IPF002-0-041021*	0.362	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	1.06	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	0.988	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	30
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	548	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	2.08	30
C0048137	DL2-F01-IPF004-0-041021	0.348	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*	0.518	40	ND	30	2.80	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	0.408	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	41.8	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.

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	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	0.228	30	3.36	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	0.606	50	0.644	30	NR	NR
C0048150 Rep	DBC-F01-IPF003-0-041021*	0.662	50	0.624	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	8.98	30	NR	NR
C0048153	DBC-F01-IPW003-0-041021	0.736	30	6.78	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
C0048155	DOU-F01-IPF002-0-041021	8.78	40	0.702	30	NR	NR
C0048155 Rep	DOU-F01-IPF002-0-041021*	8.28	40	0.720	30	NR	NR
C0048156	DOU-F01-IPF003-0-041021	5.88	40	1.02	30	NR	NR
C0048156 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	0.986	30	NR	NR
C0048158 Rep	DOU-F01-IPF005-0-041021*	4.56	30	0.882	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	ND	30	120	50
C0048161 Rep	DL2-F01-MSW001-0-041021*	ND	30	ND	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

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)**

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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	88.0	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	188	30
C0048165 Rep	DL2-F01-MSW005-0-041021*	ND	30	ND	30	174	30
C0048450	DBC-F01-MSW001-0-041104	0.868	30	8.32	30	NR	NR
C0048450 Rep	DBC-F01-MSW001-0-041104*	0.852	30	8.32	30	NR	NR
C0048451	DBC-F01-MSW002-0-041104	0.868	30	3.43	30	NR	NR
C0048451 Rep	DBC-F01-MSW002-0-041104*	0.864	30	3.35	30	NR	NR
C0048452	DBC-F01-MSW003-0-041104	0.880	30	3.62	30	NR	NR
C0048452 Rep	DBC-F01-MSW003-0-041104*	0.784	30	3.64	30	NR	NR
C0048453	DBC-F01-MSW004-0-041104	0.884	30	9.72	30	NR	NR
C0048453 Rep	DBC-F01-MSW004-0-041104*	0.880	30	9.80	30	NR	NR
C0048454	DBC-F01-MSW005-0-041104	0.832	30	2.77	30	NR	NR
C0048454 Rep	DBC-F01-MSW005-0-041104*	0.832	30	2.66	30	NR	NR
C0048455	DBC-F01-MSF001-0-041104	0.820	30	3.58	30	NR	NR
C0048455 Rep	DBC-F01-MSF001-0-041104*	0.788	30	3.48	30	NR	NR
C0048456	DBC-F01-MSF002-0-041104	0.768	30	1.84	30	NR	NR
C0048456 Rep	DBC-F01-MSF002-0-041104*	0.780	30	1.90	30	NR	NR
C0048457	DBC-F01-IPF004-0-041104	0.504	30	0.584	30	142	30
C0048457 Rep	DBC-F01-IPF004-0-041104*	0.620	30	0.540	30	152	30
C0048458	DBC-F01-IPF005-0-041104	1.60	30	2.16	30	NR	NR
C0048458 Rep	DBC-F01-IPF005-0-041104*	1.64	30	2.18	30	NR	NR
C0048459	DBC-F01-IPW004-0-041104	0.484	30	3.28	30	NR	NR
C0048459 Rep	DBC-F01-IPW004-0-041104*	0.444	30	3.28	30	NR	NR
C0048460	DBC-F01-IPW005-0-041104	0.572	30	2.10	30	NR	NR
C0048460 Rep	DBC-F01-IPW005-0-041104*	0.532	30	2.10	30	NR	NR
C0048461	DOU-F01-IPW003-0-041104	3.12	30	6.00	30	NR	NR
C0048461 Rep	DOU-F01-IPW003-0-041104*	3.05	30	5.92	30	NR	NR
C0048462	DOU-F01-IPW004-0-041104	3.48	30	7.12	30	NR	NR
C0048462 Rep	DOU-F01-IPW004-0-041104*	3.11	30	6.84	30	NR	NR
C0048463	DOU-F01-IPW005-0-041104	2.97	30	5.12	30	NR	NR
C0048463 Rep	DOU-F01-IPW005-0-041104*	2.90	30	5.32	30	NR	NR
C0048464	DL2-F01-IPW002-0-041104	1.04	30	ND	30	6.20	40
C0048464 Rep	DL2-F01-IPW002-0-041104*	0.980	30	ND	30	6.60	40
C0048465	DL1-F01-MSW001-0-041104	1.20	30	0.898	30	NR	NR
C0048465 Rep	DL1-F01-MSW001-0-041104*	1.24	30	0.932	30	NR	NR

*Laboratory Duplicates

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)**

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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	DL1-F01-MSW002-0-041104	1.30	30	1.72	30	NR	NR
C0049485 Rep	DL1-F01-MSW002-0-041104*	1.15	30	1.54	30	NR	NR
C0049487	DL1-F01-MSW003-0-041104	0.580	30	1.13	30	NR	NR
C0049487 Rep	DL1-F01-MSW003-0-041104*	0.528	30	1.14	30	NR	NR
C0049488	DL1-F01-MSW004-0-041104	1.22	30	0.808	30	181	30
C0049488 Rep	DL1-F01-MSW004-0-041104*	1.12	30	0.880	30	186	30
C0049489	DL1-F01-MSW005-0-041104	1.22	30	1.18	30	NR	NR
C0049489 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	0.824	30	NR	NR
C0049471 Rep	DL1-F01-MSF002-0-041104*	ND	30	0.804	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	0.678	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	0.558	30	17.4	30
C0049475 Rep	DL1-F01-IPW001-0-041104*	0.870	30	0.744	30	18.9	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	0.518	30	15.8	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	50.6	30
C0049478 Rep	DL1-F01-IPW004-0-041104*	ND	30	0.248	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	DL1-F01-IPF003-0-041104	ND	30	0.265	30	20.9	30
C0049480 Rep	DL1-F01-IPF003-0-041104*	ND	30	0.262	30	21.4	30
C0049481	DL1-F01-IPF004-0-041104	ND	30	ND	30	7.80	30
C0049481 Rep	DL1-F01-IPF004-0-041104*	ND	30	ND	30	7.36	30
C0049482	DL1-F01-IPF005-0-041104	ND	30	0.373	30	8.72	30
C0049482 Rep	DL1-F01-IPF005-0-041104*	ND	30	0.386	30	8.58	30
C0049483	DL3-F01-MSW001-0-041105	ND	30	ND	30	138	30
C0049483 Rep	DL3-F01-MSW001-0-041105*	ND	30	ND	30	137	30
C0049484	DL3-F01-MSW002-0-041105	ND	30	ND	30	180	30
C0049484 Rep	DL3-F01-MSW002-0-041105*	ND	30	ND	30	182	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.

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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	148	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	118	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	0.366	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	0.562	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	250	30
C0049497 Rep	DMC-F01-MSW004-0-041105*	0.233	30	1.68	30	209	30
C0049498	DMC-F01-MSW005-0-041105	ND	30	0.980	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	0.448	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

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.

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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-IPW005-0-041215	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	6.60	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*	4.66	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	5.26	40
C0056466	DOU-F01-MSF003-0-041216	1.72	30	6.56	30	NR	NR
C0056466 Rep	DOU-F01-MSF003-0-041216*	1.73	30	6.68	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	1.66	30	NR	NR
C0056468 Rep	DBC-F01-MSF005-0-041216*	ND	30	1.59	30	NR	NR
C0056469	DOU-F01-MSF002-0-041216	0.868	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)**

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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*	0.880	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*	2.28	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	1680	50
C0048154	DOU-F01-IPF001-0-041021	586	30
C0048155	DOU-F01-IPF002-0-041021	636	30
C0048158	DOU-F01-IPF005-0-041021	544	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	12800	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	808	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	698	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
C0049498	DMC-F01-MSW005-0-041105	580	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	8980	50
C0056469	DOU-F01-MSF002-0-041216	640	30
C0056471	DOU-F01-MSF002-1-041216	692	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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		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	DL3-101-CFW001-0-041216	ND	40	ND	40	1.82	50
C0056480 Rep	DL3-101-CFW001-0-041216*	ND	40	ND	40	1.82	50
C0056481	DMC-101-CFW001-0-041216	ND	30	ND	30	6.16	50
C0056481 Rep	DMC-101-CFW001-0-041216*	ND	30	ND	30	6.12	50
C0056482	DL2-101-CFW001-0-041216	ND	30	ND	30	1.81	50
C0056482 Rep	DL2-101-CFW001-0-041216*	ND	30	ND	30	1.77	50
C0056483	DBC-101-CFW001-0-041215	ND	30	ND	30	4.48	30
C0056483 Rep	DBC-101-CFW001-0-041215*	ND	30	ND	30	4.44	30
C0056484	DL1-101-CFW001-0-041215	ND	30	ND	30	8.80	40
C0056484 Rep	DL1-101-CFW001-0-041215*	ND	30	ND	30	8.84	40
C0056485	DOU-101-CFW001-0-041216	ND	30	0.202	30	11.4	50
C0056485 Rep	DOU-101-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)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPF001-0-041021 (C0040126 Spk C, 10 ppb)	10	0.309	8.56	83	ND	8.28	83	5.16	12.8	74
DL3-F01-IPF001-0-041021 (C0040126 Spk F, 100 ppb)	100	0.309	62.4	82	ND	73.2	73	5.16	78.4	73
DL3-F01-IPF002-0-041021 (C0040127 Spk D, 10 ppb)	10	0.265	8.52	83	ND	7.48	75	4.16	10.3	81
DL3-F01-IPF002-0-041021 (C0040127 Spk G, 100 ppb)	100	0.265	88.8	88	ND	79.8	80	4.16	80.8	77
DMC-F01-IPF001-0-041021 (C0040128 Spk E, 10 ppb)	10	0.330	4.48	42	ND	5.34	52	7.40	9.96	26
DMC-F01-IPF001-0-041021 (C0040128 Spk H, 100 ppb)	100	0.330	85.4	86	ND	74.8	75	7.40	81.8	74
DMC-F01-IPF002-0-041021 (C0040129 Spk C, 10 ppb)	10	0.712	7.88	70	ND	8.60	86	14.1	29.8	156
DMC-F01-IPF002-0-041021 (C0040129 Spk H, 100 ppb)	100	0.712	78.0	77	ND	98.0	98	14.1	104	80
DMC-F01-IPW001-0-041021 (C0040130 Spk G, 10 ppb)	10	1.08	7.60	86	ND	7.84	78	70.0	71.8	*
DMC-F01-IPW001-0-041021 (C0040130 Spk I, 100 ppb)	100	1.08	84.8	84	ND	80.0	80	70.0	131	81
DMC-F01-IPW002-0-041021 (C0040131 Spk E, 10 ppb)	10	0.888	6.76	68	ND	7.86	80	34.4	44.8	*
DMC-F01-IPW002-0-041021 (C0040131 Spk J, 100 ppb)	100	0.888	80.8	80	ND	74.8	76	34.4	101	87
DMC-F01-MSW001-0-041021 (C0040132 Spk E, 10 ppb Spk)	10	0.218	8.78	86	0.824	9.44	88	270	270	*
DMC-F01-MSW001-0-041021 (C0040132 Spk F, 100 ppb Spk)	100	0.218	81.2	81	0.824	89.2	83	270	375	106
DMC-F01-MSW002-0-041021 ^a (C0040133 Spk G, 99 ppb Spk)	50	ND ^a	45.2	90	ND ^a	52.0	104	530	470	*
DMC-F01-MSW002-0-041021 ^a (C0040133 Spk H, 808 ppb Spk)	500	ND ^a	432	86	ND ^a	482	96	530	924	79
DL2-F01-IPF001-0-041021 (C0040134 Spk F, 10 ppb Spk)	10	0.880	5.80	51	ND	6.88	88	3.45	8.36	49
DL2-F01-IPF001-0-041021 (C0040134 Spk K, 100 ppb Spk)	100	0.880	46.4	46	ND	58.8	58	3.45	56.4	53
DL2-F01-IPF002-0-041021 (C0040135 Spk G, 10 ppb Spk)	10	0.816	5.84	48	ND	6.40	84	4.72	8.56	38
DL2-F01-IPF002-0-041021 (C0040135 Spk L, 100 ppb Spk)	100	0.816	84.8	84	ND	78.8	79	4.72	72.4	88

*Sample residue exceeds the spiking level significantly; therefore, an accurate recovery value cannot be calculated.

^a 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^a = 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 I.

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 (%)
DL2-F01-IPF003-0-041021 (C0040135 Spk C, 10 ppb Spikes)	10	0.247	7.00	88	ND	7.88	80	2.01	8.20	72
DL2-F01-IPF003-0-041021 (C0040136 Spk H, 100 ppb Spikes)	100	0.247	82.8	83	ND	73.2	73	2.01	74.0	72
DL2-F01-IPF004-0-041021 (C0040137 Spk D, 10 ppb Spikes)	10	0.346	6.84	65	ND	7.60	76	4.52	11.3	66
DL2-F01-IPF004-0-041021 (C0040138 Spk L, 100 ppb Spikes)	100	0.346	67.6	67	ND	76.8	77	4.52	60.4	78
DL2-F01-IPF005-0-041021 (C0040139 Spk E, 10 ppb Spikes)	10	0.338	6.88	65	ND	7.68	77	2.46	9.68	72
DL2-F01-IPF005-0-041021 (C0040140 Spk J, 100 ppb Spikes)	100	0.338	68.4	68	ND	80.8	81	2.46	82.0	80
DL2-F01-IPW001-0-041021 (C0040139 Spk F, 10 ppb Spikes)	10	0.326	7.04	67	ND	7.60	76	5.86	13.9	80
DL2-F01-IPW001-0-041021 (C0040139 Spk K, 100 ppb Spikes)	100	0.326	86.0	66	ND	77.2	77	5.86	65.2	79
DL2-F01-MSF001-0-041021 (C0040140 Spk G, 10 ppb Spikes)	10	0.333	7.36	70	ND	8.20	82	77.2	78.0	*
DL2-F01-MSF001-0-041021 (C0040140 Spk L, 100 ppb Spikes)	100	0.333	64.6	64	ND	77.8	78	77.2	158	81
DL2-F01-MSF002-0-041021 (C0040141 Spk C, 10 ppb Spikes)	10	0.408	6.88	65	ND	7.48	75	94.8	101	*
DL2-F01-MSF002-0-041021 (C0040141 Spk H, 100 ppb Spikes)	100	0.408	61.6	61	ND	68.0	66	94.8	158	81
DL2-F01-MSF003-0-041021 (C0040142 Spk D, 10 ppb Spikes)	10	0.416	6.52	61	ND	6.76	68	41.6	41.2	*
DL2-F01-MSF003-0-041021 (C0040142 Spk L, 100 ppb Spikes)	100	0.416	64.8	64	ND	70.8	71	41.6	104	62
DL2-F01-MSF004-0-041021 (C0040143 Spk E, 10 ppb Spikes)	10	0.345	7.06	67	ND	7.88	78	83.6	91.2	*
DL2-F01-MSF004-0-041021 (C0040143 Spk J, 100 ppb Spikes)	100	0.345	71.6	71	ND	81.2	81	83.6	167	83
DL1-F01-IPF005-0-041021 (C0040144 Spk F, 10 ppb Spikes)	10	0.432	7.44	70	0.281	8.16	79	33.4	40.0	*
DL1-F01-IPF005-0-041021 (C0040144 Spk K, 100 ppb Spikes)	100	0.432	67.2	67	0.281	78.4	78	33.4	108.0	73
DL3-F01-IPF003-0-041021 (C0040145 Spk G, 10 ppb Spikes)	10	0.448	7.20	66	ND	8.08	81	3.56	10.8	73
DL3-F01-IPF003-0-041021 (C0040145 Spk L, 100 ppb Spikes)	100	0.448	68.2	69	ND	81.8	82	3.56	80.0	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 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.

