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3M

CERTIFIED MAIL

May 30, 2006

Document Processing Center
EPA East – Room 6428 Attn: Section 8(e)
Office of Pollution Prevention and Toxics
U.S. EPA
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001

Re: TSCA 8(e) Substantial Risk Notice: Sulfonate-based and Carboxylic-based Fluorochemicals, Docket Nos. 8EHQ-1180-374; 8EHQ-0381-0394; 8EHQ-0598-373 [check these numbers] - Results from Analysis of Groundwater, Sediments, Surface Water, Fish and Clams in the Decatur, Alabama Area

To whom it may concern:

3M is submitting this notice to supplement its previous submissions on sulfonyl and carboxylic-based fluorochemicals. More specifically, the data contained in this submittal have been generated as part of a site-related environmental assessment for fluorochemicals that 3M is performing for its 3M Decatur, Alabama manufacturing facility. As part of this effort, 3M has recently received the enclosed final analytical reports for various sampling activities conducted in the vicinity of our Decatur manufacturing facility:

Property Adjacent to 3M Decatur Facility

3M Environmental Laboratory Report No. E06-0199 – Analysis of PFOA, PFOS, PFHS, and PFBS in Aqueous Samples: 3M Decatur Offsite Sampling Event (BPP Sample Sites)

Exygen Study No. P0000760, Interim Report #23, E05-0209, Analysis of Sediment Samples

Exygen Study No. P0001131, Interim Report #23, E05-0210, Analysis of Sediment Samples

Tennessee River Biota Sampling

Exygen Study No. P0001131, Interim Report #10, E05-0210, Analysis of Fish and Clam Samples

The first three reports contain data for samples collected from property adjacent and immediately to the west of the 3M Decatur site. More specifically, three locations within an open water marsh area were sampled for surface water and sediments. The identification numbers for these locations are:

Surface Water
DAL-SWS-BPP01-0-060413
DAL-SWS-BPP02-0-060413
DAL-SWS-BPP03-0-060413

Sediments
DAL-SD-BPP01-0-060421
DAL-SD-BPP02-0-060421
DAL-SD-BPP03-0-060421

In general, the BPP01 location represented the eastern end of the marsh, closest to inlet water being supplied by a nearby well. The BPP02 location was towards the northern edge of the marsh and the BPP03 location towards the south and eastern portion of the marsh, near the outlet to adjacent wetlands.

In addition to the samples referenced above, groundwater from a well located on the western adjacent property and used to supply the marsh with water was also sampled. This location is identified as DAL-GWS-BPWELL-0-060413 in the first report listed above.

There are a two additional items pertaining to Report No. E06-0199 that merit discussion. First, the report represents a subreport for a series of samples collected adjacent to, but off of, the 3M Decatur facility. Analytical results for additional off-site samples are pending but not final at this time. Second, there are several results that have been presented as estimated minimum values based on associated quality control samples that did not demonstrate adequate method performance. We are working to better quantify these results and may have revised values when the entire laboratory report is finalized.

The fourth report listed above contains analytical data for fish and clams collected in late 2004 from the Tennessee River near the Decatur, AL area. Again, these laboratory results were just recently finalized. The enclosed figure provides sample locations corresponding to the results tabulated in the analytical report.

While 3M does not believe that any of these data taken alone or cumulatively meet the "substantial risk" reporting threshold, we nevertheless recognize the ongoing work by U.S. EPA to assess fluorochemical exposure pathways. Therefore, we are placing these results in the 8(e) docket as a supplement to previous submissions.

If you have any questions, please do not hesitate to contact Gary Hohenstein at (651) 778-5150.

Sincerely,

Katherine E. Reed

Katherine E. Reed
Staff Vice President
Environmental, Health and Safety Operations

Subreport

Analysis of PFOA, PFOS, PFHS, and PFBS in Aqueous Samples: 3M Decatur Offsite Sampling Event (BPP Sample Sites)

Laboratory Request Number: E06-0199

Method Requirement: 3M Method ETS-8-154.1 (modified)

Testing Laboratory

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Requester

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ACCREDITED

The testing reported herein meet the requirements of ISO/IEC 17025-1999 "General Requirements for the Competence of Testing and Calibration Laboratories", in accordance with the A2LA Certificate #2052-01. Testing that complies with this International Standard also operate in accordance with ISO 9001/ISO 9002 (1994).

Certificate #2052-01

3M Environmental Laboratory
3M Environmental Laboratory Manager: William K. Reagen, Ph.D.
3M Technical Lead: Michelle Malinsky, Ph.D.
Report Author: Michelle Malinsky, Ph.D.

Analytical Subreport E06-0199

Surface Water Samples BPP01, BPP02, and BPP03;
Ground Water Sample BPWell

Decatur Offsite Sampling Event
Report Date: May 1, 2006

1 Introduction/Summary

The 3M Environmental Laboratory extracted and analyzed ground water and surface water samples collected by Weston Solutions personnel on April 12 and 13, 2006 near the 3M Decatur facility. Samples were returned to the 3M Environmental Laboratory for analysis of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHS) and perfluorobutane sulfonate (PFBS) under laboratory project number E06-0199 using modified 3M Environmental Laboratory Method ETS 8-154.1 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates in Water by Solid Phase Extraction and High Performance Liquid Chromatography/Mass Spectrometry".

Per the client's request, an initial subreport is being issued for the following four sample locations only:

- DAL SWS BPP01
- DAL SWS BPP02
- DAL SWS BPP03
- DAL GWS BPWELL

This report will contain information relevant to these four sample collection points only. These results will be reissued in a comprehensive final project report that includes results for all samples collected under E06-0199.

The 3M Environmental Laboratory prepared sets of sample containers for thirty-two separate sampling locations. At a minimum, each sample set consisted of a field sample, field sample duplicate, low field spike and high field spike. For some locations, a third mid-level field spike was prepared. Each empty container was marked with a "fill to here" line which corresponded to a final volume of 450 mL. Containers reserved for field matrix spikes were fortified with an appropriate matrix spike solution containing the four target analytes prior to being sent to the field for sample collection. The spike amounts prepared for each of the four locations presented here will be provided later in this report.

Certificate #2052-01

The testing reported herein meet the requirements of ISO/IEC 17025-1999 "General Requirements for the Competence of Testing and Calibration Laboratories", in accordance with the A2LA Certificate #2052-01. Testing that complies with this International Standard also operate in accordance with ISO

Samples were initially extracted and analyzed on April 19, 2006. Samples were diluted, reextracted, and analyzed on April 21, 2006. Laboratory matrix spikes were prepared, diluted, extracted, and analyzed on April 25, 2006 in the analytical batch initiated on April 24, 2006.

Table 1 below summarizes the sample results. All results for quality control samples prepared and analyzed with the samples will be reported and discussed elsewhere in this report. All samples were diluted 1:50 in ASTM Type I water prior to extraction.

Table 1. Sample Results Summary.

3M LIMS ID	*Sample Description*	*(1) PFOA Concentration (ng/mL)*	*(2) PFOS Concentration (ng/mL)*	*(1) PFHS Concentration (ng/mL)*	*(1) PFBS Concentration (ng/mL)*
E06-0199-055	DAL SWS BPP01 0 060413	293	(2) >423	53.3	6.98
E06-0199-056	DAL SWS BPP01 DB 060413	307	(2) >380	54.3	7.17
	Average	***300***	***(2) >402***	***53.8***	***7.08***
	%RPD Sample/Sample Dup	***4.7***	***NA***	***1.8***	***2.7***
E06-0199-060	DAL SWS BPP02 0 060413	206	(2) >295	38.2	4.30
E06-0199-061	DAL SWS BPP02 DB 060413	219	(2) >314	39.8	4.72
	Average	***212***	***(2) >304***	***39.0***	***4.66***
	%RPD Sample/Sample Dup	***6.1***	***NA***	***4.1***	***2.6***
E06-0199-065	DAL SWS BPP03 0 060413	219	(2) >272	39.1	4.69
E06-0199-066	DAL SWS BPP03 DB 060413	214	(2) >256	40.1	5.05
	Average	***216***	***(2) >264***	***39.6***	***4.87***
	%RPD Sample/Sample Dup	***2.3***	***NA***	***2.5***	***7.4***
E06-0199-070	DAL GWS BPWELL 0 060413	(2) >774	(2) >1460	(2) >145	17.2
E06-0199-071	DAL GWS BPWELL DB 060413	(2) >779	(2) >1170	(2) >145	17.6
	Average	***(2) >776***	***(2) >1320***	***(2) >145***	***17.4***
	%RPD Sample/Sample Dup	***NA***	***NA***	***NA***	***2.3***

(1) The analytical method uncertainty for PFOA, PFHS, and PFBS was 100±11%, 100±5.8%, and 100±8.8%, respectively based on method accuracy and precision. See Section 3.7 for additional explanation. The limit of quantitation (LOQ) *for these samples only* was 2.50 ng/mL (PFOA) and 1.25 ng/mL (PFHS and PFBS). Results and average values are rounded to three significant figures according to EPA rounding rules. Percent relative difference (%RPD) values are rounded to two significant figures. Because of rounding, values may vary slightly from those listed in the raw data.

(2) ***All PFOS sample results presented here should be considered estimated minimum values as quality control samples (field matrix spikes and lab matrix spikes) associated with these four samples did not demonstrate adequate method performance. The accuracy of the PFOS results could be within an order of magnitude. %RPD values not calculated. BPWell results for PFOA and PFHS should also be considered estimated minimums as field matrix spike recoveries did not meet method acceptance criteria of 100±30%. The analytical accuracy of the PFOA and PFHS estimated values for this sample only could be within an order of magnitude. %RPD values not calculated.***

2 Methods - Analytical and Preparatory

2.1 Sample Collection

Samples were collected in Nalgene™ (low-density polyethylene) bottles prepared at the 3M Environmental Laboratory. Prior to sample collection, bottles designated for field matrix spikes were spiked in the laboratory with a known volume of an appropriate matrix spiking solution containing the

four analytes of interest (PFOA, PFOS, PFHS and PFBS). Collected sample bottles were received at the laboratory at ambient conditions on April 19, 2006. Table 2 below details the samples collected and spikes added to each bottle.

Table 2. Sample Collection and Spike Information.

3M LIMS ID#	Weston Sample Description	3M Bottle Number	Bottle Designation	Nominal Spike Level (ng/mL)	GPO Location Comment
E06-0199-055	DAL SWS BPP01 0 060413	109	Sample	NA	FSIA Drainage
E06-0199-056	DAL SWS BPP01 DB 060413	110	Sample Dup	NA	FSIA Drainage
E06-0199-057	DAL SWS BPP01 LS 060413	111	FMS Low	1.0	FSIA Drainage
E06-0199-058	DAL SWS BPP01 MS 060413	112	FMS Mid	10	FSIA Drainage
E06-0199-059	DAL SWS BPP01 HS 060413	113	FMS High	100	FSIA Drainage
E06-0199-060	DAL SWS BPP02 0 060413	119	Sample	NA	FSIA Drainage
E06-0199-061	DAL SWS BPP02 DB 060413	120	Sample Dup	NA	FSIA Drainage
E06-0199-062	DAL SWS BPP02 LS 060413	121	FMS Low	1.0	FSIA Drainage
E06-0199-063	DAL SWS BPP02 MS 060413	122	FMS Mid	10	FSIA Drainage
E06-0199-064	DAL SWS BPP02 HS 060413	123	FMS High	100	FSIA Drainage
E06-0199-065	DAL SWS BPP03 0 060413	114	Sample	NA	FSIA Drainage
E06-0199-066	DAL SWS BPP03 DB 060413	115	Sample Dup	NA	FSIA Drainage
E06-0199-067	DAL SWS BPP03 LS 060413	116	FMS Low	1.0	FSIA Drainage
E06-0199-068	DAL SWS BPP03 MS 060413	117	FMS Mid	10	FSIA Drainage
E06-0199-069	DAL SWS BPP03 HS 060413	118	FMS High	100	FSIA Drainage
E06-0199-070	DAL GWS BPWELL 0 060413	72	Sample	NA	BP Property
E06-0199-071	DAL GWS BPWELL DB 060413	73	Sample Dup	NA	BP Property
E06-0199-072	DAL GWS BPWELL LS 060413	74	FMS Low	10	BP Property
E06-0199-073	DAL GWS BPWELL MS 060413	75	FMS Mid	100	BP Property
E06-0199-074	DAL GWS BPWELL HS 060413	76	FMS High	1000	BP Property

DAL = Decatur, AL
SWS = Surface Water Sample
GWS = Ground Water Sample
0 = Sample
DB = Duplicate
LS = Low Spike
MS = Mid Spike
HS = High Spike

2.2 Extraction

All samples, calibration standards, and associated quality control samples were extracted using a modified procedure of ETS-8-154.1 "Determination of Perfluorinated Acids, Alcohols, Amides, and Sulfonates in Water by Solid Phase Extraction and High Performance Liquid Chromatography/Mass Spectrometry". Briefly, 40 mL of sample were loaded onto a pre-conditioned Waters C18 solid phase extraction (SPE) cartridge (Sep-Pak, 1.0 g, 6 cc) using a vacuum manifold. The loaded SPE cartridges were then eluted with 5 mL of methanol. This extraction procedure concentrates the samples by a factor of eight. (Initial volume = 40 mL, final volume = 5 mL). Lab control spikes extracted in the same manner cross-validate all the method modifications/deviations from ETS-8-154.1. See Section 3.6 for additional information.