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)
DL3-F01-IPF004-0-041021 (C0040146 Spk G, 10 ppb Spike)	10	0.784	6.84	81	ND	10.6	108	0.382	8.96	86
DL3-F01-IPF004-0-041021 (C0040146 Spk H, 100 ppb Spike)	100	0.784	68.6	88	ND	86.4	88	0.382	81.2	81
DL3-F01-IPF006-0-041021 (C0040147 Spk D, 10 ppb Spike)	10	1.53	5.44	36	ND	9.16	92	5.48	11.7	62
DL3-F01-IPF006-0-041021 (C0040147 Spk L, 100 ppb Spike)	100	1.53	60.4	59	ND	93.2	93	5.48	66.8	81
DBC-F01-IPF001-0-041021 (C0040148 Spk E, 10 ppb Spike)	10	1.34	6.24	46	0.662	10.8	101	NR	NR	NR
DBC-F01-IPF001-0-041021 (C0040148 Spk J, 100 ppb Spike)	100	1.34	56.4	56	0.662	86.8	86	NR	NR	NR
DBC-F01-IPF002-0-041021 (C0040149 Spk F, 10 ppb Spike)	10	1.43	5.84	44	ND	9.36	94	110	113	*
DBC-F01-IPF002-0-041021 (C0040149 Spk K, 100 ppb Spike)	100	1.43	59.2	58	ND	88.0	88	110	189	79
DBC-F01-IPF003-0-041021 (C0040150 Spk G, 10 ppb Spike)	10	0.896	6.72	57	0.644	10.4	98	NR	NR	NR
DBC-F01-IPF003-0-041021 (C0040150 Spk L, 100 ppb Spike)	100	0.896	60.8	68	0.644	88.8	88	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0040151 Spk C, 10 ppb Spike)	10	0.864	7.08	82	2.21	8.88	75	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0040151 Spk H, 100 ppb Spike)	100	0.864	70.0	89	2.21	86.0	96	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0040152 Spk D, 10 ppb Spike)	10	0.700	7.56	88	9.12	16.2	91	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0040152 Spk I, 100 ppb Spike)	100	0.700	68.6	89	9.12	86.0	87	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0040153 Spk E, 10 ppb Spike)	10	0.736	9.32	78	8.76	16.5	97	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0040153 Spk J, 100 ppb Spike)	100	0.736	70.8	70	8.76	80.8	84	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0040154 Spk F, 10 ppb Spike)	10	4.36	10.9	65	1.02	8.48	86	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0040154 Spk K, 100 ppb Spike)	100	4.36	76.8	72	1.02	86.0	85	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0040155 Spk G, 10 ppb Spike)	10	8.76	13.1	63	0.792	9.72	89	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0040155 Spk L, 100 ppb Spike)	100	8.76	78.0	89	0.792	80.0	79	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0040156 Spk C, 10 ppb Spike)	10	5.88	12.8	69	1.02	10.8	96	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0040156 Spk H, 100 ppb Spike)	100	5.88	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-analyse 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			C8 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DOU-F01-JPF004-0-041021 (C0040157 Spk D, 10 ppb Spiked)	10	6.12	13.2	71	2.30	12.0	97	NR	NR	NR
DOU-F01-JPF004-0-041021 (C0040157 Spk I, 100 ppb Spiked)	100	6.12	76.4	70	2.30	88.0	94	NR	NR	NR
DOU-F01-JPF005-0-041021 (C0040158 Spk E, 10 ppb Spiked)	10	4.56	12.2	76	0.956	11.2	102	NR	NR	NR
DOU-F01-JPF005-0-041021 (C0040158 Spk J, 100 ppb Spiked)	100	4.56	76.0	71	0.956	85.8	86	NR	NR	NR
DOU-F01-JPW001-0-041021 (C0040159 Spk F, 10 ppb Spiked)	10	2.89	10.2	73	0.948	10.7	98	NR	NR	NR
DOU-F01-JPW001-0-041021 (C0040159 Spk K, 100 ppb Spiked)	100	2.89	72.4	70	0.948	83.8	83	NR	NR	NR
DOU-F01-JPW002-0-041021 (C0040160 Spk G, 10 ppb Spiked)	10	18.4	34.4	180	3.88	13.2	95	NR	NR	NR
DOU-F01-JPW002-0-041021 (C0040160 Spk L, 100 ppb Spiked)	100	18.4	94.8	76	3.88	88.2	86	NR	NR	NR
DL2-F01-M6SW001-0-041021 (C0040161 Spk I, 10 ppb Spiked)	10	0.203	9.32	91	ND	9.88	97	120	126	*
DL2-F01-M6SW001-0-041021 (C0040161 Spk J, 100 ppb Spiked)	100	0.203	76.8	77	ND	86.2	85	120	177	87
DL2-F01-M6SW002-0-041021 (C0040162 Spk K, 10 ppb Spiked)	10	ND	9.66	97	ND	9.12	91	102	110	*
DL2-F01-M6SW002-0-041021 (C0040162 Spk L, 100 ppb Spiked)	100	ND	88.4	88	ND	93.6	94	102	165	83
DL2-F01-M6SW003-0-041021 (C0040163 Spk C, 10 ppb Spiked)	10	ND	9.34	92	ND	9.08	91	92.4	103	*
DL2-F01-M6SW003-0-041021 (C0040163 Spk D, 100 ppb Spiked)	100	ND	61.2	61	ND	95.2	95	92.4	182	90
DL2-F01-M6SW004-0-041021 (C0040164 Spk E, 10 ppb Spiked)	10	ND	7.36	79	ND	7.26	73	86.0	75.6	*
DL2-F01-M6SW004-0-041021 (C0040164 Spk F, 100 ppb Spiked)	100	ND	82.0	82	ND	93.6	94	86.0	166	76
DL2-F01-M6SW005-0-041021 (C0040165 Spk G, 10 ppb Spiked)	10	ND	7.96	80	ND	8.06	81	168	135	*
DL2-F01-M6SW005-0-041021 (C0040165 Spk H, 100 ppb Spiked)	100	ND	90.8	91	ND	100	103	168	234	86
DBC-F01-M6SW001-0-041104 (C0040166 Spk C, 10 ppb Spiked)	10	0.898	10.3	94	8.32	17.6	93	NR	NR	NR
DBC-F01-M6SW001-0-041104 (C0040166 Spk D, 100 ppb Spiked)	100	0.898	90.4	90	8.32	113	106	NR	NR	NR

*Sample residue exceeds the reporting 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			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)	Amount Found in Sample (ng/g)	Amount Recovered (ng/g)	Recovery (%)
DBC-F01-MSW002-0-041104 (C0040401 Spk E, 10 ppb Spike)	10	0.988	9.88	91	3.43	12.8	94	NR	NR	NR
DBC-F01-MSW002-0-041104 (C0040401 Spk F, 100 ppb Spike)	100	0.988	94.8	94	3.43	113	110	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0040402 Spk G, 10 ppb Spike)	10	0.890	9.92	91	3.62	13.8	100	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0040402 Spk H, 100 ppb Spike)	100	0.890	89.0	85	3.62	104	100	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0040403 Spk I, 10 ppb Spike)	10	0.984	9.98	91	9.72	19.6	98	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0040403 Spk J, 100 ppb Spike)	100	0.984	97.2	96	9.72	124	114	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0040404 Spk K, 10 ppb Spike)	10	0.832	8.20	74	2.77	10.2	74	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0040404 Spk L, 100 ppb Spike)	100	0.832	81.6	81	2.77	108	106	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0040405 Spk M, 10 ppb Spike)	10	0.820	8.80	88	3.58	13.7	101	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0040405 Spk N, 100 ppb Spike)	100	0.820	88.4	88	3.58	108	102	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0040406 Spk O, 10 ppb Spike)	10	0.788	9.92	92	1.94	12.5	108	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0040406 Spk P, 100 ppb Spike)	100	0.788	80.8	90	1.94	102	100	NR	NR	NR
DBC-F01-IPF004-0-041104 (C0040407 Spk Q, 10 ppb Spike)	10	0.504	10.5	100	0.584	11.8	110	142	152	*
DBC-F01-IPF004-0-041104 (C0040407 Spk R, 100 ppb Spike)	100	0.504	94.8	94	0.584	108	106	142	254	112
DBC-F01-IPF005-0-041104 (C0040408 Spk S, 10 ppb Spike)	10	1.80	10.4	88	2.15	12.2	101	NR	NR	NR
DBC-F01-IPF005-0-041104 (C0040408 Spk T, 100 ppb Spike)	100	1.80	80.8	89	2.15	105	103	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0040409 Spk U, 10 ppb Spike)	10	0.484	9.72	92	3.28	13.0	97	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0040409 Spk V, 100 ppb Spike)	100	0.484	87.6	87	3.28	104	101	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0040410 Spk W, 10 ppb Spike)	10	0.572	10.4	98	2.10	13.2	111	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0040410 Spk X, 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

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 I.

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 (ppb)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ppb wet wt.)	Amount Recovered (ppb wet wt.)	Recovery (%)	Amount Found in Sample (ppb wet wt.)	Amount Recovered (ppb wet wt.)	Recovery (%)	Amount Found in Sample (ppb wet wt.)	Amount Recovered (ppb wet wt.)	Recovery (%)
DOU-F01-IPW003-0-041104 (C0040404 Spk K, 10 ppb Spike)	10	3.12	13.1	100	8.00	16.3	103	NR	NR	NR
DOU-F01-IPW003-0-041104 (C0040401 Spk L, 100 ppb Spike)	100	3.12	68.0	85	8.00	107	101	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0040402 Spk C, 10 ppb Spike)	10	3.49	12.8	93	7.12	17.4	103	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0040402 Spk D, 100 ppb Spike)	100	3.49	68.4	85	7.12	108	101	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0040403 Spk E, 10 ppb Spike)	10	2.97	10.3	75	5.12	13.5	84	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0040403 Spk F, 100 ppb Spike)	100	2.97	53.6	81	5.12	109	104	NR	NR	NR
DL2-F01-IPW003-0-041104 (C0040404 Spk G, 10 ppb Spike)	10	1.04	9.48	84	ND	10.4	104	8.20	14.3	61
DL2-F01-IPW003-0-041104 (C0040404 Spk H, 100 ppb Spike)	100	1.04	80.0	79	ND	97.6	98	8.20	124	116
DL1-F01-MSW001-0-041104 (C0040405 Spk I, 10 ppb Spike)	10	1.20	10.6	94	0.898	11.3	104	NR	NR	NR
DL1-F01-MSW001-0-041104 (C0040405 Spk J, 100 ppb Spike)	100	1.20	69.6	88	0.898	112	111	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0040406 Spk K, 10 ppb Spike)	10	1.30	9.76	85	1.72	12.4	107	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0040406 Spk L, 100 ppb Spike)	100	1.30	80.4	79	1.72	100	98	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0040407 Spk C, 10 ppb Spike)	10	0.580	6.62	84	1.13	10.2	91	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0040407 Spk D, 100 ppb Spike)	100	0.580	65.2	85	1.13	96.5	98	NR	NR	NR
DL1-F01-MSW004-0-041104 (C0040408 Spk E, 10 ppb Spike)	10	0.908	6.52	76	0.908	9.76	89	181	201	*
DL1-F01-MSW004-0-041104 (C0040408 Spk F, 100 ppb Spike)	100	0.908	84.8	84	0.908	96.6	99	181	289	108
DL1-F01-MSW005-0-041104 (C0040409 Spk G, 10 ppb Spike)	10	1.18	9.80	78	1.18	10.4	92	NR	NR	NR
DL1-F01-MSW005-0-041104 (C0040409 Spk H, 100 ppb Spike)	100	1.18	82.4	81	1.18	96.8	98	NR	NR	NR
DL1-F01-MSF001-0-041104 (C0040470 Spk I, 10 ppb Spike)	10	ND	7.78	78	0.315	8.52	92	283	303	*
DL1-F01-MSF001-0-041104 (C0040470 Spk J, 100 ppb Spike)	100	ND	78.4	78	0.315	80.8	90	283	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	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		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 (C0040471 Spk K, 10 ppb Spike)	10	ND	8.80	88	0.624	10.5	88	NR	NR	NR
DL1-F01-MSF002-0-041104 (C0040471 Spk L, 100 ppb Spike)	100	ND	84.8	85	0.824	98.4	98	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0040472 Spk E, 10 ppb Spike)	10	0.203	8.78	88	0.852	10.2	95	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0040472 Spk D, 100 ppb Spike)	100	0.203	84.4	84	0.852	95.2	95	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0040473 Spk E, 10 ppb Spike)	10	ND	8.86	89	0.532	10.4	89	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0040473 Spk F, 100 ppb Spike)	100	ND	88.8	87	0.532	101	100	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0040474 Spk G, 10 ppb Spike)	10	ND	8.64	86	0.684	10.1	94	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0040474 Spk H, 100 ppb Spike)	100	ND	82.4	82	0.684	94.0	83	NR	NR	NR
DL1-F01-IPW001-0-041104 (C0040475 Spk I, 10 ppb Spike)	10	0.772	8.64	79	0.556	8.72	92	17.4	26.4	90
DL1-F01-IPW001-0-041104 (C0040475 Spk J, 100 ppb Spike)	100	0.772	84.0	83	0.556	97.8	97	17.4	113	98
DL1-F01-IPW002-0-041104 (C0040476 Spk K, 10 ppb Spike)	10	0.468	8.88	82	0.536	9.88	94	15.7	27.4	117
DL1-F01-IPW002-0-041104 (C0040476 Spk L, 100 ppb Spike)	100	0.468	78.4	76	0.536	88.0	87	15.7	100	84
DL1-F01-IPW003-0-041104 (C0040477 Spk C, 10 ppb Spike)	10	ND	9.08	91	0.208	9.44	92	8.38	18.7	73
DL1-F01-IPW003-0-041104 (C0040477 Spk D, 100 ppb Spike)	100	ND	90.4	90	0.208	106	106	8.38	109	100
DL1-F01-IPW004-0-041104 (C0040478 Spk E, 10 ppb Spike)	10	ND	8.48	85	0.238	9.80	98	58.6	72.0	*
DL1-F01-IPW004-0-041104 (C0040478 Spk F, 100 ppb Spike)	100	ND	71.8	72	0.238	84.8	85	58.6	140	80
DL1-F01-IPF002-0-041104 (C0040479 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 (C0040479 Spk H, 100 ppb Spike)	100	ND	86.4	86	0.222	98.4	98	11.1	108	97
DL1-F01-IPF003-0-041104 (C0040480 Spk I, 10 ppb Spike)	10	ND	8.80	88	0.285	10.8	103	20.9	30.5	98
DL1-F01-IPF003-0-041104 (C0040480 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 I.
 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				C5 Sulfonate PFHS				C8 Sulfonate PFOS			
	Amount Spiked (ng/g)	Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)	Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)	Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)		
DL-1-F01-SPF004-0-041104 (C0040401 Spk K, 10 ppb Spikes)	10	ND	8.00	80	ND	9.48	95	7.80	15.8	82		
DL-1-F01-SPF004-0-041104 (C0040401 Spk L, 100 ppb Spikes)	100	ND	84.8	85	ND	98.8	98	7.80	102	84		
DL-1-F01-SPF006-0-041104 (C0040402 Spk C, 10 ppb Spikes)	10	ND	7.44	74	0.373	9.00	88	8.72	15.9	72		
DL-1-F01-SPF006-0-041104 (C0040402 Spk D, 100 ppb Spikes)	100	ND	84.8	85	0.373	98.4	98	8.72	102	83		
DL3-F01-MBW001-0-041105 (C0040403 Spk E, 10 ppb Spikes)	10	ND	8.38	84	ND	9.20	82	138	185	*		
DL3-F01-MBW001-0-041105 (C0040403 Spk F, 100 ppb Spikes)	100	ND	85.6	86	ND	98.8	100	138	256	119		
DL3-F01-MBW002-0-041105 (C0040404 Spk G, 10 ppb Spikes)	10	ND	8.44	84	ND	9.82	98	180	150	*		
DL3-F01-MBW002-0-041105 (C0040404 Spk H, 100 ppb Spikes)	100	ND	80.4	80	ND	106	108	180	283	103		
DL3-F01-MBW003-0-041105 (C0040405 Spk I, 10 ppb Spikes)	10	ND	8.80	88	ND	8.68	98	146	154	*		
DL3-F01-MBW003-0-041105 (C0040405 Spk J, 100 ppb Spikes)	100	ND	88.4	88	ND	102	102	146	264	108		
DL3-F01-MBW004-0-041105 (C0040406 Spk K, 10 ppb Spikes)	10	ND	8.48	85	0.286	9.88	94	196	225	*		
DL3-F01-MBW004-0-041105 (C0040406 Spk L, 100 ppb Spikes)	100	ND	88.4	88	0.286	102	102	186	301	103		
DL3-F01-MBW005-0-041105 (C0040407 Spk C, 10 ppb Spikes)	10	ND	9.58	96	0.386	11.1	107	173	180	*		
DL3-F01-MBW005-0-041105 (C0040407 Spk D, 100 ppb Spikes)	100	ND	100	100	0.386	105	106	173	246	73		
DL3-F01-MBF001-0-041105 (C0040408 Spk E, 10 ppb Spikes)	10	ND	9.48	95	ND	11.4	114	118	138	*		
DL3-F01-MBF001-0-041105 (C0040408 Spk F, 100 ppb Spikes)	100	ND	84.8	85	ND	108	108	118	234	118		
DL3-F01-MBF002-0-041105 (C0040409 Spk G, 10 ppb Spikes)	10	ND	9.88	98	ND	10.4	104	118	85.8	*		
DL3-F01-MBF002-0-041105 (C0040409 Spk H, 100 ppb Spikes)	100	ND	88.6	90	ND	103	103	118	218	100		
DL3-F01-MBF003-0-041105 (C0040410 Spk I, 10 ppb Spikes)	10	ND	9.32	93	ND	11.0	110	75.6	82.0	*		
DL3-F01-MBF003-0-041105 (C0040410 Spk J, 100 ppb Spikes)	100	ND	80.8	81	ND	107	107	75.6	180	114		

*Sample matrix 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				C5 Sulfonate PFHS				C8 Sulfonate PFOS			
	Amount Spiked (ng/g)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)		
DL3-F01-MSF004-0-041105 (C0040401 Spk K, 10 ppb Spike)	10	ND	8.80	88	ND	11.0	110	130	144	*		
DL3-F01-MSF004-0-041105 (C0040401 Spk L, 100 ppb Spike)	100	ND	90.4	90	ND	104	104	130	236	108		
DL3-F01-MSF005-0-041105 (C0040402 Spk C, 10 ppb Spike)	10	ND	8.92	89	ND	10.3	103	81.8	87.2	*		
DL3-F01-MSF005-0-041105 (C0040402 Spk D, 100 ppb Spike)	100	ND	85.6	86	ND	98.4	98	81.8	181	98		
DMC-F01-IPW003-0-041105 (C0040403 Spk E, 10 ppb Spike)	10	ND	8.28	83	0.308	9.52	91	61.2	78.0	*		
DMC-F01-IPW003-0-041105 (C0040403 Spk F, 100 ppb Spike)	100	ND	84.4	84	0.386	99.6	98	61.2	155	95		
DMC-F01-IPW004-0-041105 (C0040404 Spk G, 10 ppb Spike)	10	0.300	8.04	77	0.308	9.28	80	66.8	107	*		
DMC-F01-IPW004-0-041105 (C0040404 Spk H, 100 ppb Spike)	100	0.300	90.4	90	0.396	104	104	66.8	168	98		
DMC-F01-IPW005-0-041105 (C0040405 Spk I, 10 ppb Spike)	10	ND	8.98	90	0.270	10.1	98	84.4	75.8	*		
DMC-F01-IPW005-0-041105 (C0040405 Spk J, 100 ppb Spike)	100	ND	88.4	88	0.270	105	106	84.4	163	88		
DMC-F01-MSW003-0-041105 (C0040406 Spk K, 10 ppb Spike)	10	ND	9.64	96	0.662	11.0	104	354	389	*		
DMC-F01-MSW003-0-041105 (C0040406 Spk L, 100 ppb Spike)	100	ND	92.4	92	0.582	108	108	354	484	110		
DMC-F01-MSW004-0-041105 (C0040407 Spk C, 10 ppb Spike)	10	0.318	9.52	92	1.90	12.1	102	258	258	*		
DMC-F01-MSW004-0-041105 (C0040407 Spk D, 100 ppb Spike)	100	0.318	82.4	82	1.90	101	98	258	313	54		
DMC-F01-MSW005-0-041105 (C0040408 Spk E, 10 ppb Spike)	10	ND	8.16	92	0.880	11.8	108	NR	NR	NR		
DMC-F01-MSW005-0-041105 (C0040408 Spk F, 100 ppb Spike)	100	ND	93.8	94	0.980	118	117	NR	NR	NR		
DMC-F01-MSF002-0-041105 (C0040409 Spk G, 10 ppb Spike)	10	ND	8.38	84	ND	10.4	104	NR	NR	NR		
DMC-F01-MSF002-0-041105 (C0040409 Spk H, 100 ppb Spike)	100	ND	87.8	88	ND	104	104	NR	NR	NR		
DMC-F01-MSF003-0-041105 (C0040410 Spk I, 10 ppb Spike)	10	ND	8.20	82	ND	10.4	104	66.8	108	*		
DMC-F01-MSF003-0-041105 (C0040410 Spk J, 100 ppb Spike)	100	ND	87.2	87	ND	110	110	66.8	184	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	Amount Spiked (ng/g)	C4 Sulfolane PFBS			C8 Sulfolane PFHS			C8 Sulfolane PFOS		
		Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DMC-F01-MSF004-0-041105 (C0048901 Spk K, 10 ppb Spike)	10	ND	8.76	88	0.254	11.1	108	116	123	*
DMC-F01-MSF004-0-041105 (C0048901 Spk L, 100 ppb Spike)	100	ND	86.0	86	0.254	107	107	116	226	110
DMC-F01-MSF005-0-041105 (C0048902 Spk C, 10 ppb Spike)	10	ND	8.52	85	0.420	12.2	118	146	193	*
DMC-F01-MSF005-0-041105 (C0048902 Spk D, 100 ppb Spike)	100	ND	83.6	84	0.420	110	110	146	280	114
DMC-F01-IPF003-0-041105 (C0048903 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	10.6	106	7.92	16.0	81
DMC-F01-IPF003-0-041105 (C0048903 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	106	106	7.92	101	83
DMC-F01-IPF004-0-041105 (C0048904 Spk G, 10 ppb Spike)	10	ND	7.92	79	ND	10.7	107	44.0	56.2	*
DMC-F01-IPF004-0-041105 (C0048904 Spk H, 100 ppb Spike)	100	ND	83.2	83	ND	107	107	44.0	134	90
DMC-F01-IPF005-0-041105 (C0048905 Spk I, 10 ppb Spike)	10	ND	7.88	79	ND	10.3	103	46.4	54.4	*
DMC-F01-IPF005-0-041105 (C0048905 Spk J, 100 ppb Spike)	100	ND	75.8	76	ND	96.4	96	46.4	121	75
DL2-F01-IPW005-0-041216 (C0054454 Spk E, 10 ppb Spike)	10	ND	8.44	84	ND	10.0	100	49.2	59.2	100
DL2-F01-IPW005-0-041216 (C0054454 Spk F, 100 ppb Spike)	100	ND	83.2	83	ND	102	102	49.2	142	93
DL2-F01-IPW006-0-041216 (C0054455 Spk C, 10 ppb Spike)	10	ND	8.00	80	ND	11.5	115	13.2	22.7	95
DL2-F01-IPW006-0-041216 (C0054455 Spk D, 100 ppb Spike)	100	ND	84.8	85	ND	107	107	13.2	121	108
DOU-F01-MSW002-0-041216 (C0054456 Spk I, 10 ppb Spike)	10	0.206	6.92	67	1.72	10.9	82	NR	NR	NR
DOU-F01-MSW002-0-041216 (C0054456 Spk J, 100 ppb Spike)	100	0.206	67.2	67	1.72	92.8	91	NR	NR	NR
DL2-F01-IPW004-0-041216 (C0054457 Spk C, 10 ppb Spike)	10	ND	8.08	81	ND	10.4	104	6.80	14.6	90
DL2-F01-IPW004-0-041216 (C0054457 Spk D, 100 ppb Spike)	100	ND	76.8	77	ND	96.2	95	6.80	97.2	90
DOU-F01-MSW003-0-041216 (C0054458 Spk K, 10 ppb Spike)	10	ND	8.12	81	10.4	19.4	80	NR	NR	NR
DOU-F01-MSW003-0-041216 (C0054458 Spk L, 100 ppb Spike)	100	ND	68.4	68	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 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			C5 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DLS-F01-IPW004-0-041130 (C0000409 Spk E, 10 ppb Spiked)	10	ND	7.52	75	ND	11.0	110	7.92	17.3	94
DLS-F01-IPW004-0-041130 (C0000409 Spk F, 100 ppb Spiked)	100	ND	86.4	86	ND	118	118	7.92	114	106
DOU-F01-MSW001-0-041216 (C0000408 Spk G, 10 ppb Spiked)	10	7.06	14.6	75	15.4	27.5	121	NR	NR	NR
DOU-F01-MSW001-0-041216 (C0000408 Spk H, 100 ppb Spiked)	100	7.06	83.2	76	25.4	122.0	107	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0000401 Spk E, 10 ppb Spiked)	10	4.72	10.8	81	21.7	29.3	76	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0000401 Spk F, 100 ppb Spiked)	100	4.72	72.8	89	21.7	104	82	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0000402 Spk G, 10 ppb Spiked)	10	0.364	7.06	67	1.34	8.52	72	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0000402 Spk H, 100 ppb Spiked)	100	0.364	72.0	72	1.34	87.6	86	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0000403 Spk G, 10 ppb Spiked)	10	1.41	8.80	72	8.40	17.8	62	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0000403 Spk H, 100 ppb Spiked)	100	1.41	72.0	71	8.40	94.0	88	NR	NR	NR
DLS-F01-IPW002-0-041130 (C0000404 Spk E, 10 ppb Spiked)	10	ND	7.92	79	ND	9.84	90	12.0	16.6	48
DLS-F01-IPW002-0-041130 (C0000404 Spk F, 100 ppb Spiked)	100	ND	81.2	81	ND	110	110	12.0	105	93
DLS-F01-IPW003-0-041130 (C0000405 Spk G, 10 ppb Spiked)	10	ND	7.98	80	ND	8.98	89	8.44	12.3	69
DLS-F01-IPW003-0-041130 (C0000405 Spk H, 100 ppb Spiked)	100	ND	84.4	84	ND	102	102	8.44	92.0	87
DOU-F01-MSF003-0-041216 (C0000406 Spk K, 10 ppb Spiked)	10	1.72	9.00	73	8.56	14.3	77	NR	NR	NR
DOU-F01-MSF003-0-041216 (C0000406 Spk L, 100 ppb Spiked)	100	1.72	89.8	89	8.56	107	100	NR	NR	NR
DEC-F01-MSF006-1-041216 (C0000407 Spk G, 10 ppb Spiked)	10	0.220	8.36	81	1.78	11.5	67	NR	NR	NR
DEC-F01-MSF006-1-041216 (C0000407 Spk H, 100 ppb Spiked)	100	0.220	83.6	83	1.78	96.6	95	NR	NR	NR
DEC-F01-MSF006-0-041216 (C0000408 Spk K, 10 ppb Spiked)	10	ND	8.36	84	1.88	11.4	97	NR	NR	NR
DEC-F01-MSF006-0-041216 (C0000408 Spk L, 100 ppb Spiked)	100	ND	80.8	81	1.88	98.6	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 (ppb)	Amount Found in Sample (ppb)	Recovery (%)	Amount Spiked (ppb)	Amount Found in Sample (ppb)	Recovery (%)	Amount Spiked (ppb)	Amount Found in Sample (ppb)	Recovery (%)
DOU-F01-MSF002-0-041216 (C0086468 Spk B, 10 ppb Spike)	10	0.888	8.24	84	4.98	14.5	98	NR	NR
DOU-F01-MSF002-0-041216 (C0086468 Spk H, 100 ppb Spike)	100	0.888	83.6	83	4.96	104	96	NR	NR
DL3-F01-IPW001-0-041130 (C0086470 Spk C, 10 ppb Spike)	10	ND	7.80	78	0.246	0.80	96	9.04	17.2
DL3-F01-IPW001-0-041130 (C0086470 Spk D, 100 ppb Spike)	100	ND	82.4	82	0.246	101	101	9.04	102
DOU-F01-MSF002-1-041216 (C0086471 Spk I, 10 ppb Spike)	10	0.840	9.20	84	4.68	14.5	98	NR	NR
DOU-F01-MSF002-1-041216 (C0086471 Spk J, 100 ppb Spike)	100	0.840	82.0	81	4.68	103	98	NR	NR
DOU-F01-MSF004-0-041216 (C0086472 Spk K, 10 ppb Spike)	10	0.792	7.76	70	2.35	8.80	66	NR	NR
DOU-F01-MSF004-0-041216 (C0086472 Spk L, 100 ppb Spike)	100	0.792	81.2	80	2.35	86.8	83	NR	NR
DOU-F01-MSF001-0-041216 (C0086473 Spk E, 10 ppb Spike)	10	2.18	11.1	89	10.9	20.8	98	NR	NR
DOU-F01-MSF001-0-041216 (C0086473 Spk F, 100 ppb Spike)	100	2.18	82.8	81	10.9	104	93	NR	NR
DBC-F01-MSF003-0-041216 (C0086474 Spk G, 10 ppb Spike)	10	ND	8.48	86	2.09	11.8	98	NR	NR
DBC-F01-MSF003-0-041216 (C0086474 Spk M, 100 ppb Spike)	100	ND	84.0	84	2.09	108	104	NR	NR
DBC-F01-MSF004-0-041216 (C0086475 Spk I, 10 ppb Spike)	10	ND	7.72	77	3.87	12.8	90	NR	NR
DBC-F01-MSF004-0-041216 (C0086475 Spk J, 100 ppb Spike)	100	ND	75.8	78	3.87	98.8	95	NR	NR
DMC-F01-MSF001-0-041216 (C0086476 Spk C, 10 ppb Spike)	10	ND	9.08	91	0.420	9.08	87	NR	NR
DMC-F01-MSF001-0-041216 (C0086476 Spk D, 100 ppb Spike)	100	ND	88.0	88	0.420	102	102	NR	NR
DL1-F01-IPW005-0-041216 (C0086477 Spk I, 10 ppb Spike)	10	0.253	7.40	71	0.844	8.96	93	68.8	83.2
DL1-F01-IPW005-0-041216 (C0086477 Spk J, 100 ppb Spike)	100	0.253	72.4	72	0.844	88.8	88	68.8	145
DL2-F01-IPW003-0-041201 (C0086478 Spk K, 10 ppb Spike)	10	ND	7.04	70	ND	9.04	90	7.48	13.7
DL2-F01-IPW003-0-041201 (C0086478 Spk L, 100 ppb Spike)	100	ND	71.8	72	ND	92.0	92	7.48	84.0