The samples and sample duplicates for BPP01, BPP02, and BPP03 were initially extracted undiluted on April 19, 2006. Initial analysis of the undiluted samples exceeded the upper limit of quantiation (25 ng/mL nominal) for PFOA and PFOS. Likewise, the sample and sample duplicate for BPWELL were initially extracted on April 19, 2006. The final extracts were diluted 1:10 prior to analysis. Analysis of these diluted extracts also exceeded the upper limit of quantitation. All the samples, sample duplicates, and field matrix spikes were reextracted on April 21, 2006. For BPP01, BPP02, and BPP03, the samples and associated QC were diluted 1:50 using ASTM Type I water prior to extraction. For BPWELL, the sample, sample duplicate, low spike and mid spike were diluted 1:50, while the high spike was diluted 1:500.

2.3 Analysis

All sample and quality control extracts were analyzed for PFOA, PFOS, PFHS, and PFBS using high performance liquid chromatography/ tandem mass spectrometry (HPLC/MS/MS). Pertinent instrument parameters, the liquid chromatography gradient program, and the specific mass transitions analyzed are described in the tables below.

Table 3. Instrument Parameters.

Instrument Name	ETSStan
Liquid Chromatograph	Agilent 1100
Guard column	Betasil C18 (2.1 mm.X100 mm), 5 µm
Analytical column	Betasil C18 (2.1 mm X 100 mm), 5 µm
Injection Volume	5 µL
Mass Spectrometer	Applied Biosystems API 4000
Ion Source	Turbo Spray
Electrode	Z-spray
Polarity	Negative
Software	Analyst 1.4.1

Table 4. Liquid Chromatography Gradient Program.

Step Number	*Total Time (min)*	*Flow Rate (µL/min)*	*Percent A (2 mM ammonium acetate)*	*Percent B (Methanol)*
0	0	300	80.0	20.0
1	1.0	300	80.0	20.0
2	14.5	300	10.0	90.0
3	15.5	300	10.0	90.0
4	16.5	300	80.0	20.0
5	20.0	300	80.0	20.0

Table 5. Mass Transitions.

Analyte	Mass Transition Q1/Q3	Dwell Time (msec)
[1]PFBS	299/80	125
	299/99	125
[1]PFHS	399/80	125
	399/99	125
[1]PFOS	499/130	125
	499/99	125
	499/80	125
[1]PFOA	413/369	125
	413/219	125
	413/169	125

(1) The individual transitions were summed to produce a "total ion chromatogram" (TIC). The TICs were used for quantitation.

3 Data Analysis

3.1 Calibration

Calibration standards were prepared by spiking known amounts of stock solutions containing the target analytes into 40 mL of ASTM type I water. Each spiked water standard was then extracted in the same manner as the collected samples. A total of twelve spiked standards ranging from 0.025 ng/mL to 25 ng/mL (nominal) were prepared. A quadratic, 1/x weighted, calibration curve was used to fit the data for each analyte. The data were not forced through zero during the fitting process. Calculating the standard concentration using the peak area counts and the resultant calibration curve confirmed accuracy of each curve point. Each extracted calibration standard used to generate the final calibration curve met the method calibration accuracy requirement of 100±25%. The correlation coefficients (r) were greater than 0.999 for all analytes.

3.2 Limit of Quantitation (LOQ)

The LOQ for this analysis, as defined in ETS-8-154.1, is the lowest non-zero calibration standard in the curve in which the area counts are at least twice those of the method and solvent blank(s). The LOQs for PFOA, PFOS, PFHS, and PFBS were 0.0500 ng/mL, 0.0999 ng/mL, 0.0250 ng/mL, and 0.0250 ng/mL, respectively. Note: these LOQ values have not been corrected for sample specific dilution factors. Area count comparison for the method blanks and the lowest calibration standard will be provided in Section 3.5.1.

3.3 System Suitability

Replicate injections of the 1 ng/mL extracted calibration standard were analyzed at the beginning and end of the analytical sequence to demonstrate overall system suitability. All analytes met method acceptance criteria of less than 5% relative standard deviation (RSD) for peak area and less than 2% RSD for retention time for both opening and closing system suitability injections.

3.4 Continuing Calibration

During the course of the analytical sequence, several continuing calibration verification samples (CCVs) were analyzed to confirm that the instrument response and the initial calibration curve were still in control. One PFOS CCV (139%) and one PFHS CCV (153%) exceeded method acceptance criteria of 100±25% for accuracy. Both of these non-compliant CCVs followed a contaminated solvent blank and the high recovery is most likely carryover from the previous injection. A method deviation has been

issued. The overall impact to the data quality objectives for the sample results is considered minimal as all sample injections and sample related QC injections were bracketed by compliant CCVs.

3.5 Blanks

Three types of blanks were prepared and analyzed with the samples: method blanks, solvent blanks, and field/trip blanks. Each blank type is described below.

3.5.1 Method Blanks

Several method blanks were prepared by loading 40 mL of ASTM Type I water onto a C18 SPE cartridge and eluting with 5 mL of methanol using the same extraction procedure as the samples. Method blanks were prepared to evaluate the levels of background contamination in the overall extraction process (reagent water, glassware, SPE cartridges, etc.) The calibration curve was prepared on a separate day as the samples. (Calibration standards: April 10, 2006; diluted samples: April 21, 2006). The method blanks prepared with the calibration standards as well as with the samples were both analyzed during the April 21, 2006 analysis. Additional lab matrix spikes were prepared and analyzed in a separate analytical run initiated on April 24, 2006. Again, the method blanks prepared with the calibration curve and the method blanks prepared with the spikes were analyzed. Method blank area count comparisons to the LOQ standard are provided in the Attachments section at the end of this report.

3.5.2 Solvent Blanks

Several methanol solvent blanks were analyzed to assess system contamination and/or instrument carryover. For the April 21, 2006 analysis, three separate vials of methanol solvent blanks were contaminated and produced area counts of the target analytes greater than one-half those of the LOQ standard. A method deviation has been issued. The solvent blank area count comparison for the April 21, 2006 is provided in the Attachments section at the end of this report. For the lab matrix spikes analyzed on April 24, 2006, only the initial solvent blanks injected before the opening system suitabilities and the solvent blanks analyzed after the highest calibration standard exhibited area counts greater than one-half the LOQ standard.

3.5.3 Field/Trip Blanks

Prior to sample collection, seven sets of trip/field blanks were prepared. A trip blank consists of a sample container filled with 450 mL of ASTM Type I water, sealed, and shipped to the sample collection site along with the empty containers. The trip blank serves as an additional method blank that accounts for any storage conditions and/or holding time issues that the samples may experience. Results of the trip blank analyses will be provided in the final comprehensive report.

3.6 Lab Control Spikes (LCSs)

Lab control spikes at nominal concentrations of 0.050 ng/mL, 5.0 ng/mL, 100 ng/mL, and 1000 ng/mL spikes were prepared and analyzed each day samples were extracted and analyzed. The 0.050 ng/mL and 5.0 ng/mL LCSs were prepared by spiking known amounts of the analytes into 40 mL of ASTM Type I water to produce the desired concentration. The spiked water samples were then extracted and analyzed in the same manner as the samples. For the 100 ng/mL and 1000 ng/mL LCSs, target analytes were added to 450 mL of ASTM Type I water to produce the final desired concentration. An aliquot of the LCS was then diluted 1:50 or 1:500 with ASTM Type I water prior to extraction, in the same manner as the diluted samples. This technique was done to demonstrate that the dilution procedure used for the samples was able to produce accurate results. Analysis of replicate LCSs at the various levels cross-validates the analytical method, as used here, for any modifications/deviations from ETS 8-154.1. Additionally, LCS results will be used to determine overall method uncertainty in Section 3.7. Table 6 summarizes the LCS recovery results.

$$\text{LCS Percent Recovery} = \frac{\text{Calculated Concentration}}{\text{Spike Concentration}} * 100\%$$

$$\text{LCS\% RSD} = \frac{\text{standard deviation LCS replicates}}{\text{average LCS recovery}} * 100\%$$

Table 6. [1]Lab Control Spike Results.

		PFOA			PFOS			PFHS			PFBS		
Sample Description	Lab ID	Spiked Conc. (ng/mL)	Calc. Conc. (ng/mL)	% Recovery	Spiked Conc. (ng/mL)	Calc. Conc. (ng/mL)	% Recovery	Spiked Conc. (ng/mL)	Calc. Conc. (ng/mL)	% Recovery	Spiked Conc. (ng/mL)	Calc. Conc. (ng/mL)	% Recovery
0.05 ppb LCS	LCS-060410-1	0.0500	<0.0500	[2]NA	0.0500	<0.0999	[2]NA	0.0500	0.0504	101	0.0499	0.0505	101
0.05 ppb LCS	LCS-060410-2	0.0500	<0.0500	[2]NA	0.0500	<0.0999	[2]NA	0.0500	0.0480	96.0	0.0499	0.0456	91.3
0.05 ppb LCS	LCS-060410-3	0.0500	0.0517	104	0.0500	<0.0999	[2]NA	0.0500	0.0483	96.6	0.0499	0.0480	96.1
5.0 ppb LCS	LCS-060410-4	5.00	4.93	98.6	5.00	4.93	98.7	5.00	4.98	99.6	4.99	4.94	98.9
5.0 ppb LCS	LCS-060410-5	5.00	4.86	97.2	5.00	5.01	100	5.00	4.94	98.8	4.99	4.88	97.7
5.0 ppb LCS	LCS-060410-6	5.00	5.30	106	5.00	5.05	101	5.00	4.98	99.6	4.99	4.94	98.9
0.05 ppb LCS	LCS-060421-1	0.0500	<0.05	[2]NA	0.0500	<0.0999	[2]NA	0.0500	0.0501	100	0.0499	0.0426	85.3
0.05 ppb LCS	LCS-060421-2	0.0500	<0.05	[2]NA	0.0500	<0.0999	[2]NA	0.0500	0.0485	97.0	0.0499	0.0427	85.5
0.05 ppb LCS	LCS-060421-3	0.0500	0.0514	103	0.0500	<0.0999	[2]NA	0.0500	0.145	[3]289	0.0499	0.0441	88.3
5.0 ppb LCS	LCS-060421-4	5.00	5.02	100	5.00	4.53	90.7	5.00	4.88	97.6	4.99	4.80	96.1
5.0 ppb LCS	LCS-060421-5	5.00	4.81	96.2	5.00	4.34	86.9	5.00	4.79	95.8	4.99	4.71	94.3
5.0 ppb LCS	LCS-060421-6	5.00	4.82	96.4	5.00	4.49	89.9	5.00	4.90	98.0	4.99	4.76	95.3
100 ppb LCS	LCS-060421-7	100.0	95.0	95.0	99.9	87.3	87.4	100	98.1	98.1	99.9	98.5	98.6
100 ppb LCS	LCS-060421-8	100.0	99.7	99.7	99.9	89.2	89.3	100	100.0	100.0	99.9	100.0	100
100 ppb LCS	LCS-060421-9	100.0	96.9	96.9	99.9	88.5	88.6	100	99.4	99.4	99.9	98.0	98.1
1000 ppb LCS	LCS-060421-10	1000	928	92.8	999	855	85.6	1000	940	94.0	999	909	91.0
1000 ppb LCS	LCS-060421-11	1000	977	97.7	999	899	90.0	1000	988	98.8	999	973	97.4
1000 ppb LCS	LCS-060421-12	1000	1030	103	999	929	93.0	1000	1020	102	999	1040	104
0.05 ppb LCS	LCS-060425-1	0.0500	0.0689	[4]138	0.0500	0.0631	[4]126	0.0500	0.0578	116	0.0499	0.0563	113
0.05 ppb LCS	LCS-060425-2	0.0500	0.0600	120	0.0500	0.152	[5]304	0.0500	0.0592	118	0.0499	0.0507	102
5.0 ppb LCS	LCS-060425-3	5.00	4.91	98.2	5.00	4.79	95.9	5.00	4.98	99.6	4.99	4.89	97.9
5.0 ppb LCS	LCS-060425-4	5.00	4.87	97.3	5.00	4.94	98.9	5.00	5.08	102	4.99	4.86	97.3
100 ppb LCS	LCS-060425-5	100.0	95.3	95.3	99.9	84.5	84.6	100	95.9	95.9	99.9	95.2	95.3
100 ppb LCS	LCS-060425-6	100.0	98.6	98.6	99.9	89.8	89.9	100	96.8	96.8	99.9	96.6	96.7
1000 ppb LCS	LCS-060425-7	1000	977	97.7	999	878	87.9	1000	978	97.8	999	988	98.9
1000 ppb LCS	LCS-060425-8	1000	972	97.2	999	849	85.0	1000	966	96.6	999	975	97.6
Average %Recovery ±%RSD		*101%±9.7%*			*93.1%±10%*			*99.8%±5.6%*			*96.8%±5.8%*		

(1) All results and average values listed to three significant figures according to EPA rounding rules. %RSD values given to two significant figures. Due to rounding, values may vary slightly from those in the raw data.

(2) Low end of the calibration curve disabled for PFOS and PFOA due to solvent blank contamination. Recovered LCS concentrations were below the established LOQ and are not reported.

(3) High recovery due to instrument carryover from the previous injection of a contaminated methanol blank. Recovery excluded from average and %RSD calculations.

(4) Recovery was outside the method acceptance criteria of 100±25%; however, the recovery was included in the calculation of the overall average and %RSD.

(5) Recovery was outside the method acceptance criteria of 100±25%; the recovery value was not included in the calculation of the overall average and %RSD as it was clearly a statistical outlier. The 5 ppb, 100 ppb, and 1000 ppb LCSs are more representative of the sample concentrations for the results reported in this subreport. Impact to data quality objectives is minimal.

3.7 Analytical Method Uncertainty

Both the accuracy (percent recovery) and precision (%RSD) of the lab control spikes were used to estimate the overall method's analytical uncertainty for a given analyte. For example, the overall accuracy and precision for PFOA based on LCS results was 101%±9.7%. The measured precision (%RSD) is then used to determine the range of the accuracy.