*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)	Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)	Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)		Amount Found in Sample (ng/g wet wt.)	Amount Recovered (ng/g wet wt.)	Recovery (%)	
DL3-F01-SPW005-0-041201 (C0000479 Spk 1, 10 ppb Spike)	10	ND	8.16	82	ND	8.96	90		4.88	14.0	91	
DL3-F01-SPW005-0-041201 (C0000479 Spk 2, 100 ppb Spike)	100	ND	94.8	95	ND	97.2	97		4.88	94.4	90	
Average:				79	Average:				Average:			
Standard Deviation:				13	Standard Deviation:				Standard Deviation:			

*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 V. Matrix Spike Recovery of PFOS in Re-extracted Fish Samples

Sample Description	Amount Spiked (ng/g)	C8 Sulfonate PFOS		
		Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DBC-F01-IPW001-0-041021 (C0048151 Spk C, 1000 ppb)	1000	2080	2880	78
DBC-F01-IPW002-0-041021 (C0048162 Spk D, 5000 ppb)	5000	3330	8680	107
DBC-F01-IPW003-0-041021 (C0048153 Spk E, 1000 ppb)	1000	1680	3180	150
DOU-F01-IPF001-0-041021 (C0048154 Spk F, 1000 ppb)	1000	586	1780	116
DOU-F01-IPF002-0-041021 (C0048155 Spk G, 1000 ppb)	1000	638	1740	110
DOU-F01-IPF005-0-041021 (C0048156 Spk H, 1000 ppb)	1000	544	1650	111
DOU-F01-IPW002-0-041021 (C0048160 Spk I, 1000 ppb)	1000	1210	2610	140
DBC-F01-MSW001-0-041104 (C0048490 Spk J, 10,000 ppb)	10000	9480	23200	137
DBC-F01-MSW002-0-041104 (C0048451 Spk K, 10,000 ppb)	10000	7520	16800	91
DBC-F01-MSW003-0-041104 (C0048452 Spk L, 10,000 ppb)	10000	12600	23200	108
DBC-F01-MSW004-0-041104 (C0048453 Spk M, 5000 ppb)	5000	3440	8520	102
DBC-F01-MSW005-0-041104 (C0048454 Spk N, 10,000 ppb)	10000	5240	15200	100
DBC-F01-MSF001-0-041104 (C0048455 Spk O, 10,000 ppb)	10000	5240	14400	92
DBC-F01-MSF002-0-041104 (C0048456 Spk P, 10,000 ppb)	10000	4760	15200	104
DBC-F01-IPF005-0-041104 (C0048458 Spk Q, 1000 ppb)	1000	608	1600	98
DBC-F01-IPW004-0-041104 (C0048459 Spk R, 1000 ppb)	1000	1230	2590	136
DBC-F01-IPW005-0-041104 (C0048460 Spk S, 5000 ppb)	5000	2620	6520	78
DOU-F01-IPW003-0-041104 (C0048461 Spk T, 1000 ppb)	1000	2270	2640	37*
DOU-F01-IPW004-0-041104 (C0048462 Spk U, 1000 ppb)	1000	1740	2740	100
DOU-F01-IPW005-0-041104 (C0048463 Spk V, 5000 ppb)	5000	2130	7600	109
DL1-F01-MSW001-0-041104 (C0048465 Spk W, 1000 ppb)	1000	968	1860	86
DL1-F01-MSW002-0-041104 (C0048466 Spk X, 1000 ppb)	1000	708	1780	107
DL1-F01-MSW003-0-041104 (C0048467 Spk Y, 1000 ppb)	1000	698	1930	123
DL1-F01-MSW005-0-041104 (C0048468 Spk Z, 1000 ppb)	1000	604	1680	106

*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)**

Sample Description	Amount Spiked (ng/g)	C8 Sulfonate PFOS		
		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	666	1090	112
DL1-F01-MSF005-0-041104 (C0048472 Spk G, 1000 ppb)	1000	432	1670	124
DL1-F01-MSF006-0-041104 (C0048474 Spk H, 1000 ppb)	1000	740	1830	109
DMC-F01-MSW005-0-041105 (C0048489 Spk I, 1000 ppb)	1000	580	1780	122
DMC-F01-MSF002-0-041105 (C0048498 Spk J, 1000 ppb)	1000	620	1500	88
DOU-F01-MSW002-0-041216 (C0058446 Spk K, 10,000 ppb)	10000	26200	32700	45
DOU-F01-MSW003-0-041216 (C0058458 Spk L, 8000 ppb)	5000	4040	10200	123
DOU-F01-MSW001-0-041216 (C0058460 Spk C, 10000 ppb)	10000	7600	25000	174*
DOU-F01-MSW004-0-041216 (C0058481 Spk D, 8000 ppb)	5000	5120	9880	96
DOU-F01-MSW005-0-041216 (C0058482 Spk E, 1000 ppb)	1000	496	1780	126
DOU-F01-MSF005-0-041216 (C0058483 Spk F, 1000 ppb)	1000	720	1620	110
DOU-F01-MSF003-0-041216 (C0058486 Spk G, 1000 ppb)	1000	846	1720	87
DBC-F01-MSF006-1-041216 (C0058487 Spk H, 10000 ppb)	10000	9320	32400	230*
DBC-F01-MSF006-0-041216 (C0058488 Spk I, 10000 ppb)	10000	8960	24100	151
DOU-F01-MSF002-0-041216 (C0058489 Spk J, 1000 ppb)	1000	640	1720	108
DOU-F01-MSF002-1-041216 (C0058471 Spk K, 1000 ppb)	1000	692	1950	126
DOU-F01-MSF004-0-041216 (C0058472 Spk L, 1000 ppb)	1000	1620	2700	88
DOU-F01-MSF001-0-041216 (C0058473 Spk C, 8000 ppb)	5000	2070	7960	118
DBC-F01-MSF003-0-041216 (C0058474 Spk D, 10000 ppb)	10000	4640	15500	108
DBC-F01-MSF004-0-041216 (C0058475 Spk E, 10000 ppb)	10000	9280	24500	152
DMC-F01-MSF001-0-041216 (C0058476 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	293	1340	106
DOU-F01-IPF003-0-041021 (C0048155 Spk I, 1000 ppb)	1000	359	1400	104

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

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-01-CPW001-G-041216 (C0000400 Spk C, 10 ppb)	10	ND	5.98	80	ND	6.18	82	1.82	6.32	46
DL3-01-CPW001-G-041216 (C0000400 Spk C, 100 ppb)	100	ND	82.4	82	ND	95.5	96	1.82	85.2	63
DMC-01-CPW001-G-041216 (C0000401 Spk E, 10 ppb)	10	ND	9.04	80	ND	9.44	94	6.18	11.3	51
DMC-01-CPW001-G-041216 (C0000401 Spk F, 100 ppb)	100	ND	82.8	83	ND	98.8	98	6.18	92.8	87
DL2-01-CPW001-G-041216 (C0000402 Spk G, 10 ppb)	10	ND	7.28	73	ND	7.84	78	1.81	7.52	57
DL2-01-CPW001-G-041216 (C0000402 Spk H, 100 ppb)	100	ND	83.6	84	ND	101	101	1.81	88.4	87
DBC-01-CPW001-G-041216 (C0000403 Spk I, 10 ppb)	10	ND	10.3	103	ND	10.8	108	4.48	13.0	88
DBC-01-CPW001-G-041216 (C0000403 Spk J, 100 ppb)	100	ND	84.4	84	ND	87.2	87	4.48	88.4	82
DL1-01-CPW001-G-041216 (C0000404 Spk K, 10 ppb)	10	ND	8.98	90	ND	9.32	93	8.80	16.0	82
DL1-01-CPW001-G-041216 (C0000404 Spk L, 100 ppb)	100	ND	86.2	86	ND	103	103	8.80	96.8	88
DOU-01-CPW001-G-041216 (C0000405 Spk M, 10 ppb)	10	ND	8.48	85	0.202	8.32	81	11.4	17.2	88
DOU-01-CPW001-G-041216 (C0000405 Spk N, 100 ppb)	100	ND	84.4	84	0.202	95.0	98	11.4	98.0	87
Average:		84			83			73		
Standard Deviation:		10			13			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

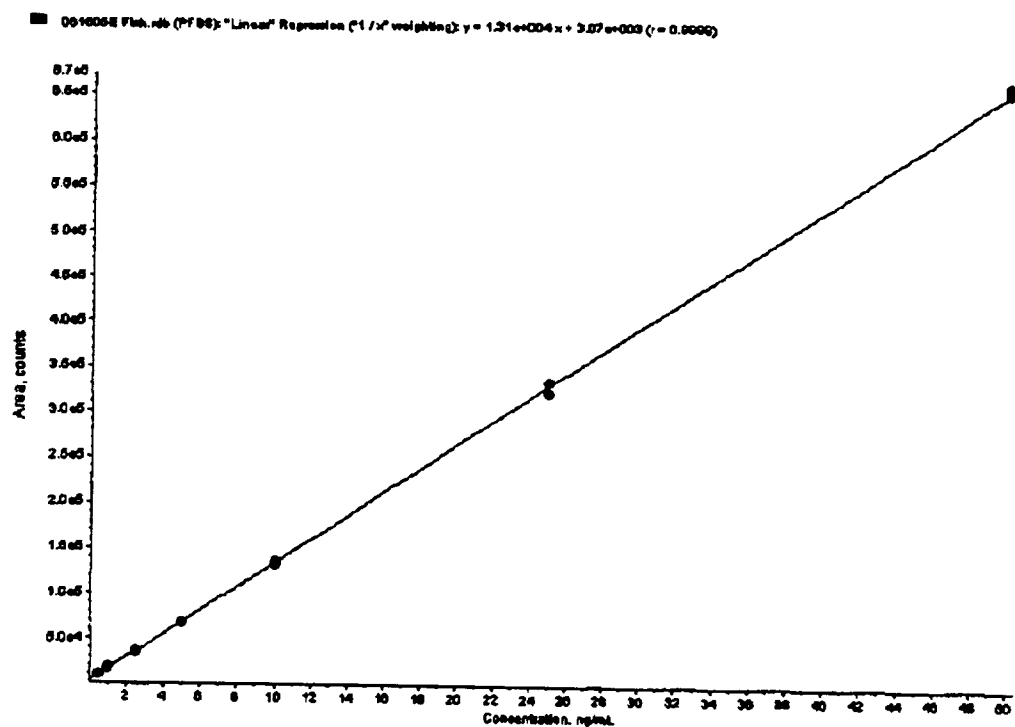
Figure 1. Typical Calibration Curve for PFBS in Methanol

Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.0005 $\mu\text{g/mL}$ and 0.001 $\mu\text{g/mL}$, Respectively

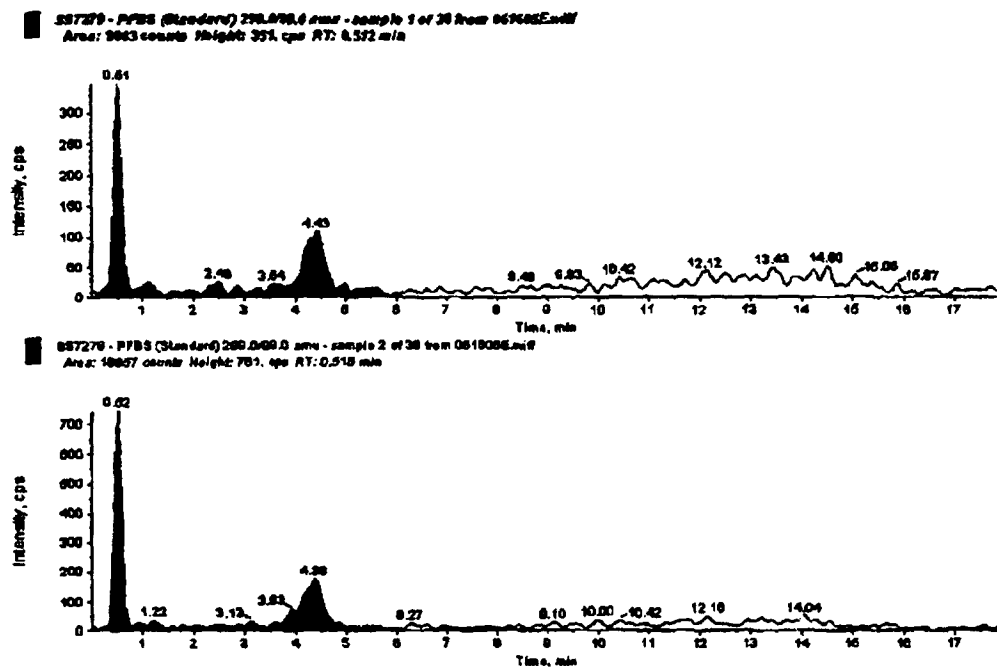


Figure 3. PFBS in Control Fish Blank, 0.00625 $\mu\text{g/mL}$ Fortified Control Fish Spk A, and 0.025 $\mu\text{g/mL}$ Fortified Control Fish Spk B, Respectively

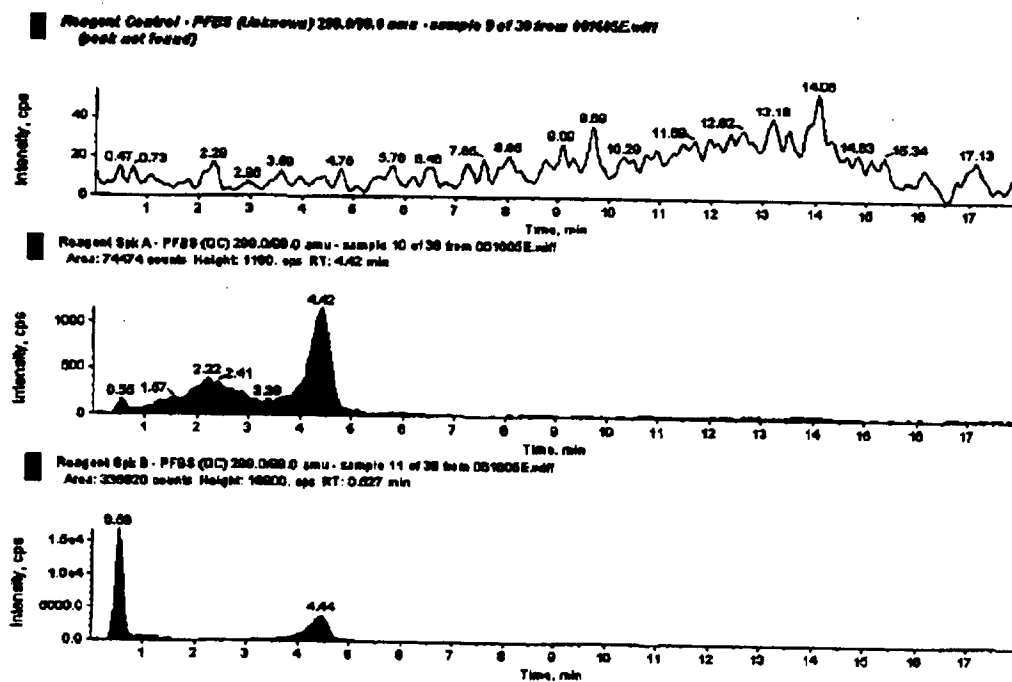


Figure 4. Chromatogram Representing a Fish Sample Analyzed for PFBS (Exygen ID: C0049478, Data Set:061605E)

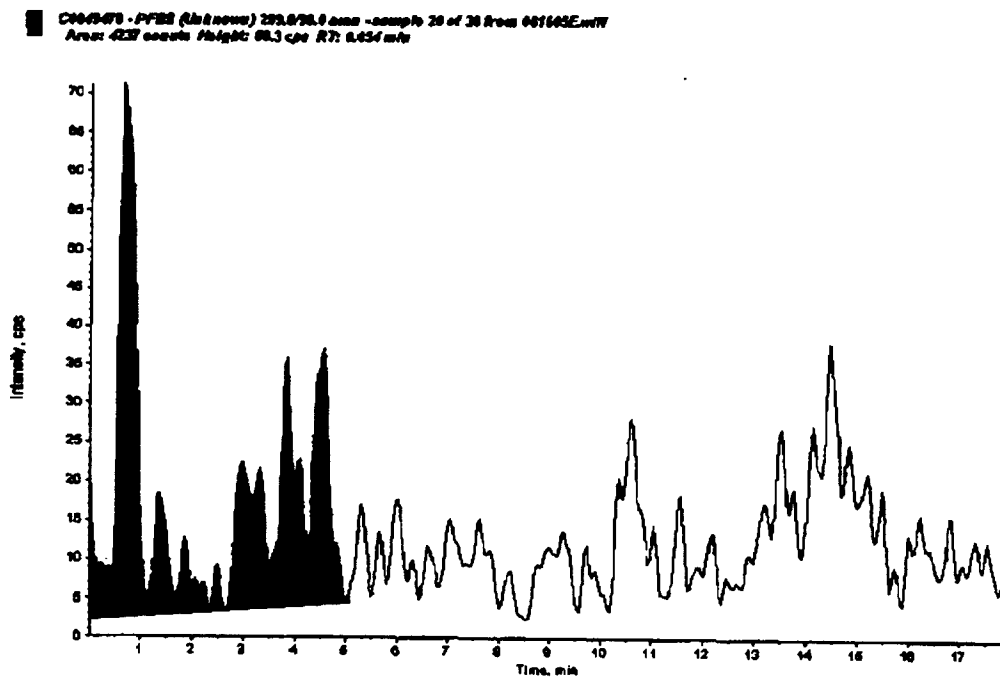


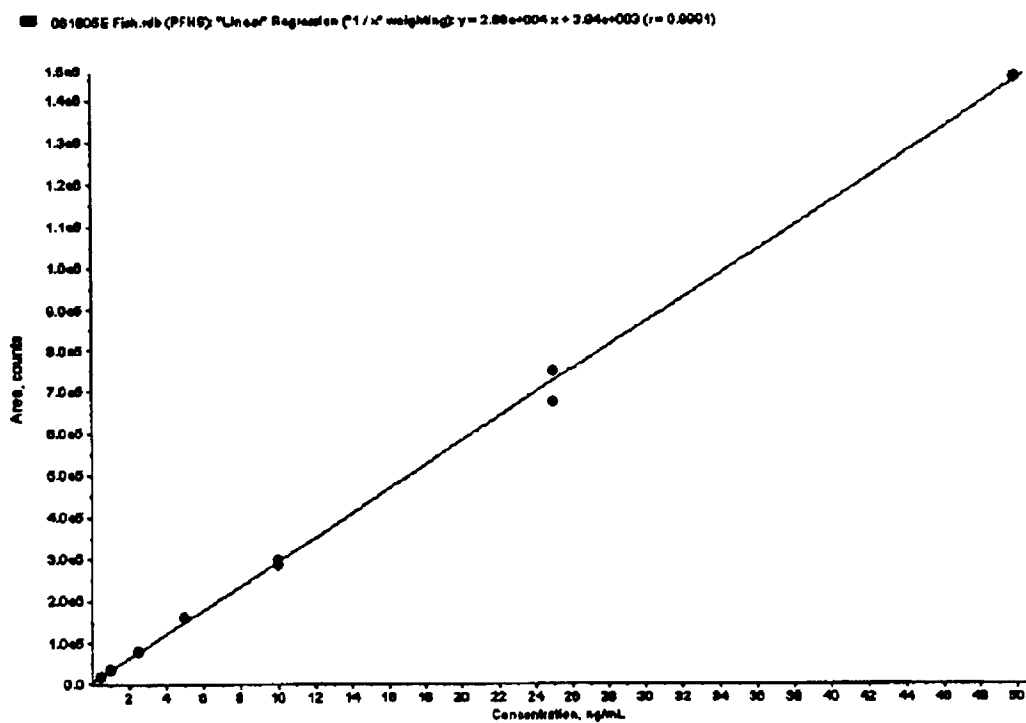
Figure 5. Typical Calibration Curve for PFHS in Methanol

Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.0005 $\mu\text{g/mL}$ and 0.001 $\mu\text{g/mL}$, Respectively

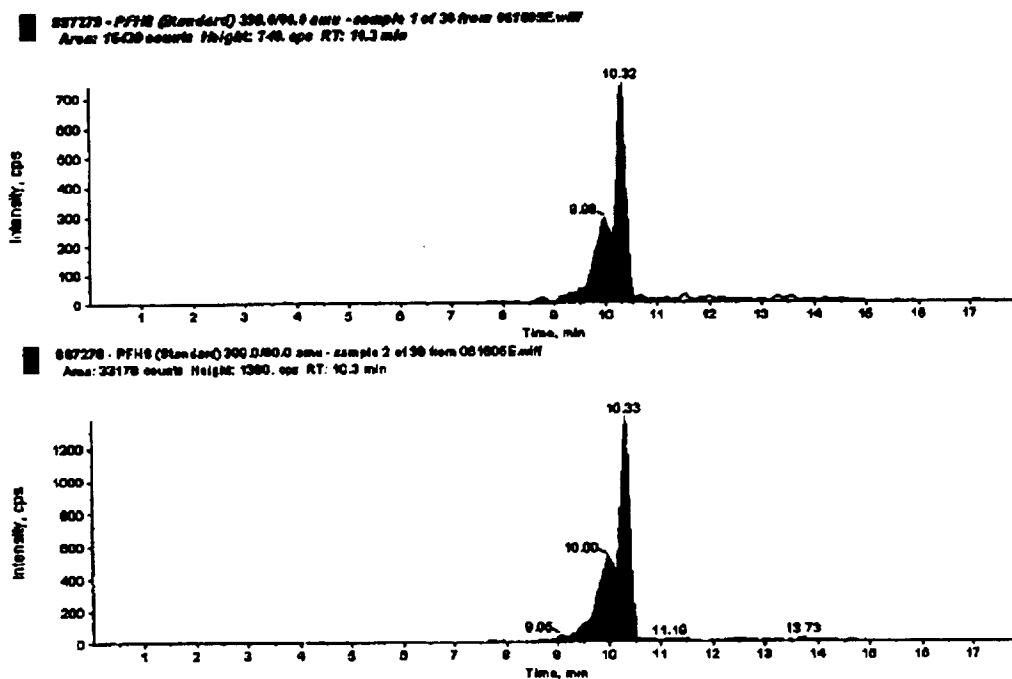


Figure 7. PFHS in Control Fish Blank, 0.00625 $\mu\text{g/mL}$ Fortified Control Fish Spk A, and 0.025 $\mu\text{g/mL}$ Fortified Control Fish Spk B, Respectively

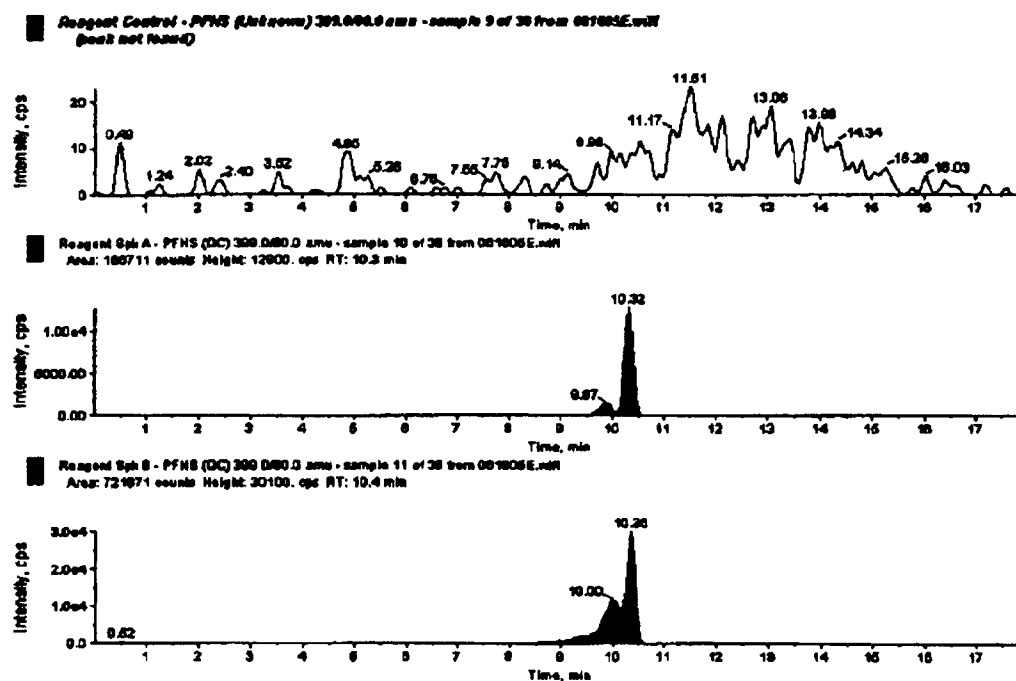


Figure 3. Chromatogram Representing a Fish Sample Analyzed for PFHS (Exygen ID: C0049478, Data Set: 061605E)