Example:

101*(0.097) = 9.84
101+ 9.84 =111.1; 101 – 9.84 = 91.45

Thus, LCS accuracy results range from 91.45% to 111.1%. The absolute difference of the low and high ends of this range, when compared to 100%, are then calculated.

111.1%-100 = 11.1%
100%-91.45 = 8.54%.

The most conservative (largest) absolute difference is then used as the analytical uncertainty for the given analyte. Therefore, the analytical uncertainty for PFOA is given as 100±11% (two significant figures) for these results. For PFOS, PFHS, and PFBS, the analytical method uncertainty is 100±16%, 100±5.8%, and 100±8.8%, respectively. It should be emphasized that the analytical method uncertainty described above **only** applies to the method and the procedures performed within. Additional quality control samples specific to the sample matrix, in the form of field matrix spikes and lab matrix spikes, demonstrate that the individual **sample** results are accurate to within 100±30%, the targeted data quality objectives.

3.8 Field Matrix Spikes (FMS)

At a minimum, low and high field matrix spikes were collected at each sampling point to verify that the analytical method is applicable to the collected matrix. For some locations, a mid-level spike was also collected. Field matrix spike recoveries within method acceptance criteria of 100±30% confirm that "unknown" components in the sample matrix do not interfere with the extraction and analysis of the analytes of interest. Field matrix spikes will be presented in the next section with the sample data. The field matrix spike should be at least 50% of the endogenous sample concentration to be considered an appropriate spike level for the given sample matrix.

$$\text{FMS Recovery} = \frac{(\text{Sample Concentration of FMS} - \text{Average Concentration: Field Sample \& Field Sample Dup.})}{\text{Spike Concentraton}} * 100\%$$

3.9 Lab Matrix Spikes (LMS)

If the sample and sample duplicate produced concentrations where none of the associated field matrix spikes were at least 50% of the endogenous concentration, then laboratory matrix spikes (LMS) were prepared. LMSs were prepared by spiking a known amount of target analytes into a measured aliquot of the sample matrix. The spiked matrix was then diluted, if necessary, and extracted in the same manner as the samples. LMS recoveries within 100±30% demonstrated that the analytical method was appropriate for the given sample matrix **only** in the absence of an appropriate field matrix spike.

4 Data Summary

The data tables below summarize the sample results, field matrix spike recoveries, and laboratory matrix spike recoveries for the four locations (BPP01, BPP02, BPP03, and BPWell). Each table provides the average concentration and the relative percent difference (RPD) of the sample and sample duplicate.

BPP01
The endogenous concentration for BPP01 was at least twice the spike concentration for the low spike (1 ng/mL), mid spike (10 ng/mL) and high spike (100 ng/mL) for both PFOA and PFOS. A laboratory matrix spike at 500 ng/mL was prepared and analyzed. The LMS recovery met method acceptance criteria of 100±30% for PFOA (79.6%), but not for PFOS (60.6%). **Therefore, the sample results for PFOS should be considered estimated minimums with a sample analytical accuracy within an order of magnitude as the analytical method has not been demonstrated to be suitable for the given sample matrix.** FMS and/or LMS recoveries within 100±30% for PFOA, PFHS, and PFBS demonstrate that the analytical method is appropriate for the given sample matrix for these analytes.

BPP02
The endogenous concentration for BPP02 was at least twice the spike concentration for the low spike (1 ng/mL) and mid spike (10 ng/mL) for both PFOA and PFOS as well as the high spike (100 ng/mL) for PFOS only. A laboratory matrix spike at 250 ng/mL was prepared and analyzed. The LMS recovery met method acceptance criteria of 100±30% for PFOA (75.0%), but not PFOS (59.8%). **Therefore, the sample results for PFOS should be considered estimated minimums with a sample analytical accuracy within an order of magnitude as the analytical method has not been demonstrated to be suitable for the given sample matrix.** FMS and/or LMS recoveries within 100±30% for PFOA, PFHS, and PFBS demonstrate that the analytical method is appropriate for the given sample matrix for these analytes.

BPP03
The endogenous concentration for BPP03 was at least twice the spike concentration for the low spike (1 ng/mL) and mid spike (10 ng/mL) for both PFOA and PFOS as well as the high spike (100 ng/mL) for PFOS only. A laboratory matrix spike at 250 ng/mL was prepared and analyzed. The LMS recovery met method acceptance criteria of 100±30% for PFOA (73.8%), but not PFOS (60.8%). **Therefore, the sample results for PFOS should be considered estimated minimums with a sample analytical accuracy within an order of magnitude as the analytical method has not been demonstrated to be suitable for the given sample matrix.** The high level PFBS FMS exceeded method acceptance criteria of 100±30% (134%). However, the PFBS results for this sample are still considered accurate within 100±30% because the criteria exceedence was minimal (within 4%) and mid level FMS and LMS recoveries were excellent, 101% and 90.2%, respectively. High level FMS and LMS recoveries within 100±30% for PFOA and PFHS also demonstrate that the analytical method is appropriate for the given sample matrix for these two analytes as well.

BPWell
The endogenous concentration for BPWell was at least twice the spike concentration for the low spike (10 ng/mL) for PFOA, PFOS, PHFS, and PFBS as well as the mid spike for PFOA, PFOS, and PFHS. Although the high level spike (1000 ng/mL) was an appropriate level for all four analytes, the recoveries were consistently less than 30% for all analytes, with PFOS showing no measuring recovery. The high FMS was reextracted to rule out a potential preparatory error during the extraction process. The second extraction produced similar recoveries as the first. A laboratory matrix spike at 1000 ng/mL was also prepared. The LMS recovery met method acceptance criteria of 100±30% for PFOA (85.4%), PFHS(93.5%), and PFBS(97.2%) but not for PFOS (13.5%). **Therefore, the sample results for PFOS should be considered estimated minimums with a sample analytical accuracy within an order of magnitude as the analytical method has not been demonstrated to be suitable for the given sample matrix. Although the LMS produces acceptable recoveries for PFOA and PFHS, the sample results should be considered estimated minimums as no appropriate supplementary FMS recovery was available.** Low and mid level FMS and LMS recoveries within 100±30% for PFBS demonstrate that the analytical method is appropriate for the given sample matrix for this analyte only. Further method development for the BP Well sample matrix will be performed to see if improved PFOA, PFOS, and PFHS recoveries can be achieved.

Table 7. [1]BPP01 Results.

		PFOA		PFOS		PFHS		PFBS	
3M LIMS ID	Description	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery
E06-0199-055	DAL SWS BPP01 0 060413	293	NA	423	NA	53.3	NA	6.98	NA
E06-0199-056	DAL SWS BPP01 DB 060413	307	NA	380	NA	54.3	NA	7.17	NA
E06-0199-057	DAL SWS BPP01 LS 060413	292	[2]NR	330	[2]NR	53.4	[2]NR	7.44	[2]NR
E06-0199-058	DAL SWS BPP01 MS 060413	328	[2]NR	484	[2]NR	66.6	[2]NR	18.8	117
E06-0199-059	DAL SWS BPP01 HS 060413	361	[2]NR	365	[2]NR	136	82.2	93.8	86.8
LMS-060425-1	DAL SWS BPP01 0 060413 500ppb LMS	698	79.6	704	[3]60.6	485	86.2	444	87.5
Average Concentration (ng/mL) ± %RPD		300±4.7%		[4]>402		53.8±1.9%		7.08±2.7%	

NA= Not Applicable.

(1) Table displays rounded values for all concentrations and percent recovery values (3 significant figures), %RPD values (2 significant figures). Recovery and RPD values may vary slightly from the values in the raw data. All samples and associated FMSs were diluted 1:50 in ASTM Type I water prior to extraction. LMS was diluted 1:100 prior to extraction.

(2) NR=Not reportable. FMS level not appropriate for the given matrix. The endogenous sample concentration was at least twice spike level.

(3) LMS recovery outside of method acceptance criteria of 100±30%.

(4) PFOS sample results should be considered estimated minimums only. Sample matrix specific QC did not meet method criteria. The analytical sample accuracy is arbitrarily estimated to within an order of magnitude. %RPD values not reported.

Table 8. [1]BPP02 Results.

		PFOA		PFOS		PFHS		PFBS	
3M LIMS ID	*Description*	*Conc. (ng/mL)*	*% Recovery*	*Conc. (ng/mL)*	*% Recovery*	*Conc. (ng/mL)*	*% Recovery*	*Conc. (ng/mL)*	*% Recovery*
E06-0199-060	DAL SWS BPP02 0 060413	206	NA	295	NA	38.2	NA	4.6	NA
E06-0199-061	DAL SWS BPP02 DB 060413	219	NA	314	NA	39.8	NA	4.72	NA
E06-0199-062	DAL SWS BPP02 LS 060413	234	[2]NR	388	[2]NR	42.3	[2]NR	5.9	[2]NR
E06-0199-063	DAL SWS BPP02 MS 060413	220	[2]NR	346	[2]NR	48.0	[2]NR	14.4	97.5
E06-0199-064	DAL SWS BPP02 HS 060413	314	102	437	[2]NR	134	95.0	102	97.5
LMS-060425-2	DAL SWS BPP02 0 060413 250ppb	400	75.0	454	[3]59.8	256	86.8	223	87.4
Average Concentration (ng/mL) ±%RPD		***212±6.1%***		***[4]>304***		***39.0±4.1%***		***4.66±2.6%***	

NA=Not Applicable.

(1) Table displays rounded values for all concentrations and percent recovery values (3 significant figures), %RPD values (2 significant figures). Recovery and RPD values may vary slightly from the values in the raw data. All samples and associated QC were diluted 1:50 in ASTM Type I water prior to extraction.

(2) NR=Not reportable. FMS level not appropriate for the given matrix. Endogenous concentration was at least twice spike level.

(3) LMS recovery outside of method acceptance criteria of 100±30%

(4) PFOS sample results should be considered estimated minimums only. Sample matrix specific QC did not meet method criteria. The analytical sample accuracy is arbitrarily estimated to within an order of magnitude. %RPD values not reported.

Table 9. BPP03 Results.

		PFOA		PFOS		PFHS		PFBS	
3M LIMS ID	Description	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery
E06-0199-065	DAL SWS BPP03 0 060413	219	NA	272	NA	39.1	NA	4.69	NA
E06-0199-066	DAL SWS BPP03 DB 060413	214	NA	256	NA	40.1	NA	5.05	NA
E06-0199-067	DAL SWS BPP03 LS 060413	211	[2]NR	258	[2]NR	40.2	[2]NR	5.76	[2]NR
E06-0199-068	DAL SWS BPP03 MS 060413	228	[2]NR	255	[2]NR	50.0	[2]NR	15.0	101
E06-0199-069	DAL SWS BPP03 HS 060413	324	108	315	[2]NR	143	103	139	[3]134
LMS-060425-3	DAL SWS BPP03 0 060413 250ppb LMS	401	73.8	416	[4]60.8	262	89.0	230	90.2
Average Concentration (ng/mL) ±%RPD		216±2.3%		[5]>264		39.6±2.5%		4.87±7.4%	

NA=Not Applicable.

(1) Table displays rounded values for all concentrations and percent recovery values (3 significant figures), %RPD values (2 significant figures). Recovery and RPD values may vary slightly from the values in the raw data. All samples and associated QC were diluted 1:50 in ASTM Type I water prior to extraction.

(2) NR=Not reportable. FMS level not appropriate for the given matrix. Endogenous concentration was at least twice spike level.

(3) FMS recoveries outside method acceptance criteria of 100±30%.

(4) LMS recovery outside of method acceptance criteria of 100±30%.

(5) PFOS sample results should be considered estimated minimums only. Sample matrix specific QC did not meet method criteria. The analytical sample accuracy is arbitrarily estimated to within an order of magnitude. %RPD values not reported.

Table 10. [1]BPWell results.

		PFOA		PFOS		PFHS		PFBS	
3M LIMS ID	Description	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery	Conc. (ng/mL)	% Recovery
E06-0199-070	DAL GWS BPWELL 0 060413	774	NA	1460	NA	145	NA	17.2	NA
E06-0199-071	DAL GWS BPWELL DB 060413	779	NA	1170	NA	145	NA	17.6	NA
E06-0199-072	DAL GWS BPWELL LS 060413	723	[2]NR	960	[2]NR	152	[2]NR	26.9	95.1
E06-0199-073	DAL GWS BPWELL MS 060413	821	[2]NR	1020	[2]NR	244	[2]NR	122	105
[3]E06-0199-074	DAL GWS BPWELL HS 060413	1020	[5]24.4	1200	[6]NMR	375	[5]23.0	286	[5]26.9
[4]E06-0199-074	DAL GWS BPWELL HS 060413	987	[5]21.0	1320	[6]NMR	348	[5]20.3	254	[5]23.7
LMS-060425-4	DAL GWS BPWELL 0 060413 1000ppb LMS	1630	85.4	1450	[7]13.5	1080	93.5	980	97.2
Average Concentration (ng/mL) ± %RPD		[8]>776		[8]>1320		[8]>145		17.4±2.3%	

NA=Not Applicable.

(1) Table displays rounded values for all concentrations and percent recovery values (3 significant figures), %RPD values (2 significant figures). Recovery and RPD values may vary slightly from the values in the raw data. The sample, sample duplicate, low FMS, and mid FMS were diluted 1:50 in ASTM Type I water prior to extraction. The high FMS and 1000 ppb LMS were diluted 1:500 prior to extraction.

(2) NR=Not reportable. FMS level not appropriate for the given matrix. Endogenous concentration was at least twice spike level.

(3) FMS extracted on 4-21-2006 with the samples.

(4) FMS re-extracted on 4-25-0006.

(5) FMS recovery outside of method acceptance criteria of 100±30%.

(6) NMR = No measurable recovery.

(7) LMS recovery outside method acceptance criteria of 100±30%.

(8) PFOA, PFOS, and PFHS sample results should be considered estimated minimums only. Field matrix spikes did not meet method criteria. The analytical sample accuracy is arbitrarily estimated to within an order of magnitude. %RPD values not reported.

5 Conclusion

Sample matrix QC samples for the four samples reported herein did not meet method acceptance criteria of 100±30% for PFOS. ***Therefore, the values listed in Table 1 and Tables 7-10 for PFOS should be considered estimated minimums. The sample analytical accuracy is arbitrarily estimated to be within an order of magnitude.*** Further method development is needed for this matrix as the QC results demonstrated that unknown matrix components preclude accurate analysis with the method as performed here. Field matrix spikes yielding recoveries within 100±30% demonstrated that the analytical method was suitable for the given sample matrices for PFOA, PFHS, and PFBS for BPP01, BPP02, and BPP03. The analytical method uncertainty for these analytes was presented in Table1. ***PFOA and PFHS results for BPWell should also be considered estimated minimums with a sample analytical accuracy within an order of magnitude as no acceptable field matrix spikes recoveries were produced.***

6 Data / Sample Retention

All remaining sample and associated project data (hardcopy and electronic) will be archived according to 3M Environmental Laboratory standard operating procedures.

7 Signatures

Michelle D. Malinsky, Ph.D., 3M Technical Lead — Date: 5/1/06

Kent R. Lindstrom, 3M Technical Reviewer — Date: 05/01/06

William K. Reagen, Ph.D., Environmental Laboratory Management — Date: 05/01/06

The 3M Environmental Laboratory's Quality Assurance Unit has audited the data and report for this project.

Quality Assurance Representative — Date: 5-1-06

8 Attachments

8.1 Method Blank/LOQ Area Count Comparison.

Table 11. Method Blank/LOQ Area Count Comparison: April 21, 2006.

Analytical Run: April 21, 2006			*Area Counts*			
Datafile	*Sample Description*	*Sample Name*	*PFOA*	*PFOS*	*PFHS*	*PFBS*
Method Blanks Prepared with the Extracted Curve						
s060421a025	Method Blank-1	MB-060410-1	55982	3413	1591	2760
s060421a026	Method Blank-2	MB-060410-2	18376	1082	668	2001
s060421a027	Method Blank-3	MB-060410-3	16127	1718	1970	2336
s060421a028	Method Blank-4	MB-060410-4	22714	1116	639	1305
s060421a029	Method Blank-5	MB-060410-5	21587	792	1686	2109
s060421a030	Method Blank-6	MB-060410-6	25928	4338	2077	5711
s060421a031	Method Blank-7	MB-060410-7	21060	2143	2436	3561
s060421a032	Method Blank-8	MB-060410-8	16383	1776	1660	4235
Method Blanks Prepared with the Extracted Samples						
s060421a045	Method Blank-1	MB-060421-1	12933	1437	[1]**121128**	277
s060421a046	Method Blank-2	MB-060421-2	12809	1446	[1]**129379**	715
s060421a047	Method Blank-3	MB-060421-3	13875	2228	[1]**66834**	595
s060421a048	Method Blank-4	MB-060421-4	22242	2053	[1]**42902**	1052
s060421a049	Method Blank-5	MB-060421-5	19675	1762	[1]**33923**	826
s060421a050	Method Blank-6	MB-060421-6	17774	1568	[1]**13105**	2071
s060421a051	Method Blank-7	MB-060421-7	13843	1084	6059	[2]**19419**
s060421a052	Method Blank-8	MB-060421-8	14073	1492	5570	450
Area Counts of the LOQ standard			163373	14600	13176	25766
Area Counts of the LOQ standard*0.5			81686.5	7300	6588	12883
Concentration of LOQ standard (ng/mL)			0.0500	0.0999	0.025	0.025

(1) Method blanks injected immediately after a highly contaminated solvent blank. Carryover present. Values excluded when establishing the LOQ for PFHS.

(2) Method blank determined to be a statistical outlier at the 99% confidence level using Dixon's Q-test. Value excluded when establishing the LOQ for PFBS.

Table 12. Method Blank/LOQ Area Count Comparison: April 24, 2006.

Analytical Run: April 24, 2006			Area Counts			
Datafile	Sample Description	Sample Name	PFOA	PFOS	PFHS	PFBS
Method Blanks Prepared with the Extracted Curve						
s060424a025	Method Blank-1	MB-060410-1	[(1)]36625	3514	2029	3078
s060424a026	Method Blank-2	MB-060410-2	[(1)]42239	2274	2890	3938
s060424a027	Method Blank-3	MB-060410-3	25302	2950	1147	2248
s060424a028	Method Blank-4	MB-060410-4	22151	804	1197	1638
s060424a029	Method Blank-5	MB-060410-5	30401	2565	1955	2593
s060424a030	Method Blank-6	MB-060410-6	17458	827	1275	5242
s060424a031	Method Blank-7	MB-060410-7	23074	1195	1974	3516
s060424a032	Method Blank-8	MB-060410-8	22049	1192	1512	4294
Method Blanks Prepared with the Extracted Laboratory Matrix Spikes						
s060424a090	Method Blank-1	MB-060425-1	30448	2301	1778	1906
s060424a091	Method Blank-2	MB-060425-2	15006	1266	1311	2990
s060424a092	Method Blank-3	MB-060425-3	11262	991	1039	1703
s060424a093	Method Blank-4	MB-060425-4	11867	836	707	6142
s060424a094	Method Blank-5	MB-060425-5	16236	1024	717	2723
s060424a095	Method Blank-6	MB-060425-6	15924	1107	589	5446
s060424a096	Method Blank-7	MB-060425-7	22758	1530	1038	4359
s060424a097	Method Blank-8	MB-060425-8	19284	1971	1135	1677
Area Counts of the LOQ standard			36060	6724	12166	27032
Area Counts of the LOQ standard*0.5			18030	3362	6083	13516
Concentration of LOQ standard (ng/mL)			0.025	0.04	0.025	0.025

(1) Value excluded for the LOQ determination for the given set. Area counts are very close to one-half the LOQ value. Prior analysis of this extract demonstrated area counts below one-half the 25 ppt standard. LMSs analyzed on this day are well above the LOQ.

8.2 Solvent Blank/LOQ Comparison.

Table 13. Area Counts of Solvent Blank.

		Area Counts			
File	Vial Position	PFOA	PFOS	PFHS	PFBS
s060421a001	91	70233	3067	1853	192
s060421a002	91	16249	2196	1851	563
s060421a003	91	18222	1642	567	471
s060421a009	91	48287	4524	1505	447
s060421a010	92	(1)**84762**	5006	2424	889
s060421a023	92	48308	4192	4524	6609
s060421a024	92	(1)**84666**	5317	4738	7007
s060421a035	93	(1)**162166**	(1)**283547**	(1)**52950**	(1)**41582**
s060421a037	93	23806	(1)**8904**	2706	2357
s060421a042	93	15939	2135	1693	1823
s060421a044	94	49169	4298	(1)**274354**	1118
s060421a055	94	12685	1141	(1)**328841**	547
s060421a057	94	47071	3382	(1)**257946**	946
s060421a068	95	53698	2525	5237	1137
s060421a070	95	74125	5907	6588	975
s060421a081	95	62139	2124	3945	1225
s060421a083	95	68329	3139	3951	1149
s060421a094	96	76799	4192	3512	1054
s060421a096	96	77269	3358	3905	1173
s060421a107	97	14824	1166	852	335
s060421a109	97	19120	1763	1499	1289
s060421a120	97	9918	1028	1024	698
s060421a122	98	24599	1589	2321	3275
s060421a127	98	37573	1305	6127	2190
s060421a129	99	18673	1100	1909	937
s060421a135	99	31743	1944	1472	632
LOQ Standard		163373	14600	13176	25766
½*LOQ Standard		81687	7300	6588	12883

(1) Area counts of the solvent blank were greater than ½*the LOQ standard area counts.

INTERIM REPORT # 10 - Analysis of Fish and Clam Samples

STUDY TITLE

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

DATA REQUIREMENTS

EPA TSCA Good Laboratory Practice Standards 40 CFR 792

STUDY DIRECTOR

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INTERIM REPORT COMPLETION DATE

May 09, 2006

PERFORMING LABORATORY

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STUDY SPONSOR

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PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 132

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number P0001131, entitled "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," conducted for 3M Company, is being performed in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by Exygen Research.

John Flaherty
Principal Investigator
Exygen Research

5/9/06
Date

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

5/10/06
Date

Michael A. Santoro
Sponsor Representative
3M Company

5/12/06
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number P0001131, entitled, "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program". All reviewed phases[1] were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director
18. Raw Data Review and Interim Analytical Report Review	08/01-05/05	09/20/05	09/21/05	09/21/05
26. Raw Data Review and Interim Analytical Report Review	05/03/06	05/08/06	05/09/06	05/09/06

Lydia Shaffer
Technical Lead, Quality Assurance Unit

05/09/06
Date

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

CERTIFICATION OF AUTHENTICITY

This interim report, for Exygen Study Number P0001131, is a true and complete representation of the raw data.

Submitted by: Exygen Research
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Principal Investigator, Exygen:

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Date

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:

Name	Title
John Flaherty	Vice President
Karen Risha	Laboratory Supervisor
Paul Connolly	Technical Lead-LC/MS
Amy Sheehan	Associate Scientist
Christine Edwards	Technician
Brittany Kravets	Technician
Mindy Cressley	Technician
Krista Gallant	Technician
Scott Crain	Technician
Eric Hoover	Technician
Carlyle Horrell	Intern Technician
Shawn Robb	Technical Lead - Facilities
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Eric Edwards	Sample Custodian
Edward Carns	Sample Custodian

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF APPENDICES

Page

1.0 SUMMARY

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

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

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

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

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

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

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

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

F = Filet tissue sample
W = Whole body sample

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

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

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

Examples of this sample ID system are:

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

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

2.0 OBJECTIVE

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

3.0 INTRODUCTION

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

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

4.0 ANALYTICAL TEST SAMPLES

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

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

5.0 REFERENCE MATERIAL

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

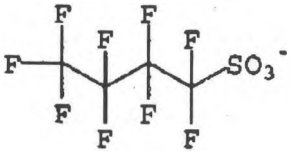
The available information for the reference materials is listed below. PFBS and PFHS were stored frozen and PFOS was stored refrigerated.

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

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

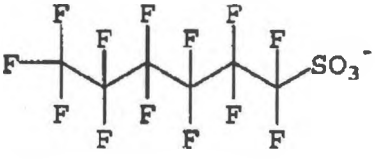
PFBS

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



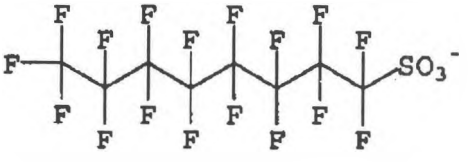
PFHS

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



PFOS

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



6.0 DESCRIPTION OF ANALYTICAL METHOD

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

6.1 Extraction Procedure

6.1.1 Sample Preparation

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

6.1.2 Glassware Preparation

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

6.1.3 Column Preparation

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

6.1.4 Sample Extraction

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

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

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

6.2 Preparation of Standards and Fortification Solutions

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

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

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

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

Conc. of Fort Solution (µg/mL)[1]	Fort Volume (mL)	Volume of Fortified Sample (mL)	Final Conc. of Calibration Std. (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.01	10	100	0.001
0.005	10	100	0.0005

[1] of PFBS, PFHS and PFOS

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

6.3 Chromatography

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

6.4 Instrument Sensitivity

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

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

Instrument: API 4000 Biomolecular Mass Analyzer

Interface: Turbo Ion Spray Liquid Introduction Interface

Computer: DELL OptiPlex GX400

Software: Windows NT, Analyst 1.4.1

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

HP Column Oven

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

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

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFBS	negative	299 → 99	~4.7 min.
PFHS	negative	399 → 80	~10.8 min.
PFOS	negative	499 → 80	~12.9 min.

6.6 Quantitation and Example Calculation

Fifteen microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x fit weighted linear regression) by Analyst software using six concentrations of standards. The concentration was determined from the following equations.
Equation 1 calculated the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program.