■ C0049478 - PFHS (Unknown) 399.4/96.0 amu - sample 20 of 20 from M1605E.mw
Area: 29070 counts Height: 1738 cps RT: 10.3 min

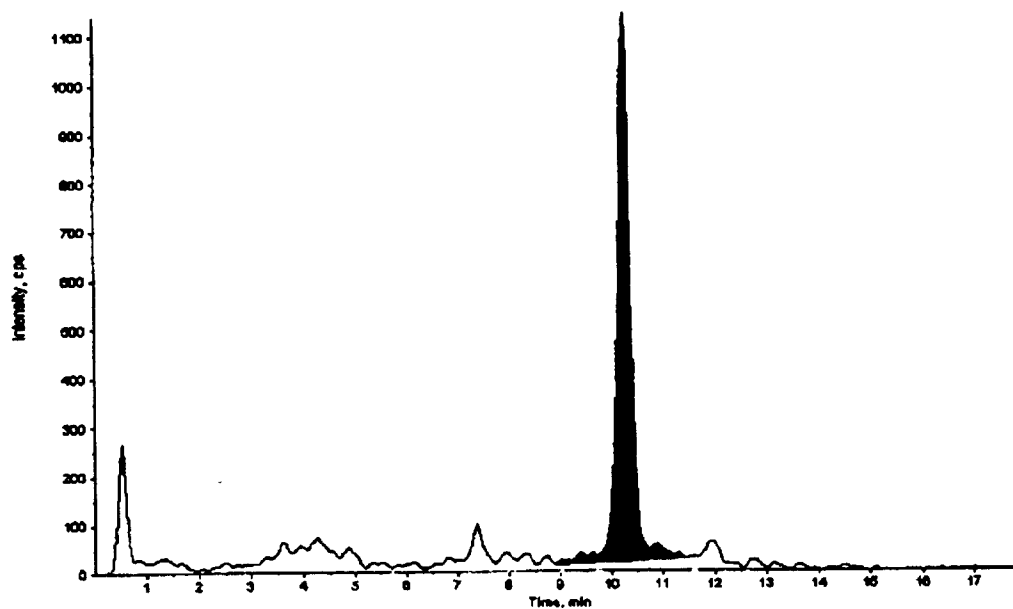


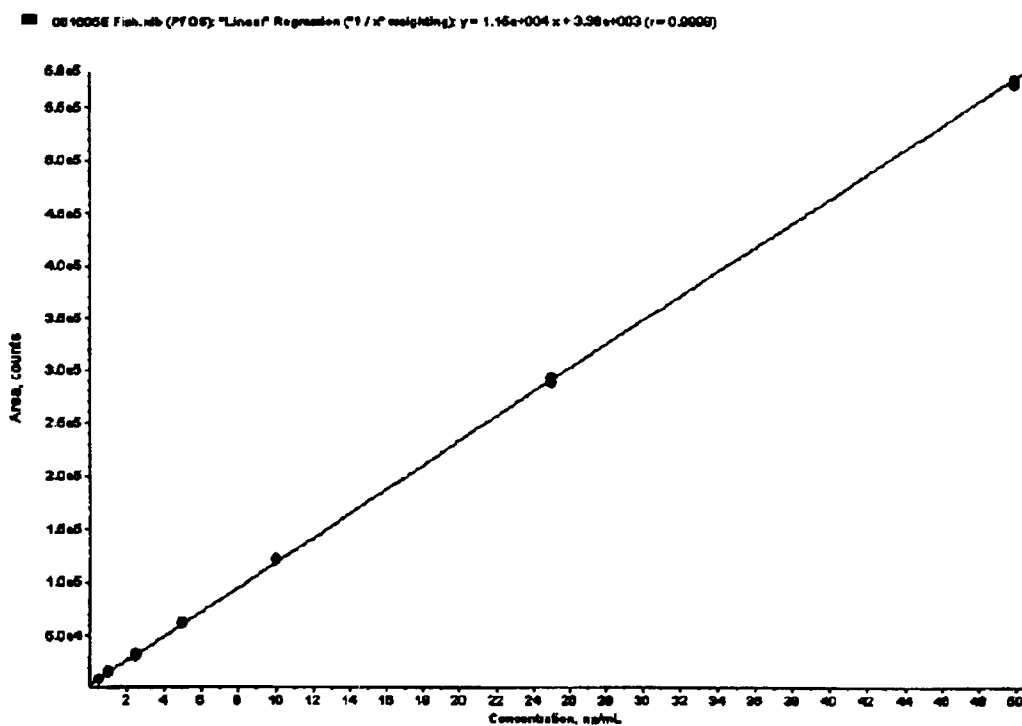
Figure 9. Typical Calibration Curve for PFOS in Methanol

Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.0005 $\mu\text{g/mL}$ and 0.001 $\mu\text{g/mL}$, Respectively

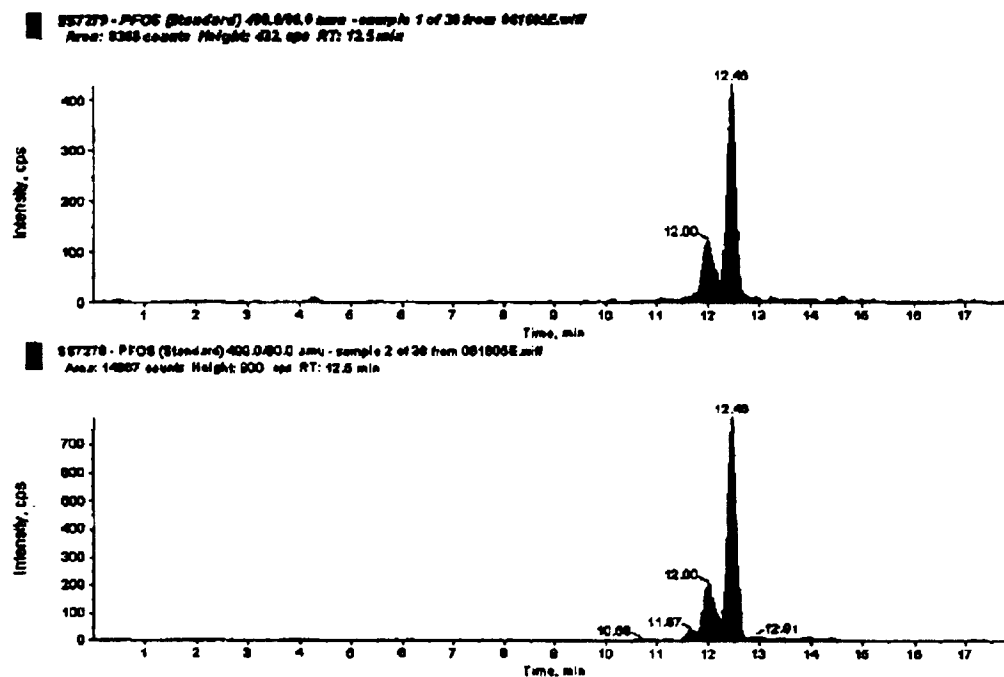
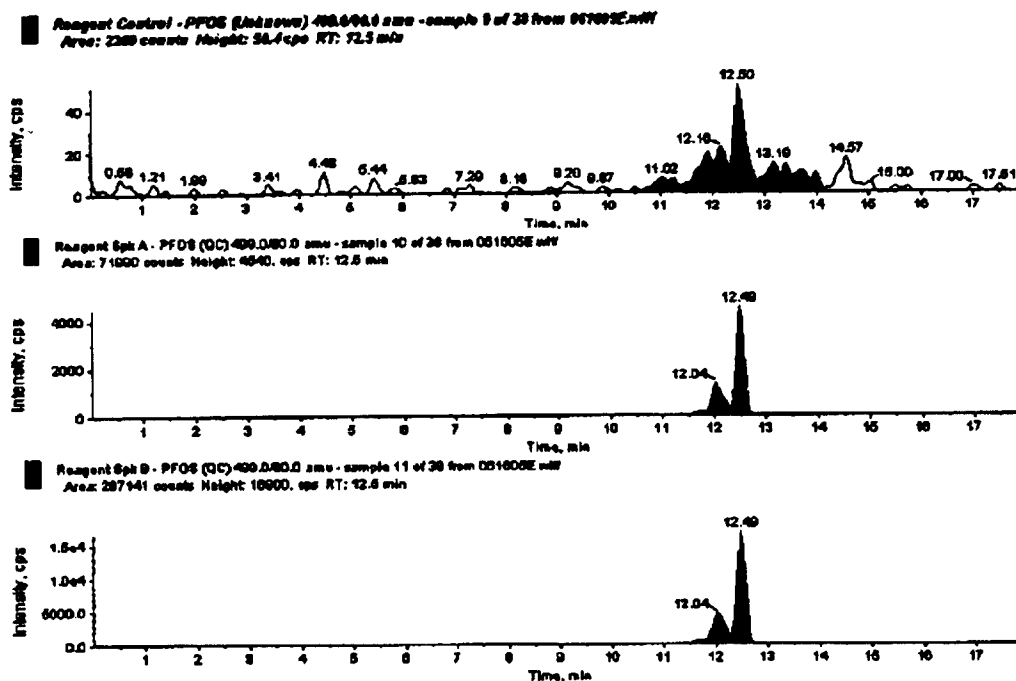


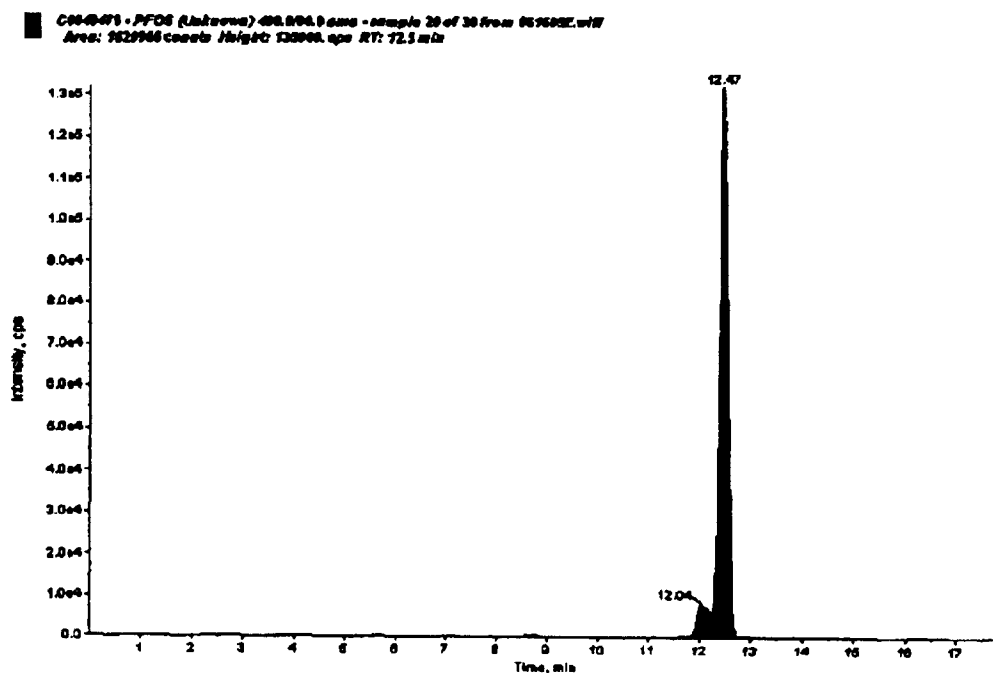
Figure 11. PFOS in Control Fish Blank, 0.00625 $\mu\text{g/mL}$ Fortified Control Fish Spk A, and 0.025 $\mu\text{g/mL}$ Fortified Control Fish Spk B, Respectively



Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

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**

Page 1 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

DISTRIBUTION:

- 1) Jaishirha 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

Page 2 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

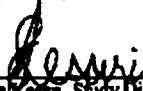
Exygen Protocol Number: P0001131

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


Jaimin K. Kesh, 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

11/29/04
Date


Richard A. Grazzini, President, Facility Management
Exygen Research

21 - OCT - 2004
Date


Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research

11/29/04
Date

Page 3 of 65

Exygen Protocol Number: P0001131

TABLE OF CONTENTS

TITLE PAGE	1
DISTRIBUTION	2
PROTOCOL APPROVAL	3
TABLE OF CONTENTS	4
INTRODUCTION	5
TEST MATERIALS	5
OBJECTIVE	6
TESTING FACILITY	6
STUDY DIRECTOR	7
SPONSOR REPRESENTATIVE	7
PRINCIPAL INVESTIGATOR	7
PROPOSED EXPERIMENTAL START AND TERMINATION DATES	7
IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM	8
SAMPLE PROCUREMENT, RECEIPT AND RETENTION	8
SAMPLE IDENTIFICATION	9
ANALYTICAL PROCEDURE SUMMARY	9
VERIFICATION OF ANALYTICAL PROCEDURE	9
METHOD FOR CONTROL OF BIAS	11
STATISTICAL METHODS	11
GLP STATEMENT	11
REPORT	11
SAFETY AND HEALTH	12
AMENDMENTS TO PROTOCOL	13
DATA RECORD KEEPING	13
QUALITY ASSURANCE	14
RETENTION OF DATA AND ARCHIVING	14
APPENDIX I. ANALYTICAL METHODS	15

Page 4 of 65

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.