Equation 1:

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

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

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

Equation 2:

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

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

Equation 3:

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

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

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

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

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

From equation 1:

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

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

From equation 2:

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

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

From equation 3:

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

$$= 85\ \%$$

7.0 EXPERIMENTAL DESIGN

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

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

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

8.0 RESULTS

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

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

9.0 CONCLUSIONS

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

10.0 RETENTION OF DATA AND SAMPLES

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

TABLES

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048126	DL3-F01-IPF001-0-041021	0.309	40	ND	30	5.16	30
C0048126 Rep	DL3-F01-IPF001-0-041021*	0.318	40	ND	30	5.64	30
C0048127	DL3-F01-IPF002-0-041021	0.266	40	ND	30	4.16	40
C0048127 Rep	DL3-F01-IPF002-0-041021*	0.382	40	ND	30	4.56	40
C0048128	DMC-F01-IPF001-0-041021	0.330	50	ND	50	7.40	50
C0048128 Rep	DMC-F01-IPF001-0-041021*	0.366	50	ND	50	8.36	50
C0048129	DMC-F01-IPF002-0-041021	0.712	30	ND	30	14.1	50
C0048129 Rep	DMC-F01-IPF002-0-041021*	0.792	30	ND	30	17.0	50
C0048130	DMC-F01-IPW001-0-041021	1.08	40	ND	30	70.0	40
C0048130 Rep	DMC-F01-IPW001-0-041021*	1.06	40	0.297	30	58.4	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	0.346	40	ND	30	4.52	40
C0048137 Rep	DL2-F01-IPF004-0-041021*	0.330	40	ND	30	4.12	40
C0048138	DL2-F01-IPF005-0-041021	0.338	40	ND	30	2.46	30
C0048138 Rep	DL2-F01-IPF005-0-041021*	0.318	40	ND	30	2.80	30
C0048139	DL2-F01-IPW001-0-041021	0.328	30	ND	30	5.88	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	41.6	40
C0048142 Rep	DL2-F01-MSF003-0-041021*	0.376	40	ND	40	40.0	40
C0048143	DL2-F01-MSF004-0-041021	0.345	30	ND	30	83.6	30
C0048143 Rep	DL2-F01-MSF004-0-041021*	0.349	30	ND	30	83.2	30

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

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048144	DL1-F01-IPF001-0-041021	0.432	30	0.261	30	33.4	40
C0048144 Rep	DL1-F01-IPF001-0-041021*	0.428	30	0.252	30	32.6	40
C0048145	DL3-F01-IPF003-0-041021	0.448	30	ND	30	3.55	30
C0048145 Rep	DL3-F01-IPF003-0-041021*	0.440	30	0.229	30	3.35	30
C0048146	DL3-F01-IPF004-0-041021	0.784	40	ND	30	0.382	30
C0048146 Rep	DL3-F01-IPF004-0-041021*	0.900	40	ND	30	0.230	30
C0048147	DL3-F01-IPF005-0-041021	1.53	60	ND	30	5.48	40
C0048147 Rep	DL3-F01-IPF005-0-041021*	1.07	60	ND	30	5.64	40
C0048148	DBC-F01-IPF001-0-041021	1.34	50	0.692	30	NR	NR
C0048148 Rep	DBC-F01-IPF001-0-041021*	1.36	50	0.680	30	NR	NR
C0048149	DBC-F01-IPF002-0-041021	1.43	50	ND	30	110	30
C0048149 Rep	DBC-F01-IPF002-0-041021*	0.756	50	ND	30	109	30
C0048150	DBC-F01-IPF003-0-041021	0.996	50	0.644	30	NR	NR
C0048150 Rep	DBC-F01-IPF003-0-041021*	0.652	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	8.96	30	NR	NR
C0048153	DBC-F01-IPW003-0-041021	0.736	30	6.76	30	NR	NR
C0048153 Rep	DBC-F01-IPW003-0-041021*	0.760	30	7.44	30	NR	NR
C0048154	DOU-F01-IPF001-0-041021	4.36	40	1.02	30	NR	NR
C0048154 Rep	DOU-F01-IPF001-0-041021*	4.24	40	0.972	30	NR	NR
C0048155	DOU-F01-IPF002-0-041021	6.76	40	0.792	30	NR	NR
C0048155 Rep	DOU-F01-IPF002-0-041021*	6.28	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	0.956	30	NR	NR
C0048158 Rep	DOU-F01-IPF005-0-041021*	4.36	30	0.892	30	NR	NR
C0048159	DOU-F01-IPW001-0-041021	2.89	30	0.948	30	NR	NR
C0048159 Rep	DOU-F01-IPW001-0-041021*	2.81	30	0.964	30	NR	NR
C0048160	DOU-F01-IPW002-0-041021	18.4	50	3.69	30	NR	NR
C0048160 Rep	DOU-F01-IPW002-0-041021*	19.3	50	3.70	30	NR	NR
C0048161	DL2-F01-MSW001-0-041021	0.203	30	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

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NR = Not reported due to quality control result failures.

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048163	DL2-F01-MSW003-0-041021	ND	30	ND	30	92.4	30
C0048163 Rep	DL2-F01-MSW003-0-041021*	ND	30	ND	30	91.6	30
C0048164	DL2-F01-MSW004-0-041021	ND	30	ND	30	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	168	30
C0048165 Rep	DL2-F01-MSW005-0-041021*	ND	30	ND	30	174	30
C0049450	DBC-F01-MSW001-0-041104	0.868	30	8.32	30	NR	NR
C0049450 Rep	DBC-F01-MSW001-0-041104*	0.852	30	8.32	30	NR	NR
C0049451	DBC-F01-MSW002-0-041104	0.868	30	3.43	30	NR	NR
C0049451 Rep	DBC-F01-MSW002-0-041104*	0.884	30	3.35	30	NR	NR
C0049452	DBC-F01-MSW003-0-041104	0.860	30	3.62	30	NR	NR
C0049452 Rep	DBC-F01-MSW003-0-041104*	0.784	30	3.64	30	NR	NR
C0049453	DBC-F01-MSW004-0-041104	0.884	30	9.72	30	NR	NR
C0049453 Rep	DBC-F01-MSW004-0-041104*	0.880	30	9.80	30	NR	NR
C0049454	DBC-F01-MSW005-0-041104	0.832	30	2.77	30	NR	NR
C0049454 Rep	DBC-F01-MSW005-0-041104*	0.832	30	2.66	30	NR	NR
C0049455	DBC-F01-MSF001-0-041104	0.820	30	3.58	30	NR	NR
C0049455 Rep	DBC-F01-MSF001-0-041104*	0.788	30	3.46	30	NR	NR
C0049456	DBC-F01-MSF002-0-041104	0.788	30	1.94	30	NR	NR
C0049456 Rep	DBC-F01-MSF002-0-041104*	0.780	30	1.90	30	NR	NR
C0049457	DBC-F01-IPF004-0-041104	0.504	30	0.584	30	142	30
C0049457 Rep	DBC-F01-IPF004-0-041104*	0.520	30	0.540	30	152	30
C0049458	DBC-F01-IPF005-0-041104	1.60	30	2.15	30	NR	NR
C0049458 Rep	DBC-F01-IPF005-0-041104*	1.64	30	2.18	30	NR	NR
C0049459	DBC-F01-IPW004-0-041104	0.484	30	3.28	30	NR	NR
C0049459 Rep	DBC-F01-IPW004-0-041104*	0.444	30	3.28	30	NR	NR
C0049460	DBC-F01-IPW005-0-041104	0.572	30	2.10	30	NR	NR
C0049460 Rep	DBC-F01-IPW005-0-041104*	0.532	30	2.10	30	NR	NR
C0049461	DOU-F01-IPW003-0-041104	3.12	30	6.00	30	NR	NR
C0049461 Rep	DOU-F01-IPW003-0-041104*	3.05	30	5.92	30	NR	NR
C0049462	DOU-F01-IPW004-0-041104	3.49	30	7.12	30	NR	NR
C0049462 Rep	DOU-F01-IPW004-0-041104*	3.11	30	6.84	30	NR	NR
C0049463	DOU-F01-IPW005-0-041104	2.97	30	5.12	30	NR	NR
C0049463 Rep	DOU-F01-IPW005-0-041104*	2.90	30	5.32	30	NR	NR
C0049464	DL2-F01-IPW002-0-041104	1.04	30	ND	30	8.20	40
C0049464 Rep	DL2-F01-IPW002-0-041104*	0.960	30	ND	30	6.60	40
C0049465	DL1-F01-MSW001-0-041104	1.20	30	0.896	30	NR	NR
C0049465 Rep	DL1-F01-MSW001-0-041104*	1.24	30	0.932	30	NR	NR

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NR = Not reported due to quality control result failures.

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049466	DL1-F01-MSW002-0-041104	1.30	30	1.72	30	NR	NR
C0049466 Rep	DL1-F01-MSW002-0-041104*	1.15	30	1.54	30	NR	NR
C0049467	DL1-F01-MSW003-0-041104	0.560	30	1.13	30	NR	NR
C0049467 Rep	DL1-F01-MSW003-0-041104*	0.528	30	1.14	30	NR	NR
C0049468	DL1-F01-MSW004-0-041104	1.22	30	0.908	30	181	30
C0049468 Rep	DL1-F01-MSW004-0-041104*	1.12	30	0.880	30	186	30
C0049469	DL1-F01-MSW005-0-041104	1.22	30	1.18	30	NR	NR
C0049469 Rep	DL1-F01-MSW005-0-041104*	1.20	30	1.18	30	NR	NR
C0049470	DL1-F01-MSF001-0-041104	ND	30	0.315	30	293	30
C0049470 Rep	DL1-F01-MSF001-0-041104*	ND	30	0.299	30	307	30
C0049471	DL1-F01-MSF002-0-041104	ND	30	0.624	30	NR	NR
C0049471 Rep	DL1-F01-MSF002-0-041104*	ND	30	0.604	30	NR	NR
C0049472	DL1-F01-MSF003-0-041104	0.203	30	0.652	30	NR	NR
C0049472 Rep	DL1-F01-MSF003-0-041104*	ND	30	0.676	30	NR	NR
C0049473	DL1-F01-MSF004-0-041104	ND	30	0.532	30	NR	NR
C0049473 Rep	DL1-F01-MSF004-0-041104*	ND	30	0.536	30	NR	NR
C0049474	DL1-F01-MSF005-0-041104	ND	30	0.684	30	NR	NR
C0049474 Rep	DL1-F01-MSF005-0-041104*	ND	30	0.644	30	NR	NR
C0049475	DL1-F01-IPW001-0-041104	0.772	30	0.556	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	0.516	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	59.6	30
C0049478 Rep	DL1-F01-IPW004-0-041104*	ND	30	0.246	30	57.2	30
C0049479	DL1-F01-IPF002-0-041104	ND	30	0.222	30	11.1	30
C0049479 Rep	DL1-F01-IPF002-0-041104*	ND	30	0.247	30	11.2	30
C0049480	DL-1-F01-IPF003-0-041104	ND	30	0.265	30	20.9	30
C0049480 Rep	DL-1-F01-IPF003-0-041104*	ND	30	0.262	30	21.4	30
C0049481	DL-1-F01-IPF004-0-041104	ND	30	ND	30	7.60	30
C0049481 Rep	DL-1-F01-IPF004-0-041104*	ND	30	ND	30	7.36	30
C0049482	DL-1-F01-IPF005-0-041104	ND	30	0.373	30	8.72	30
C0049482 Rep	DL-1-F01-IPF005-0-041104*	ND	30	0.386	30	8.56	30
C0049483	DL3-F01-MSW001-0-041105	ND	30	ND	30	139	30
C0049483 Rep	DL3-F01-MSW001-0-041105*	ND	30	ND	30	137	30
C0049484	DL3-F01-MSW002-0-041105	ND	30	ND	30	160	30
C0049484 Rep	DL3-F01-MSW002-0-041105*	ND	30	ND	30	162	30

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049485	DL3-F01-MSW003-0-041105	ND	30	ND	30	146	30
C0049485 Rep	DL3-F01-MSW003-0-041105*	ND	30	ND	30	144	30
C0049486	DL3-F01-MSW004-0-041105	ND	30	0.285	30	198	30
C0049486 Rep	DL3-F01-MSW004-0-041105*	ND	30	0.282	30	199	30
C0049487	DL3-F01-MSW005-0-041105	ND	30	0.366	30	173	30
C0049487 Rep	DL3-F01-MSW005-0-041105*	ND	30	0.341	30	168	30
C0049488	DL3-F01-MSF001-0-041105	ND	30	ND	30	116	30
C0049488 Rep	DL3-F01-MSF001-0-041105*	ND	30	ND	30	115	30
C0049489	DL3-F01-MSF002-0-041105	ND	30	ND	30	116	30
C0049489 Rep	DL3-F01-MSF002-0-041105*	ND	30	ND	30	111	30
C0049490	DL3-F01-MSF003-0-041105	ND	30	ND	30	75.6	30
C0049490 Rep	DL3-F01-MSF003-0-041105*	ND	30	ND	30	72.0	30
C0049491	DL3-F01-MSF004-0-041105	ND	30	ND	30	130	30
C0049491 Rep	DL3-F01-MSF004-0-041105*	ND	30	ND	30	134	30
C0049492	DL3-F01-MSF005-0-041105	ND	30	ND	30	81.6	30
C0049492 Rep	DL3-F01-MSF005-0-041105*	ND	30	ND	30	83.6	30
C0049493	DMC-F01-IPW003-0-041105	ND	30	0.366	30	61.2	30
C0049493 Rep	DMC-F01-IPW003-0-041105*	ND	30	0.334	30	63.6	30
C0049494	DMC-F01-IPW004-0-041105	0.300	30	0.396	30	68.8	30
C0049494 Rep	DMC-F01-IPW004-0-041105*	0.345	30	0.464	30	69.6	30
C0049495	DMC-F01-IPW005-0-041105	ND	30	0.270	30	64.4	30
C0049495 Rep	DMC-F01-IPW005-0-041105*	ND	30	0.255	30	62.8	30
C0049496	DMC-F01-MSW003-0-041105	ND	30	0.592	30	354	30
C0049496 Rep	DMC-F01-MSW003-0-041105*	ND	30	0.560	30	330	30
C0049497	DMC-F01-MSW004-0-041105	0.318	30	1.90	30	259	30
C0049497 Rep	DMC-F01-MSW004-0-041105*	0.233	30	1.88	30	269	30
C0049498	DMC-F01-MSW005-0-041105	ND	30	0.960	30	NR	NR
C0049498 Rep	DMC-F01-MSW005-0-041105*	ND	30	0.936	30	NR	NR
C0049499	DMC-F01-MSF002-0-041105	ND	30	ND	30	NR	NR
C0049499 Rep	DMC-F01-MSF002-0-041105*	ND	30	0.367	30	NR	NR
C0049500	DMC-F01-MSF003-0-041105	ND	30	ND	30	56.8	40
C0049500 Rep	DMC-F01-MSF003-0-041105*	ND	30	ND	30	58.0	40
C0049501	DMC-F01-MSF004-0-041105	ND	30	0.254	30	116	30
C0049501 Rep	DMC-F01-MSF004-0-041105*	ND	30	0.228	30	119	30
C0049502	DMC-F01-MSF005-0-041105	ND	30	0.420	30	146	30
C0049502 Rep	DMC-F01-MSF005-0-041105*	ND	30	0.448	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