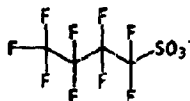
This study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

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

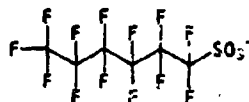
PFBS

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: SE0036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



Page 5 of 65

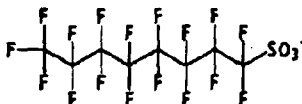
Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

PFOs

Chemical Name: Perfluorooctanesulfonate
 Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3K$)
 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
 State College, PA 16801
 Phone: (814) 272-1039

Page 6 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

STUDY DIRECTOR

Jaisirahs Kesari P.E., DEE
Weston Solutions, Inc.
1400 Weston Way
West Chester, PA 19380
Phone: (610) 701-3761
Fax: (610) 701-7401
j.kesari@westonsolutions.com

SPONSOR REPRESENTATIVE

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

PRINCIPAL INVESTIGATOR

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

Page 7 of 63

Exygen Protocol Number: P0001131

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 assess 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.

Page 8 of 65

Exygen Protocol Number: P0001131

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 pre-cleaned Sci/Spec Premier wide mouth HDPE bottles. These bottles have been routinely used for fluorochemical sample

Page 9 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

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 (¹³C PFOA). PFOA and ¹³C PFOA are included in the solutions used to spike the samples. The results for PFOA and ¹³C 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

Page 10 of 63

Exygen Protocol Number: P0001131

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).

Page 11 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

- 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).

Page 12 of 61

Exygen Protocol Number: P0001131

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., $\mu\text{g/mL}$).

Page 13 of 65

Exygen Protocol Number: P0001131

- 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.

Page 14 of 63

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"

Page 15 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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
3058 Research Drive
State College, PA 16801

Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research08/24/04
Date
John Fishery
Vice President, Operations, Exygen Research08/24/04
Date

Total Pages: 7

Page 16 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number Y0001700

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 mL/min 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 polystyrene centrifuge tubes.
- 5.5 15 mL disposable polystyrene centrifuge tubes.
- 5.6 Disposable micropipettes (20-100 µL, 100-500 µL).
- 5.7 125-mL LSWH 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 or (1g) K18 SPE cartridges.

Page 2 of 7

Page 17 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

- 5.12 SP8 vacuum manifold.
- 5.13 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.
- 6.0 Chromatographic System
- 6.1 Analytical Column: Fluoropak RP (Kjodane Scientific), 2.1 mm x 50 mm, 5 μ (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 μ L (can be increased to as much as 50 μ L).
- 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 $\rightarrow$ 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.

Page 3 of 12

Page 18 of 63

Interim Report #10-Analysis of Fish and Ciam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Manual

Method Number V0001786

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/Portification Solutions

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (assumed for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 10 µg/mL portification 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 portification 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 portification 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 portification 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 portification solutions are to be stored in a refrigerator or 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 Portification Solution (µg/L)	Portification Volume (µL)	Volume of Portified Control Sample (mL)	Final Concentration of Calibration Standard (µg/L)	Calibration Standard ID (example)
0	0	40	0	XCambdyy-0
10	100	40	25	XCambdyy-1
10	200	40	20	XCambdyy-2
10	400	40	100	XCambdyy-3
100	100	40	250	XCambdyy-4
100	200	40	500	XCambdyy-5
100	400	40	1000	XCambdyy-6

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

Page 4 of 7

Page 19 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research	Method Number: V0001780
ANALYTICAL METHOD	
Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS	
P2.3	A zero standard solution (reagent blank) must be prepared with each set of standards extracted.
P2.4	Store all extracted calibration standards in 15-mL polypropylene vials at 2°C to 6°C, up to two weeks.
P2.5	Alternate volumes and concentrations of standards may be prepared as needed.
10.0	Batch Set Up
10.1	Each batch of samples extracted (typically 30 or less) must include at least one reagent control (method blank, using HPLC water) and two reagent controls fortified at known concentrations (lab control spikes) 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 cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 3 mL of HPLC water (~2 drops/mL). 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 mixed 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 analysis by linear regression using 1/x weighting of peak area

Page 3 of 3

Page 20 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001780

ANALYTICAL METHOD

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

various calibration standard concentrations 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 ions from a parent of 413 ions. The 413 ions parent corresponds to the PFOA anion, while the daughter ion (369 ions) represents the loss of carbon dioxide.
- 13.2 Blank matrix 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 matrix not used for re-analysis.
- 13.3 Recovery 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-analyzed. 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 Hughes Box 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 assigned standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). 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 $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the run must be reanalyzed.

14.0 Calculations

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

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

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

Page 4 of 7

Page 21 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001790

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 (%) =

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

Page 7 of 7

Page 22 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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
3055 Research Drive
State College, PA 16801

Approved By:


Paul C. Kelly
Technical Leader, LC-MS, Exygen Research10/24/04
Date
Jim Palmer
Vice President, Operations, Exygen Research10/24/04
Date

Total Pages: 7

Page 23 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001701

ANALYTICAL METHODMethod 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 Spectrometer Detector (LC/MS/MS) in soil.

2.0 Safety

- 2.1 Always observe with 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 μ L, 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 20 mL disposable polystyrene centrifuge tubes.
5.5 15 mL disposable polystyrene centrifuge tubes.
5.6 Disposable microtubes (20-100 μ L, 100-200 μ L).
5.7 125-mL LSWB narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autosamplers (100-1000 μ L and 10-100 μ L), with disposable tips.
5.11 Waters Sep Pak Vac 8 or (1g) K18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Ultrasonic bath.

Page 2 of 7

Page 24 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001751

ANALYTICAL METHOD

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

- 1.14 Wide-orifice detector.
 1.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Phenomenex RP (Kymene Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-0521.00)
 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
2.0	35	65	0.3
20.0	35	65	0.3
22.5	65	35	0.3

- 6.6 Injection Volume: 10 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 LC/MS 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.

Page 1 of 1

Page 25 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number V00011781

ANALYTICAL METHOD

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

9.0: Standard Preparation

9.1: Standard Stock/Portification Solution

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFQA 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 PFQA 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 PFQA 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 PFQA 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 PFQA 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 (µg/L)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final	
			Concentration of Calibration Standard (ng/L)*	Calibration Standard ID (example)
0	0	40	0	XCumadity-0
10	100	40	25	XCumadity-1
10	200	40	50	XCumadity-2
10	400	40	100	XCumadity-3
100	100	40	250	XCumadity-4
100	200	40	500	XCumadity-5
100	400	40	1000	XCumadity-6

* The assumed concentration of the calibration standard is equal to its initial concentration, due to the concentration of the standard during the extraction (RPE).
XC = uncorrected calibration standard.

Page 4 of 7

Page 26 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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**

- 9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards analyzed.
- 9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.
- 9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.
- 10.0 Batch Set Up
- 10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.
- 11.0 Sample Extraction
- 11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
- 11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes.
- 11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.
- 11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.
- 11.5 Centrifuge for ~10 minutes at ~3000 rpm.
- 11.6 Condition the C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~2 drops). Do not let column run dry.
- 11.7 Load (desorb) the sample on the conditioned C₁₈ SPE cartridge. Discard eluate.
- 11.8 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
- 11.9 Analyze samples using electrospray LC/MS/MS.
- 12.0 Chromatography
- 12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
- 12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
- 12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of

Page 5 of 7

Page 17 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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**

extracted calibration standards may be injected at the beginning of a run 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 *Model.you 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 Chromatogram must show a peak of a daughter ion at 369 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) 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 Responses 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 Hugh Bowes 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^2) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). 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 time between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the run must be reanalyzed.

Page 4 of 7

Page 28 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001721

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sed 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 Lysat software program:

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

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

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

Recovery (%) =

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

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

$$\text{PFOA found (ppb)} = \frac{(\text{PFOA found (ng/L)} \times \text{volume extracted (0.05 L)})}{\text{sample weight (g)}}$$

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

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

Page 7 of 7

Page 29 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

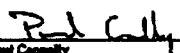
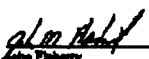
Method Number: V0001782

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

Analytical Testing Facility:

Exygen Research
3059 Research Drive
State College, PA 16801

Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research10/28/04
Date
John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Page 30 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Number	Method Number V0001763
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 Spectrometer Detector (LC/MS/MS) in sediment.
2.0	Safety
2.1	Always observe safe laboratory practices.
2.2	Consult the appropriate MSDS before handling any chemical for proper safety precautions.
3.0	Sample Requirement
3.1	At least 30 g of test sample for extraction.
3.2	No sample prewashing 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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2	A device to collect raw data for peak integration and quantitation.
5.3	A cylinder balance capable of reading to 0.00001 g.
5.4	30 mL disposable polystyrene centrifuge tubes.
5.5	15 mL disposable polystyrene centrifuge tubes.
5.6	Disposable micropipets (50-100 μ L, 100-200 μ L).
5.7	125-mL LDPE narrow-mouth bottles.
5.8	3 mL clear HPLC vial kit.
5.9	Disposable pipettes.
5.10	Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
5.11	Waters Sep Pak Var 6 or (1g) C18 SPE cartridges.
5.12	SPE vacuum manifold.

Page 2 of 7

Page 31 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0801762

ANALYTICAL METHOD

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

5.13 Voucher:
5.14 Wash-solvent diluent:
5.15 Centrifuge capable of spinning 30 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluorophase RP (Keysome Scientific), 2.1 mm x 50 mm, 5µ (P/N: E2505-022100)
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 MS/MS mode, monitoring 413 → 340 m/z for PFPA.

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.

Page 3 of 7

Page 32 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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

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 minimum 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 (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/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

Page 4 of 7

Page 33 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number W0001702

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 4°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 submitted (typically 20 or less) must include at least one unspiked control and two unspiked controls fortified at known concentrations (all tested spikes to verify procedural recovery for the batch).
- 10.2 Requirements for field and laboratory duplicate 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 34 mL of 1% acetic acid, cap, vortex and shake on a wrist action shaker for ~40 minutes.
- 11.3 Centrifuge the tubes at ~3000 rpm for ~30 minutes.
- 11.4 Condition the C₁₈ 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.
- 11.5 Load (desorb) the sample on the conditioned C₁₈ SPE cartridge. Discard eluate.
- 11.6 Add 20 mL of methanol to the effluent 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 ~30 minutes.
- 11.8 Desorb 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 C₁₈ 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.

Page 3 of 7

Page 34 of 65

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0801702

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 run.
- 12.3 An entire set of external calibration standards must be injected 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 amount 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 MetaLynx 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 after loss a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) 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/L, then a new blank sample must be obtained and the entire set must be re-analyzed.
- 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-analyzed. Any matrix spikes 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 Hughes River 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 30% of the total number of extracted standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration curves 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.

Page 6 of 7

Page 33 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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 $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the run must be reanalyzed.

14.8 Calculations

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

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

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

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

Recovery (%) =

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

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

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

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

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

Page 7 of 7

Page 36 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method Number: V0001783

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

Analytical Testing Facility:

Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

P. J. Cull
Paul Cull
Technical Leader, LC-MS, Exygen Research10/26/04
DateJohn Flannery
John Flannery
Vice President, Operations, Exygen Research10/26/04
Date

Total Pages: 8

Page 37 of 63

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	
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 Requirements	3.1 At least 20 g of test sample for extraction. 3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. 3.3 Sample collection procedures will be specified in the sampling plan for this project.
4.0 Reagents and Standards	4.1 Water - HPLC grade 4.2 Acetonitrile - HPLC grade 4.3 Carbon (120-400 mesh) - Reagent grade 4.4 Methanol - HPLC grade 4.5 Silica gel (60-200 mesh) - Reagent grade 4.6 Fluka (90-100 mesh) - Reagent grade 4.7 Supraclean LC-MS - Reagent grade 4.8 1-Octanol - HPLC grade 4.9 L-Ascorbic acid - Reagent grade 4.10 Dimethylsiloxane - Reagent grade 4.11 Toluene - Reagent grade 4.12 Acetonitrile - 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.

Page 2 of 4

Page 18 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0881781

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 Tissue/sox.
- 5.6 125 mL pear-shaped flask.
- 5.7 50 mL disposable polystyrene centrifuge tubes.
- 5.8 15 mL disposable polystyrene centrifuge tubes.
- 5.9 Disposable micropipets (50-100 μ L, 100-200 μ L).
- 5.10 125-mL LDPE fume-proof bottles.
- 5.11 2 mL clear HPLC vial kit.
- 5.12 Disposable pipettes.
- 5.13 Autopipettor (100-1000 μ L and 10-400 μ L), with disposable tips.
- 5.14 SPE tubes (20mL) (Supelco cat. no. N657177).
- 5.15 Vial action shaker.
- 5.16 Centrifuge capable of spinning 50 mL polystyrene tubes at 2000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Fluorophase RP (Kinesis Scientific), 2.1 mm x 50 mm, 5 μ (P/N: 82905-032130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 1 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: 12 μ L (can be increased to as much as 50 μ L).
- 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.

Page 2 of 8

Page 39 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Oxygen Research	Method Number V0001783
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 Retention 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% Dimethylchloroform in toluene is prepared by bringing 3 mL of Dimethylchloroform to a final volume of 10 mL with toluene.	
Alternate volumes may be prepared.	
9.0 Standard Preparation	
9.1 Standard Stock/Portioning 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.	

Page 4 of 5

Page 40 of 45

Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001741

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 stock solution.

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

Concentration of Perfluorooctanoic Acid (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.005
1.0	2.5	100	0.015
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 LC/MS 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 instrument control and two instrument controls fortified at known concentrations (this 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 (Rusby or similar, replace lid and mix well).

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

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

11.4 Vortex and condition the SPE tubes and elution the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g Barield, 2 g silica gel, 2 g octadecyl, and 1 g LC-MS. Condition the columns with 20 mL of methanol, then 30 mL of acetonitrile. Discard all washes. Do not allow the columns to dry.

11.6 Elute the 125 mL pear-shaped flasks by rinsing with the 30% dimethylformamide 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.