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
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	48.4	30
C0049505 Rep	DMC-F01-IPF005-0-041105*	ND	30	ND	30	46.0	30
C0056354	DL2-F01-MSF005-0-041216	ND	30	ND	30	49.2	30
C0056354 Rep	DL2-F01-MSF005-0-041216*	ND	30	ND	30	51.6	30
C0056455	DL2-F01-IPW006-0-041215	ND	30	ND	30	13.2	30
C0056455 Rep	DL2-F01-IPW005-0-041215*	ND	30	ND	30	13.3	30
C0056456	DOU-F01-MSW002-0-041216	0.206	40	1.72	30	NR	NR
C0056456 Rep	DOU-F01-MSW002-0-041216*	0.227	40	1.70	30	NR	NR
C0056457	DL2-F01-IPW004-0-041215	ND	30	ND	30	6.80	30
C0056457 Rep	DL2-F01-IPW004-0-041215*	ND	30	ND	30	6.80	30
C0056458	DOU-F01-MSW003-0-041216	ND	30	10.4	30	NR	NR
C0056458 Rep	DOU-F01-MSW003-0-041216*	ND	30	10.6	30	NR	NR
C0056459	DL3-F01-IPW004-0-041130	ND	30	ND	30	7.92	30
C0056459 Rep	DL3-F01-IPW004-0-041130*	ND	30	ND	30	7.52	30
C0056460	DOU-F01-MSW001-0-041216	7.08	30	15.4	30	NR	NR
C0056460 Rep	DOU-F01-MSW001-0-041216*	7.12	30	14.2	30	NR	NR
C0056461	DOU-F01-MSW004-0-041216	4.72	40	21.7	30	NR	NR
C0056461 Rep	DOU-F01-MSW004-0-041216*	4.68	40	22.5	30	NR	NR
C0056462	DOU-F01-MSW005-0-041216	0.364	30	1.34	30	NR	NR
C0056462 Rep	DOU-F01-MSW005-0-041216*	0.36	30	1.41	30	NR	NR
C0056463	DOU-F01-MSF005-0-041216	1.41	30	8.40	30	NR	NR
C0056463 Rep	DOU-F01-MSF005-0-041216*	1.43	30	8.44	30	NR	NR
C0056464	DL3-F01-IPW002-0-041130	ND	30	ND	30	12.0	50
C0056464 Rep	DL3-F01-IPW002-0-041130*	ND	30	ND	30	11.5	50
C0056465	DL3-F01-IPW003-0-041130	ND	30	ND	30	5.44	40
C0056465 Rep	DL3-F01-IPW003-0-041130*	ND	30	ND	30	5.28	40
C0056466	DOU-F01-MSF003-0-041216	1.72	30	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	1.68	30	NR	NR
C0056468 Rep	DBC-F01-MSF005-0-041216*	ND	30	1.59	30	NR	NR
C0056469	DOU-F01-MSF002-0-041216	0.868	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	8.80	30

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

		C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS [illegible]		C8 Sulfonate PFOS [illegible]	
Exygen ID	Client Sample ID	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0056471	DOU-F01-MSF002-1-041216	0.840	30	4.68	30	NR	NR
C0056471 Rep	DOU-F01-MSF002-1-041216*	0.880	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	88.8	30
C0056477 Rep	DL1-F01-IPW005-0-041216*	0.249	30	0.644	30	87.6	30
C0056478	DL2-F01-IPW003-0-041201	ND	30	ND	30	7.48	40
C0056478 Rep	DL2-F01-IPW003-0-041201*	ND	30	ND	30	7.44	40
C0056479	DL3-F01-IPW005-0-041201	ND	30	ND	30	4.88	30
C0056479 Rep	DL3-F01-IPW005-0-041201*	ND	30	ND	30	4.72	30

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

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

Exygen ID	Client Sample ID	C8 Sulfonate PFOS Perfluorooctanesulfonate Analyte Found (ng/g) Wet Weight	Assessed Accuracy (+/- %)
C0048151	DBC-F01-IPW001-0-041021	2080	30
C0048152	DBC-F01-IPW002-0-041021	3330	30
C0048153	DBC-F01-IPW003-0-041021	1660	50
C0048154	DOU-F01-IPF001-0-041021	596	30
C0048155	DOU-F01-IPF002-0-041021	836	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	12600	30
C0049453	DBC-F01-MSW004-0-041104	3440	30
C0049454	DBC-F01-MSW005-0-041104	5240	30
C0049455	DBC-F01-MSF001-0-041104	5240	30
C0049456	DBC-F01-MSF002-0-041104	4760	30
C0049458	DBC-F01-IPF005-0-041104	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	898	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	560	30
C0049499	DMC-F01-MSF002-0-041105	620	30
C0056456	DOU-F01-MSW002-0-041216	28200	60
C0056458	DOU-F01-MSW003-0-041216	4040	30
C0056460	DOU-F01-MSW001-0-041216	NR	NR
C0056461	DOU-F01-MSW004-0-041216	5120	30
C0056462	DOU-F01-MSW005-0-041216	496	30
C0056463	DOU-F01-MSF005-0-041216	720	30
C0056466	DOU-F01-MSF003-0-041216	848	30
C0056467	DBC-F01-MSF005-1-041216	NR	NR
C0056468	DBC-F01-MSF005-0-041216	8960	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

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

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPF001-0-041021 (C0048126 Spk C, 10 ppb)	10	0.309	6.56	63	ND	8.28	83	5.16	12.8	74
DL3-F01-IPF001-0-041021 (C0048126 Spk F, 100 ppb)	100	0.309	82.4	82	ND	73.2	73	5.16	78.4	73
DL3-F01-IPF002-0-041021 (C0048127 Spk D, 10 ppb)	10	0.265	6.52	63	ND	7.48	75	4.16	10.3	61
DL3-F01-IPF002-0-041021 (C0048127 Spk G, 100 ppb)	100	0.265	89.6	89	ND	79.6	80	4.16	80.8	77
DMC-F01-IPF001-0-041021 (C0048128 Spk E, 10 ppb)	10	0.330	4.48	42	ND	5.24	52	7.40	9.96	26
DMC-F01-IPF001-0-041021 (C0048128 Spk H, 100 ppb)	100	0.330	86.4	86	ND	74.8	75	7.40	81.8	74
DMC-F01-IPF002-0-041021 (C0048129 Spk C, 10 ppb)	10	0.712	7.68	70	ND	9.60	96	14.1	29.8	156
DMC-F01-IPF002-0-041021 (C0048129 Spk H, 100 ppb)	100	0.712	78.0	77	ND	96.0	96	14.1	104	90
DMC-F01-IPW001-0-041021 (C0048130 Spk D, 10 ppb)	10	1.08	7.60	35	ND	7.84	78	70.0	71.8	*
DMC-F01-IPW001-0-041021 (C0048130 Spk I, 100 ppb)	100	1.08	64.8	64	ND	80.0	80	70.0	131	61
DMC-F01-IPW002-0-041021 (C0048131 Spk E, 10 ppb)	10	0.966	5.76	58	ND	7.96	80	34.4	44.8	*
DMC-F01-IPW002-0-041021 (C0048131 Spk J, 100 ppb)	100	0.966	60.8	60	ND	74.8	75	34.4	101	67
DMC-F01-MSW001-0-041021 (C0048132 Spk E, 10 ppb Spike)	10	0.218	9.76	95	0.524	9.44	89	270	270	*
DMC-F01-MSW001-0-041021 (C0048132 Spk F, 100 ppb Spike)	100	0.218	81.2	81	0.524	93.2	93	270	375	105
DMC-F01-MSW002-0-041021[A] (C0048133 Spk G, 50 ppb Spike)	50	ND[A]	45.2	90	ND[A]	52.0	104	530	470	*
DMC-F01-MSW002-0-041021[A] (C0048133 Spk H, 500 ppb Spike)	500	ND[A]	432	86	ND[A]	482	96	530	924	79
DL2-F01-IPF001-0-041021 (C0048134 Spk F, 10 ppb Spike)	10	0.660	5.80	51	ND	6.56	66	3.45	8.36	49
DL2-F01-IPF001-0-041021 (C0048134 Spk K, 100 ppb Spike)	100	0.660	46.4	46	ND	58.8	59	3.45	56.4	53
DL2-F01-IPF002-0-041021 (C0048135 Spk G, 10 ppb Spike)	10	0.816	5.84	48	ND	6.40	64	4.72	8.56	38
DL2-F01-IPF002-0-041021 (C0048135 Spk L, 100 ppb Spike)	100	0.816	64.8	64	ND	78.8	79	4.72	72.4	68

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL2-F01-IPF003-0-041021 (C0048136 Spk C, 10 ppb Spike)	10	0.247	7.00	68	ND	7.98	80	2.01	9.20	72
DL2-F01-IPF003-0-041021 (C0048136 Spk H, 100 ppb Spike)	100	0.247	62.8	63	ND	73.2	73	2.01	74.0	72
DL2-F01-IPF004-0-041021 (C0048137 Spk D, 10 ppb Spike)	10	0.346	6.84	65	ND	7.60	76	4.52	11.3	68
DL2-F01-IPF004-0-041021 (C0048137 Spk I, 100 ppb Spike)	100	0.346	67.6	67	ND	76.8	77	4.52	80.4	76
DL2-F01-IPF005-0-041021 (C0048138 Spk E, 10 ppb Spike)	10	0.338	6.86	65	ND	7.68	77	2.46	9.68	72
DL2-F01-IPF005-0-041021 (C0048138 Spk J, 100 ppb Spike)	100	0.338	68.4	68	ND	80.6	81	2.46	82.0	80
DL2-F01-IPW001-0-041021 (C0048139 Spk F, 10 ppb Spike)	10	0.326	7.04	67	ND	7.60	76	5.86	13.9	80
DL2-F01-IPW001-0-041021 (C0048139 Spk K, 100 ppb Spike)	100	0.326	66.0	66	ND	77.2	77	5.86	85.2	79
DL2-F01-MSF001-0-041021 (C0048140 Spk G, 10 ppb Spike)	10	0.333	7.36	70	ND	8.20	82	77.2	78.0	*
DL2-F01-MSF001-0-041021 (C0048140 Spk L, 100 ppb Spike)	100	0.333	64.8	64	ND	77.6	78	77.2	158	81
DL2-F01-MSF002-0-041021 (C0048141 Spk C, 10 ppb Spike)	10	0.408	6.58	65	ND	7.48	75	94.8	101	*
DL2-F01-MSF002-0-041021 (C0048141 Spk H, 100 ppb Spike)	100	0.408	61.6	61	ND	68.0	68	94.8	158	61
DL2-F01-MSF003-0-041021 (C0048142 Spk D, 10 ppb Spike)	10	0.416	6.52	61	ND	6.76	68	41.6	41.2	*
DL2-F01-MSF003-0-041021 (C0048142 Spk I, 100 ppb Spike)	100	0.416	64.8	64	ND	70.8	71	41.6	104	62
DL2-F01-MSF004-0-041021 (C0048143 Spk E, 10 ppb Spike)	10	0.345	7.06	67	ND	7.56	76	83.6	91.2	*
DL2-F01-MSF004-0-041021 (C0048143 Spk J, 100 ppb Spike)	100	0.345	71.6	71	ND	81.2	81	83.6	167	83
DL1-F01-IPF001-0-041021 (C0048144 Spk F, 10 ppb Spike)	10	0.432	7.44	70	0.261	8.16	79	33.4	40.0	*
DL1-F01-IPF001-0-041021 (C0048144 Spk K, 100 ppb Spike)	100	0.432	67.2	67	0.261	76.4	76	33.4	106.0	73
DL3-F01-IPF003-0-041021 (C0048145 Spk G, 10 ppb Spike)	10	0.448	7.20	68	ND	8.08	81	3.55	10.8	73
DL3-F01-IPF003-0-041021 (C0048145 Spk L, 100 ppb Spike)	100	0.440	69.2	69	ND	81.6	82	3.55	80.0	76

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPF004-0-041021 (C0048146 Spk C, 10 ppb Spike)	10	0.784	6.84	61	ND	10.6	106	0.382	8.96	86
DL3-F01-IPF004-0-041021 (C0048146 Spk H, 100 ppb Spike)	100	0.784	69.6	69	ND	88.4	88	0.382	81.2	81
DL3-F01-IPF005-0-041021 (C0048147 Spk D, 10 ppb Spike)	10	1.53	5.44	39	ND	9.16	92	5.48	11.7	62
DL3-F01-IPF005-0-041021 (C0048147 Spk I, 100 ppb Spike)	100	1.53	60.4	59	ND	93.2	93	5.48	86.6	81
DBC-F01-IPF001-0-041021 (C0048148 Spk E, 10 ppb Spike)	10	1.34	6.24	49	0.662	10.8	101	NR	NR	NR
DBC-F01-IPF001-0-041021 (C0048148 Spk J, 100 ppb Spike)	100	1.34	56.4	55	0.662	86.8	86	NR	NR	NR
DBC-F01-IPF002-0-041021 (C0048149 Spk F, 10 ppb Spike)	10	1.43	5.84	44	ND	9.36	94	110	113	*
DBC-F01-IPF002-0-041021 (C0048149 Spk K, 100 ppb Spike)	100	1.43	59.2	58	ND	88.0	88	110	189	79
DBC-F01-IPF003-0-041021 (C0048150 Spk G, 10 ppb Spike)	10	0.996	6.72	57	0.644	10.4	98	NR	NR	NR
DBC-F01-IPF003-0-041021 (C0048150 Spk L, 100 ppb Spike)	100	0.996	60.6	60	0.644	88.8	88	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0048151 Spk C, 10 ppb Spike)	10	0.864	7.08	62	2.21	9.68	75	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0048151 Spk H, 100 ppb Spike)	100	0.864	70.0	69	2.21	88.0	86	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048152 Spk D, 10 ppb Spike)	10	0.700	7.56	69	9.12	18.2	91	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048152 Spk I, 100 ppb Spike)	100	0.700	69.6	69	9.12	96.0	87	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048153 Spk E, 10 ppb Spike)	10	0.736	8.32	76	6.76	16.5	97	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048153 Spk J, 100 ppb Spike)	100	0.736	70.8	70	6.76	90.8	84	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0048154 Spk F, 10 ppb Spike)	10	4.36	10.9	65	1.02	9.48	85	NR	NR	NR
DOU-F01-IPF001-0-041021 (C0048154 Spk K, 100 ppb Spike)	100	4.36	76.8	72	1.02	86.0	85	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0048155 Spk G, 10 ppb Spike)	10	8.76	13.1	63	0.792	9.72	89	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0048155 Spk L, 100 ppb Spike)	100	8.76	76.0	69	0.792	80.0	79	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0048156 Spk C, 10 ppb Spike)	10	5.86	12.8	69	1.02	10.8	98	NR	NR	NR
DOU-F01-IPF003-0-041021 (C0048156 Spk H, 100 ppb Spike)	100	5.86	81.2	75	1.02	88.4	87	NR	NR	NR

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DOU-F01-IPF004-0-041021 (C0048157 Spk D, 10 ppb Spike)	10	6.12	13.2	71	2.30	12.0	97	NR	NR	NR
DOU-F01-IPF004-0-041021 (C0048157 Spk I, 100 ppb Spike)	100	6.12	76.4	70	2.30	86.0	84	NR	NR	NR
DOU-F01-IPF005-0-041021 (C0048158 Spk E, 10 ppb Spike)	10	4.56	12.2	76	0.956	11.2	102	NR	NR	NR
DOU-F01-IPF005-0-041021 (C0048158 Spk J, 100 ppb Spike)	100	4.56	76.0	71	0.956	85.6	85	NR	NR	NR
DOU-F01-IPW001-0-041021 (C0048159 Spk F, 10 ppb Spike)	10	2.89	10.2	73	0.948	10.7	98	NR	NR	NR
DOU-F01-IPW001-0-041021 (C0048159 Spk K, 100 ppb Spike)	100	2.89	72.4	70	0.948	83.6	83	NR	NR	NR
DOU-F01-IPW002-0-041021 (C0048160 Spk G, 10 ppb Spike)	10	18.4	34.4	160	3.69	13.2	95	NR	NR	NR
DOU-F01-IPW002-0-041021 (C0048160 Spk L, 100 ppb Spike)	100	18.4	94.8	76	3.69	89.2	86	NR	NR	NR
DL2-F01-MSW001-0-041021 (C0048161 Spk I, 10 ppb Spike)	10	0.203	9.32	91	ND	8.68	87	120	126	*
DL2-F01-MSW001-0-041021 (C0048161 Spk J, 100 ppb Spike)	100	0.203	76.8	77	ND	86.2	85	120	177	57
DL2-F01-MSW002-0-041021 (C0048162 Spk K, 10 ppb Spike)	10	ND	9.66	97	ND	9.12	91	102	110	*
DL2-F01-MSW002-0-041021 (C0048162 Spk L, 100 ppb Spike)	100	ND	86.4	86	ND	93.6	94	102	165	63
DL2-F01-MSW003-0-041021 (C0048163 Spk C, 10 ppb Spike)	10	ND	9.24	92	ND	9.08	91	92.4	103	*
DL2-F01-MSW003-0-041021 (C0048163 Spk D, 100 ppb Spike)	100	ND	81.2	81	ND	95.2	95	92.4	182	90
DL2-F01-MSW004-0-041021 (C0048164 Spk E, 10 ppb Spike)	10	ND	7.56	76	ND	7.28	73	66.0	75.6	*
DL2-F01-MSW004-0-041021 (C0048164 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	93.6	94	66.0	166	76
DL2-F01-MSW005-0-041021 (C0048165 Spk G, 10 ppb Spike)	10	ND	7.96	80	ND	8.08	81	168	135	*
DL2-F01-MSW005-0-041021 (C0048165 Spk H, 100 ppb Spike)	100	ND	80.8	81	ND	100	103	168	234	66
DBC-F01-MSW001-0-041104 (C0048650 Spk C, 10 ppb Spike)	10	0.888	10.3	94	6.32	17.6	93	NR	NR	NR
DBC-F01-MSW001-0-041104 (C0048650 Spk D, 100 ppb Spike)	100	0.888	90.4	90	6.32	113	106	NR	NR	NR

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DBC-F01-MSW002-0-041104 (C0049451 Spk E, 10 ppb Spike)	10	0.888	9.96	91	3.43	12.8	94	NR	NR	NR
DBC-F01-MSW002-0-041104 (C0049451 Spk F, 100 ppb Spike)	100	0.888	94.8	94	3.43	113	110	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0049453 Spk G, 10 ppb Spike)	10	0.860	9.92	91	3.62	13.6	100	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0049452 Spk H, 100 ppb Spike)	100	0.860	86.0	85	3.62	104	100	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0049453 Spk I, 10 ppb Spike)	10	0.884	9.96	91	9.72	19.5	98	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0049453 Spk J, 100 ppb Spike)	100	0.884	97.2	96	9.72	124	114	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0049454 Spk K, 10 ppb Spike)	10	0.832	8.20	74	2.77	10.2	74	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0049454 Spk L, 100 ppb Spike)	100	0.832	91.6	91	2.77	108	105	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0049455 Spk M, 10 ppb Spike)	10	0.820	9.80	98	3.58	13.7	101	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0049455 Spk N, 100 ppb Spike)	100	0.820	98.4	98	3.58	106	102	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0049456 Spk O, 10 ppb Spike)	10	0.768	9.92	92	1.94	12.5	106	NR	NR	NR
DBC-F01-MSF002-0-041104 (C0049456 Spk P, 100 ppb Spike)	100	0.768	90.8	90	1.94	102	100	NR	NR	NR
DBC-F01-IPF004-0-041104 (C0049457 Spk C, 10 ppb Spike)	10	0.504	10.5	100	0.584	11.6	110	142	152	*
DBC-F01-IPF004-0-041104 (C0049457 Spk D, 100 ppb Spike)	100	0.504	94.8	94	0.584	108	106	142	254	112
DBC-F01-IPF005-0-041104 (C0049458 Spk E, 10 ppb Spike)	10	1.60	10.4	88	2.15	12.2	101	NR	NR	NR
DBC-F01-IPF005-0-041104 (C0049458 Spk F, 100 ppb Spike)	100	1.60	90.8	89	2.15	105	103	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0049459 Spk G, 10 ppb Spike)	10	0.484	9.72	92	3.28	13.0	97	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0049459 Spk H, 100 ppb Spike)	100	0.484	87.6	87	3.28	104	101	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0049460 Spk I, 10 ppb Spike)	10	0.572	10.4	98	2.10	13.2	111	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0049460 Spk J, 100 ppb Spike)	100	0.572	92.8	92	2.10	102	100	NR	NR	NR

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DOU-F01-IPW003-0-041104 (C0049461 Spk K, 10 ppb Spike)	10	3.12	13.1	100	8.00	16.3	103	NR	NR	NR
DOU-F01-IPW003-0-041104 (C0049461 Spk L, 100 ppb Spike)	100	3.12	86.0	85	8.00	107	101	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk C, 10 ppb Spike)	10	3.49	12.8	93	7.12	17.4	103	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk D, 100 ppb Spike)	100	3.49	86.4	85	7.12	108	101	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk E, 10 ppb Spike)	10	2.97	10.5	75	5.12	13.5	84	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk F, 100 ppb Spike)	100	2.97	83.6	81	5.12	109	104	NR	NR	NR
DL2-F01-IPW002-0-041104 (C0049464 Spk G, 10 ppb Spike)	10	1.04	9.48	84	ND	10.4	104	8.20	14.3	61
DL2-F01-IPW002-0-041104 (C0049464 Spk H, 100 ppb Spike)	100	1.04	80.0	79	ND	97.8	98	8.20	124	116
DL1-F01-MSW001-0-041104 (C0049465 Spk I, 10 ppb Spike)	10	1.20	10.6	94	0.898	11.3	104	NR	NR	NR
DL1-F01-MSW001-0-041104 (C0049465 Spk J, 100 ppb Spike)	100	1.20	99.6	98	0.898	112	111	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk K, 10 ppb Spike)	10	1.30	9.76	85	1.72	12.4	107	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk L, 100 ppb Spike)	100	1.30	90.4	79	1.72	100	98	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk C, 10 ppb Spike)	10	0.560	8.92	84	1.13	10.2	91	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk D, 100 ppb Spike)	100	0.560	85.2	85	1.13	98.8	98	NR	NR	NR
DL1-F01-MSW004-0-041104 (C0049468 Spk E, 10 ppb Spike)	10	0.908	8.52	76	0.908	9.76	89	181	201	*
DL1-F01-MSW004-0-041104 (C0049468 Spk F, 100 ppb Spike)	100	0.908	84.8	84	0.908	99.6	99	181	289	108
DL1-F01-MSW005-0-041104 (C0049469 Spk G, 10 ppb Spike)	10	1.16	8.80	76	1.16	10.4	92	NR	NR	NR
DL1-F01-MSW005-0-041104 (C0049469 Spk H, 100 ppb Spike)	100	1.16	82.4	81	1.16	98.8	98	NR	NR	NR
DL1-F01-MSF001-0-041104 (C0049470 Spk I, 10 ppb Spike)	10	ND	7.76	78	0.315	9.52	92	293	303	*
DL1-F01-MSF001-0-041104 (C0049470 Spk J, 100 ppb Spike)	100	ND	78.4	78	0.315	90.8	90	293	394	101

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0049471 Spk K, 10 ppb Spike)	10	ND	8.60	86	0.624	10.5	99	NR	NR	NR
DL1-F01-MSF002-0-041104 (C0049471 Spk L, 100 ppb Spike)	100	ND	84.8	85	0.624	98.4	98	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0049472 Spk C, 10 ppb Spike)	10	0.203	8.78	86	0.652	10.2	95	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0049472 Spk D, 100 ppb Spike)	100	0.203	84.4	84	0.652	95.2	95	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0049473 Spk E, 10 ppb Spike)	10	ND	8.86	89	0.532	10.4	99	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0049473 Spk F, 100 ppb Spike)	100	ND	86.8	87	0.532	101	100	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0049474 Spk G, 10 ppb Spike)	10	ND	8.64	86	0.684	10.1	94	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0049474 Spk H, 100 ppb Spike)	100	ND	82.4	82	0.684	94.0	93	NR	NR	NR
DL1-F01-IPW001-0-041104 (C0049475 Spk I, 10 ppb Spike)	10	0.772	8.64	79	0.556	9.72	92	17.4	26.4	90
DL1-F01-IPW001-0-041104 (C0049475 Spk J, 100 ppb Spike)	100	0.772	84.0	83	0.556	97.8	97	17.4	113	96
DL1-F01-IPW002-0-041104 (C0049476 Spk K, 10 ppb Spike)	10	0.468	8.68	82	0.536	9.96	94	15.7	27.4	117
DL1-F01-IPW002-0-041104 (C0049476 Spk L, 100 ppb Spike)	100	0.468	76.4	76	0.536	88.0	87	15.7	100	84
DL1-F01-IPW003-0-041104 (C0049477 Spk C, 10 ppb Spike)	10	ND	9.08	91	0.209	9.44	92	9.38	16.7	73
DL1-F01-IPW003-0-041104 (C0049477 Spk D, 100 ppb Spike)	100	ND	90.4	90	0.209	106	106	9.38	109	100
DL1-F01-IPW004-0-041104 (C0049478 Spk E, 10 ppb Spike)	10	ND	8.46	85	0.238	9.80	96	59.6	72.0	*
DL1-F01-IPW004-0-041104 (C0049478 Spk F, 100 ppb Spike)	100	ND	71.6	72	0.238	84.6	85	59.6	140	80
DL1-F01-IPF002-0-041104 (C0049479 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 (C0049479 Spk H, 100 ppb Spike)	100	ND	86.4	86	0.222	98.4	98	11.1	108	97
DL-1-F01-IPF003-0-041104 (C0049480 Spk I, 10 ppb Spike)	10	ND	8.80	88	0.265	10.8	103	20.9	30.5	96
DL-1-F01-IPF003-0-041104 (C0049480 Spk J, 100 ppb Spike)	100	ND	78.8	79	0.265	90.4	90	20.9	109	88