Page 5 of 8

Page 41 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001703

ANALYTICAL METHOD

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

- 11.7 Centrifuge the 30 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.
 - 11.8 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shaped flask.
 - 11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Resuspend the brown fat phase using a vortexer for ~30 seconds and run the vortexer 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 eluate from the initial extraction.
 - 11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.
 - 11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).
 - 11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.
 - 11.17 Transfer the extract 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 injections in a sample set.
 - 12.4 Use linear standard curves for quantification. Linear standard curves are generated for the analysis by linear regression using 1/x weighting of peak area versus calibration standard concentration using MetaLynx 3.3 (or equivalent) software system.

Page 4 of 6

Page 42 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research	Method Number: V0001780
ANALYTICAL METHOD	
Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS	

- (2.5) Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

- 12.1 Chromatogram must show a peak of a daughter ion at 369 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) 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-analyzed.
- 13.3 Responses 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-analyzed.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hughes River 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 (R² 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 Chem Lynx software program:

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

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

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

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

Page 7 of 8

Page 43 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001101

ANALYTICAL METHOD

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

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

Recovery (%) =

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

Page 6 of 8

Page 44 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

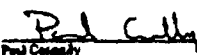
ANALYTICAL METHOD
Method Number: V0001784

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

Analytical Testing Facility:

Exygen Research
3000 Research Drive
State College, PA 16801

Approved By:


Paul Cossady
Technical Leader, LC-MS, Exygen Research10/26/04
Date
John Pomeroy
Vice President, Operations, Exygen Research
Date

Total Pages: 7

Page 45 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research	Method Number V0001754
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 quantization of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in vegetation.
2.0 Safety	2.1 Always observe safe laboratory practices. 2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.
3.0 Sample Requirement	3.1 At least 20 g of test sample for extraction. 3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. 3.3 Sample collection procedures will be specified in the sampling plan for this project.
4.0 Reagents and Standards	4.1 Water - HPLC grade 4.2 Acetonitrile - HPLC grade 4.3 Carbon (120-400 mesh) - Reagent grade 4.4 Methanol - HPLC grade 4.5 Silica gel (60-200 mesh) - Reagent grade 4.6 Fluka (60-100 mesh) - Reagent grade 4.7 Suprapur LC-MS - Reagent grade 4.8 1-Octanol - HPLC grade 4.9 L-Ascorbic acid - Reagent grade 4.10 Dimethylchlorosilane - Reagent grade 4.11 Toluene - Reagent grade 4.12 Argon/Carbon Dioxide - 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 mL/min equipped with a variable volume injector capable of injecting 1-200 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.

Page 3 of 7

Page 46 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Reagen Research Method Number V0001704

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, pear-shaped flask.
- 5.6 50 mL, disposable polystyrene centrifuge tubes.
- 5.7 15 mL, disposable polystyrene centrifuge tubes.
- 5.8 Disposable microplate (36-160uL, 100-200uL).
- 5.9 125-mL, LDPE screw-mouth bottles.
- 5.10 2 mL, clear HPLC vial lot.
- 5.11 Disposable pipettes.
- 5.12 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.13 96B tubes (20mL) (Shimadzu cat. no. MS7177).
- 5.14 Waste action station.
- 5.15 Centrifuge capable of spinning 50 mL polystyrene tubes at 2000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Phenomenex RP (Keystone Scientific), 2.1 mm $\times$ 50 mm, 5 μ (P/N: 62345-022130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 1 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 μ L (can be increased to as much as 50 μ L).
- 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 $\rightarrow$ 349 m/z for PFOA.

Page 3 of 7

Page 47 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research	Method Number V0001704
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 LC/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% Dimethylchlorosulfone in toluene is prepared by bringing 3 mL of dimethylchlorosulfone to a final volume of 10 mL with toluene.	
Alternate volumes may be prepared.	
9.0 Standard Preparation	
9.1 Standard Stock/fortification Solutions	
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.	

Page 6 of 7

Page 48 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number W0001704

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 Perfluorooctanoic Acid (ppm)	Volume (mL)	Diluted to (mL)	Final Concentration (ppm)
1.0	1.0	100	0.01
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.005	10	100	0.0005
0.1	10	100	0.01
0.001	10	100	0.0001

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 Setup

- 10.1 Each batch of samples submitted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (see control table) 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 (verify no needles, syringes lid and seal, wall).
- 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 ~3000 rpm for ~10 minutes.
- 11.4 Tuck and condition the SPE tubes and minimize the pour-shaped flasks.
- 11.5 Push the 30 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-MS. Condition the columns with 30 mL of methanol, then 30 mL of acetonitrile. Discard all wastes. Do not allow the columns to dry.
- 11.6 Simulate the 125 mL pour-shaped flasks by rinsing with the 10% dimethylsiloxane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flask 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 pour-shaped flask. Collect the eluate in the 125 mL simulated pour-shaped flask.

Page 5 of 7

Page 49 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0004754

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

- 11.8 Add 30 mL of methanol to the sample in the 50 mL centrifuge tube.
 - 11.9 Shake the sample again for ~10 minutes on a vortex mixer.
 - 11.10 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~5 minutes.
 - 11.11 Decant the extract into 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 extract 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 method samples.
 - 12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical run.
 - 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 quantification. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using Microsoft Excel (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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.

Page 6 of 7

Page 50 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001764

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 Hogg River 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 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the solvent set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ 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:

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

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

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

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 estimate the percent recovery.

Recovery (%) =

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

Page 7 of 7

Page 31 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method Number: V0001703

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFPA) in Small
Marine Mollusks by LC/MS/MS

Analysis Testing Facility:

Exygen Research
3628 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Phiberty
John Phiberty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Page 52 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Oxygen Research	Method Number V0001705
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 Requirements	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 bead processor and homogenize with dry ice. Place the samples in containers and leave open in fume storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in fume 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 vials/pipets (50-100µL, 100-200µL). 5.7 125-mL LLDPE narrow-mouth bottles. 5.8 2 mL clear HPLC vial kit.

Page 1 of 1

Page 53 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exogen Research Method Number V0001781

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 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
- 5.11 Waters Sep Paks Plus 6 or (1g) HPLC 20 µm cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Titration.
- 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 (Keyence Scientific), 2.1 mm x 50 mm, 3µ (P/N: 82304-022130)
- 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 (µL/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 30 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 Mod.: Electrospray Negative MS/MS mode, monitoring 413 → 360 m/z for PFOA.

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

Page 3 of 7

Page 54 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0004705

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 Solutions**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (verified 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 minimum 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 (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
100	2.0	100	2.0
100	2.0	100	2.0
100	1.0	100	1.0
2.0	10	100	0.2
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.

Page 4 of 7

Page 55 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

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	
10.0 Blank Set Up	
10.1	Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two spiked controls fortified at known concentrations (the 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 Preparation	
11.1	Weigh 1 g of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that chemical weights of liver may be measured depending on the sample also available for use.
11.2	Add water to the sample for a final volume of 10 mL.
11.3	Homogenize sample using a Sonomixer 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 cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~3 drops). 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 (the amount for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
12.4	Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

Page 3 of 7

Page 36 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Oxygen Study No.: P0001131

Oxygen Protocol Number: P0001131

Oxygen Research

Method Number V0001765

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

run on calibration standard concentration using Method 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 Chromatogram must show a peak of a daughter ion at 369 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LLOQ. 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 Replicate 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 Neter 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^2) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.992$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards to the subsequent set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than ± 0.5 minutes in 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

Page 6 of 7

Page 37 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001795

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Mammal Liver 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 (%) =

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

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

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

Page 1 of 7

Page 58 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001756

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Mammal Species by LC/MS/MSAnalytical Testing Facility: Exygen Research
3004 Research Drive
State College, PA 16801

Approved By:


Paul Connelly
Technical Leader, LC-MS, Exygen Research10/26/04
Date
Mike Piskary
Vice President, Operations, Exygen Research10/26/04
Date

Total Pages: 7

Page 39 of 43

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001754

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, serum samples must be allowed to completely thaw to room temperature before use.
- 3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

- 5.1 A high performance liquid chromatograph capable of pumping up to 2 mL/min equipped with a variable volume injector capable of injecting 5-200 μ L, 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 30 mL disposable polypropylene centrifuge tubes.
- 5.5 15 mL disposable polypropylene centrifuge tubes.
- 5.6 Disposable micropipettes (50-1000 μ L, 100-2000 μ L).
- 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 μ L and 10-400 μ L) with disposable tips.
- 5.11 Waters Sep Pak Vac 8 or (1g) AC18 SR5 cartridges.
- 5.12 HPLC vacuum manifold.
- 5.13 Vortexer.

Page 1 of 1

Page 60 of 61

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research Method Number V000176a

ANALYTICAL METHOD

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

- 5.14 Vial-sealant eluent.
 5.15 Cartridge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Fluorophase RP (Kymene Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82965-832130)
 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.

Alternate volumes may be prepared.

Page 3 of 7

Page 61 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001796

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 (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	1.0	100	5.0
100	1.5	100	2.8
100	1.8	100	1.0
5.0	10	100	0.5
2.8	30	100	0.2
1.0	10	100	0.1

- 9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.
- 9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

- 10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (in 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.

Page 4 of 7

Page 62 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001796

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 20 mL polypropylene centrifuge tubes (serum as needed, replace lid and mix well). Note that accurate 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 methanol and shake for ~20 minutes on a wrist-action shaker.
- 11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.
- 11.7 Remove the supernatant into a 20 mL disposable centrifuge tube and add 35 mL of water.
- 11.8 Condition the C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~2 drops). Do not let column run dry.
- 11.9 Load the sample on conditioned C₁₈ SPE cartridge. Discard eluate.
- 11.10 Elute with ~3 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 At least one 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 known standard curves for quantitation. Linear standard curves are generated for the analysis by linear regression using 1/x weighting of peak area versus calibration standard concentration using Minitab 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.

Page 5 of 7

Page 63 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001764

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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) 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-analyzed.
- 13.3 Recovery 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-analyzed. Any matrix spikes 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 NIST 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 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the affected set of samples should be re-analyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be re-analyzed.

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 data Lynx software program:

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

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.

Recovery (%) =

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

Page 6 of 7

Page 64 of 65

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001705

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 ug/mL to ppb.

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

Page 7 of 7

Page 63 of 63

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

3058 Research Drive
State College, PA 16801Phone: 814-272-1039
Fax: 814-231-1580**PROTOCOL AMENDMENT**Amendment Number: 1Effective Date: 01/19/05Exygen 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.

[Signature]
Study Director Signature

3/9/05
Date

[Signature]
Principal Investigator Signature

2/2/05
Date

[Signature]
Study Director Management Signature

N/A
Date

[Signature]
Sponsor Signature (if required)

2/16/05
Date

Exygen QAU Review LJS or LMK

LIBRARY ID: VV0001228-6

ADMINISTRATIVE FORM

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

3058 Research Drive
State College, PA 16801Phone: 814-272-1039
Fax: 814-231-1580

PROTOCOL AMENDMENT

Amendment Number: 2 Page 1 of 1
 Effective Date: 03/07/06
 Exygen Study Number P0001131 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

- 1) Report, page 11 of 65
- 2) Test Materials, page 6 of 66: 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 smaller manner ~~more often~~ *rather than*.
- 2) The API 4000 LC/MS/MS systems detect the 499 → 80 PF/IS 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

Principal Investigator Signature

Study Director Management Signature

Exygen Management Signature

Sponsor Signature (if required)

Date

Date

Date

Date

Date

Exygen QAU Review LJS 2/2/06

LIBRARY ID: Y0001228-8

ADMINISTRATIVE FORM

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

00-20-2003 02:35pm From: NESTON SOLUTIONS

T-384 P.003/004 F-427

Exygen

RESEARCH

3058 Research Drive
State College, PA 16801Phone: 814-272-1039
Fax: 814-231-1580

DEVIATION FORM

General:		Page 1 of 1	
☒ Project Specific Deviation		☐ Facility Deviation	
Date of Occurrence: 08/29/05			
Exygen Project #: P780/P1131		Deviation #: 2/2	
Client Project #: NA		Reference #: 05-133 8/2/05	
Regulatory Driver:	Deviation Type: (Include VR for methods and SOPs)		
☒ GMP	☒ Protocol		
☐ GLP	☐ Method		
☐ Other	☐ SOP		
☐ None	VR: NA		
Notebook reference: NA			
Sample Description:			
Login #: L4264/L4266		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 ☒ Other			
Impact:			
No negative impact on this study.			
Corrective Actions:			
Deviation issued.			
Signatures:			
	9/2/05		26-SEP-05
Principal Investigator	Date	Exygen Management	Date
	9/20/05	NA	
Study Director	Date	Sponsor Representative	Date
	9/20/05	NA	
Quality Assurance	Date	Sponsor Management	Date

Interim Report #10-Analysis of Fish and Clam Samples

Exygen Study No.: P0001131

08-20-2005 02:35pm From: WESTON SOLUTIONS

T-384 P.004/004 F-437

3058 Research Drive
State College, PA 16801Phone: 814-272-1039
Fax: 814-231-1580

DEVIATION FORM

Page 1 of 1

General:	
☒ Project Specific Deviation	☐ Facility Deviation
Date of Occurrence: <u>12/27/04</u>	
Exygen Project #: <u>P760/P1131</u>	Deviation #: <u>3/3</u>
Client Project #: <u>NA</u>	Reference #: <u>05-134</u> <i>8/2/05</i>
Regulatory Driver:	Deviation Type: (include V# for methods and SOPs)
☐ GMP ☒ GLP ☐ Other ☐ None	☐ Protocol ☐ Method ☒ SOP V# 202-20 Section 5.2.3 Notebook reference: NA
Sample Description:	
Login #: <u>NA</u>	Container #: <u>NA</u> Lot #: <u>NA</u>
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 sterilized glassware prepared for the fish extraction from 12/27/04 through 01/07/05.	
Cause: ☐ Preparation ☐ Analysis ☐ Instrument ☐ Client Request ☒ 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:	
<u>[Signature]</u> Principal Investigator	<u>9/20/05</u> Date
<u>[Signature]</u> Study Director	<u>9/20/05</u> Date
<u>[Signature]</u> Quality Assurance	<u>9/20/05</u> Date
<u>[Signature]</u> Exygen Management	<u>9/20/05</u> Date
<u>NA</u> Sponsor Representative	<u>NA</u> Date
<u>NA</u> Sponsor Management	<u>NA</u> Date