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL-1-F01-IPF004-0-041104 (C0049461 Spk K, 10 ppb Spike)	10	ND	8.60	80	ND	9.48	95	7.80	15.8	82
DL-1-F01-IPF004-0-041104 (C0049461 Spk L, 100 ppb Spike)	100	ND	84.8	85	ND	98.8	99	7.80	102	94
DL-1-F01-IPF005-0-041104 (C0049462 Spk C, 10 ppb Spike)	10	ND	7.44	74	0.373	9.00	86	8.72	15.9	72
DL-1-F01-IPF005-0-041104 (C0049462 Spk D, 100 ppb Spike)	100	ND	84.8	85	0.373	98.4	98	8.72	102	93
DL3-F01-MSW001-0-041105 (C0049463 Spk E, 10 ppb Spike)	10	ND	8.36	84	ND	9.20	92	139	166	*
DL3-F01-MSW001-0-041105 (C0049463 Spk F, 100 ppb Spike)	100	ND	95.6	88	ND	99.8	100	139	256	119
DL3-F01-MSW002-0-041105 (C0049464 Spk G, 10 ppb Spike)	10	ND	8.44	84	ND	9.92	99	160	150	*
DL3-F01-MSW002-0-041105 (C0049464 Spk H, 100 ppb Spike)	100	ND	90.4	90	ND	106	106	160	263	103
DL3-F01-MSW003-0-041105 (C0049465 Spk I, 10 ppb Spike)	10	ND	8.60	86	ND	9.58	96	146	154	*
DL3-F01-MSW003-0-041105 (C0049465 Spk J, 100 ppb Spike)	100	ND	86.4	86	ND	102	102	146	254	108
DL3-F01-MSW004-0-041105 (C0049466 Spk K, 10 ppb Spike)	10	ND	8.48	85	0.286	9.88	94	198	225	*
DL3-F01-MSW004-0-041105 (C0049466 Spk L, 100 ppb Spike)	100	ND	88.4	88	0.286	102	102	198	301	103
DL3-F01-MSW005-0-041105 (C0049467 Spk C, 10 ppb Spike)	10	ND	9.56	96	0.366	11.1	107	173	193	*
DL3-F01-MSW005-0-041105 (C0049467 Spk D, 100 ppb Spike)	100	ND	100	100	0.366	105	105	173	246	73
DL3-F01-MSF001-0-041105 (C0049468 Spk E, 10 ppb Spike)	10	ND	9.48	95	ND	11.4	114	116	138	*
DL3-F01-MSF001-0-041105 (C0049468 Spk F, 100 ppb Spike)	100	ND	94.8	95	ND	109	109	116	234	118
DL3-F01-MSF002-0-041105 (C0049469 Spk G, 10 ppb Spike)	10	ND	9.86	99	ND	10.4	104	116	95.8	*
DL3-F01-MSF002-0-041105 (C0049469 Spk H, 100 ppb Spike)	100	ND	89.6	90	ND	103	103	116	216	100
DL3-F01-MSF003-0-041105 (C0049470 Spk I, 10 ppb Spike)	10	ND	9.32	93	ND	11.0	110	75.6	92.0	*
DL3-F01-MSF003-0-041105 (C0049470 Spk J, 100 ppb Spike)	100	ND	90.8	91	ND	107	107	75.6	190	114

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-MSF004-0-041105 (C0049491 Spk K, 10 ppb Spike)	10	ND	8.60	86	ND	11.0	110	130	144	*
DL3-F01-MSF004-0-041105 (C0049491 Spk L, 100 ppb Spike)	100	ND	90.4	90	ND	104	104	130	238	108
DL3-F01-MSF005-0-041105 (C0049492 Spk C, 10 ppb Spike)	10	ND	8.92	89	ND	10.3	103	81.8	97.2	*
DL3-F01-MSF005-0-041105 (C0049492 Spk D, 100 ppb Spike)	100	ND	85.6	86	ND	98.4	98	81.8	181	99
DMC-F01-IPW003-0-041105 (C0049493 Spk E, 10 ppb Spike)	10	ND	8.26	83	0.366	9.52	91	81.2	79.0	*
DMC-F01-IPW003-0-041105 (C0049493 Spk F, 100 ppb Spike)	100	ND	84.4	84	0.366	99.6	99	81.2	158	95
DMC-F01-IPW004-0-041105 (C0049494 Spk G, 10 ppb Spike)	10	0.300	8.04	77	0.396	9.28	89	68.8	107	*
DMC-F01-IPW004-0-041105 (C0049494 Spk H, 100 ppb Spike)	100	0.300	90.4	90	0.396	104	104	68.8	168	99
DMC-F01-IPW005-0-041105 (C0049495 Spk I, 10 ppb Spike)	10	ND	8.98	90	0.270	10.1	98	64.4	75.8	*
DMC-F01-IPW005-0-041105 (C0049495 Spk J, 100 ppb Spike)	100	ND	88.4	88	0.270	105	105	64.4	153	89
DMC-F01-MSW003-0-041105 (C0049496 Spk K, 10 ppb Spike)	10	ND	9.84	98	0.592	11.0	104	354	389	*
DMC-F01-MSW003-0-041105 (C0049496 Spk L, 100 ppb Spike)	100	ND	92.4	92	0.592	108	106	354	464	110
DMC-F01-MSW004-0-041105 (C0049497 Spk C, 10 ppb Spike)	10	0.318	9.52	92	1.90	12.1	102	259	259	*
DMC-F01-MSW004-0-041105 (C0049497 Spk D, 100 ppb Spike)	100	0.318	82.4	82	1.90	101	99	259	313	54
DMC-F01-MSW005-0-041105 (C0049498 Spk E, 10 ppb Spike)	10	ND	9.16	92	0.980	11.8	108	NR	NR	NR
DMC-F01-MSW005-0-041105 (C0049498 Spk F, 100 ppb Spike)	100	ND	93.6	94	0.980	118	117	NR	NR	NR
DMC-F01-MSF002-0-041105 (C0049499 Spk G, 10 ppb Spike)	10	ND	8.36	84	ND	10.4	104	NR	NR	NR
DMC-F01-MSF002-0-041105 (C0049499 Spk H, 100 ppb Spike)	100	ND	97.6	98	ND	104	104	NR	NR	NR
DMC-F01-MSF003-0-041105 (C0049500 Spk I, 10 ppb Spike)	10	ND	8.20	82	ND	10.4	104	58.8	108	*
DMC-F01-MSF003-0-041105 (C0049500 Spk J, 100 ppb Spike)	100	ND	87.2	87	ND	110	110	58.8	194	137

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DMC-F01-MSF004-0-041105 (C0048601 Spk K, 10 ppb Spike)	10	ND	8.76	88	0.254	11.1	108	116	123	*
DMC-F01-MSF004-0-041105 (C0048601 Spk L, 100 ppb Spike)	100	ND	88.0	88	0.254	107	107	116	226	110
DMC-F01-MSF005-0-041105 (C0048602 Spk C, 10 ppb Spike)	10	ND	8.52	85	0.420	12.2	118	146	193	*
DMC-F01-MSF005-0-041105 (C0048602 Spk D, 100 ppb Spike)	100	ND	83.6	84	0.420	110	110	146	280	114
DMC-F01-IPF003-0-041105 (C0048603 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	10.8	108	7.92	16.0	81
DMC-F01-IPF003-0-041105 (C0048603 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	108	108	7.92	101	93
DMC-F01-IPF004-0-041105 (C0048604 Spk G, 10 ppb Spike)	10	ND	7.92	79	ND	10.7	107	44.0	56.2	*
DMC-F01-IPF004-0-041105 (C0048604 Spk H, 100 ppb Spike)	100	ND	83.2	83	ND	107	107	44.0	134	90
DMC-F01-IPF005-0-041105 (C0048605 Spk I, 10 ppb Spike)	10	ND	7.88	79	ND	10.3	103	46.4	54.4	*
DMC-F01-IPF005-0-041105 (C0048605 Spk J, 100 ppb Spike)	100	ND	75.6	76	ND	96.4	96	46.4	121	75
DL2-F01-MSF005-0-41216 (C0056364 Spk E, 10 ppb Spike)	10	ND	8.44	84	ND	10.0	100	49.2	59.2	100
DL2-F01-IPW005-0-041215 (C0056364 Spk F, 100 ppb Spike)	100	ND	83.2	83	ND	102	102	49.2	142	93
DL2-F01-IPW005-0-041215 (C0056456 Spk C, 10 ppb Spike)	10	ND	8.00	80	ND	11.5	115	13.2	22.7	95
DL2-F01-IPW005-0-041215 (C0056456 Spk D, 100 ppb Spike)	100	ND	84.8	85	ND	107	107	13.2	121	108
DOU-F01-MSW002-0-041216 (C0056458 Spk I, 10 ppb Spike)	10	0.206	8.82	87	1.72	10.9	92	NR	NR	NR
DOU-F01-MSW002-0-041216 (C0056458 Spk J, 100 ppb Spike)	100	0.206	87.2	87	1.72	92.8	91	NR	NR	NR
DL2-F01-IPW004-0-041215 (C0056457 Spk C, 10 ppb Spike)	10	ND	8.08	81	ND	10.4	104	6.80	14.6	80
DL2-F01-IPW004-0-041215 (C0056457 Spk D, 100 ppb Spike)	100	ND	76.8	77	ND	95.2	95	6.80	97.2	90
DOU-F01-MSW003-0-041216 (C0056459 Spk K, 10 ppb Spike)	10	ND	8.12	81	10.4	19.4	90	NR	NR	NR
DOU-F01-MSW003-0-041216 (C0056459 Spk L, 100 ppb Spike)	100	ND	88.4	88	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	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPW004-0-041130 (C0056459 Spk E, 10 ppb Spike)	10	ND	7.52	75	ND	11.0	110	7.92	17.3	94
DL3-F01-IPW004-0-041130 (C0056459 Spk F, 100 ppb Spike)	100	ND	66.4	66	ND	118	118	7.92	114	106
DOU-F01-MSW001-0-041216 (C0056460 Spk G, 10 ppb Spike)	10	7.06	14.6	75	15.4	27.5	121	NR	NR	NR
DOU-F01-MSW001-0-041216 (C0056460 Spk H, 100 ppb Spike)	100	7.06	83.2	76	15.4	122.0	107	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0056461 Spk E, 10 ppb Spike)	10	4.72	10.8	61	21.7	29.3	76	NR	NR	NR
DOU-F01-MSW004-0-041216 (C0056461 Spk F, 100 ppb Spike)	100	4.72	72.8	68	21.7	104	82	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0056462 Spk G, 10 ppb Spike)	10	0.364	7.08	67	1.34	8.52	72	NR	NR	NR
DOU-F01-MSW005-0-041216 (C0056462 Spk H, 100 ppb Spike)	100	0.364	72.0	72	1.34	87.6	86	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0056463 Spk C, 10 ppb Spike)	10	1.41	8.60	72	8.40	17.8	92	NR	NR	NR
DOU-F01-MSF005-0-041216 (C0056463 Spk D, 100 ppb Spike)	100	1.41	72.0	71	8.40	94.0	86	NR	NR	NR
DL3-F01-IPW002-0-041130 (C0056464 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	9.04	90	12.0	16.6	46
DL3-F01-IPW002-0-041130 (C0056464 Spk F, 100 ppb Spike)	100	ND	81.2	81	ND	110	110	12.0	105	93
DL3-F01-IPW003-0-041130 (C0056465 Spk G, 10 ppb Spike)	10	ND	7.98	80	ND	8.88	89	5.44	12.3	69
DL3-F01-IPW003-0-041130 (C0056465 Spk H, 100 ppb Spike)	100	ND	84.4	84	ND	102	102	5.44	92.0	87
DOU-F01-MSF003-0-041216 (C0056466 Spk K, 10 ppb Spike)	10	1.72	9.00	73	8.56	14.3	77	NR	NR	NR
DOU-F01-MSF003-0-041216 (C0056466 Spk L, 100 ppb Spike)	100	1.72	89.6	88	8.56	107	100	NR	NR	NR
DBC-F01-MSF005-1-041216 (C0056467 Spk C, 10 ppb Spike)	10	0.220	8.36	81	1.78	11.5	97	NR	NR	NR
DBC-F01-MSF005-1-041216 (C0056467 Spk D, 100 ppb Spike)	100	0.220	83.6	83	1.78	96.8	95	NR	NR	NR
DBC-F01-MSF005-0-041216 (C0056468 Spk K, 10 ppb Spike)	10	ND	8.36	84	1.88	11.4	97	NR	NR	NR
DBC-F01-MSF005-0-041216 (C0056468 Spk L, 100 ppb Spike)	100	ND	80.8	81	1.88	99.6	98	NR	NR	NR

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

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

NR = Not reported due to quality control result failures. Re-analysis results are shown in Table II.

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

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

Sample Description	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 (%)
DOU-F01-MSF002-0-041216 (C0056469 Spk G, 10 ppb Spike)	10	0.688	9.24	84	4.56	14.5	99	NR	NR	NR
DOU-F01-MSF002-0-041216 (C0056469 Spk H, 100 ppb Spike)	100	0.688	85.6	83	4.56	104	99	NR	NR	NR
DL3-F01-IPW001-0-041130 (C0056470 Spk C, 10 ppb Spike)	10	ND	7.80	78	0.246	9.80	96	9.04	17.2	82
DL3-F01-IPW001-0-041130 (C0056470 Spk D, 100 ppb Spike)	100	ND	82.4	82	0.246	101	101	9.04	102	93
DOU-F01-MSF002-1-041216 (C0056471 Spk I, 10 ppb Spike)	10	0.840	9.20	84	4.68	14.5	98	NR	NR	NR
DOU-F01-MSF002-1-041216 (C0056471 Spk J, 100 ppb Spike)	100	0.840	82.0	81	4.68	103	98	NR	NR	NR
DOU-F01-MSF004-0-041216 (C0056472 Spk K, 10 ppb Spike)	10	0.792	7.76	70	2.35	8.80	65	NR	NR	NR
DOU-F01-MSF004-0-041216 (C0056472 Spk L, 100 ppb Spike)	100	0.792	81.2	80	2.35	85.6	83	NR	NR	NR
DOU-F01-MSF001-0-041216 (C0056473 Spk E, 10 ppb Spike)	10	2.18	11.1	89	10.9	20.8	99	NR	NR	NR
DOU-F01-MSF001-0-041216 (C0056473 Spk F, 100 ppb Spike)	100	2.18	82.8	81	10.9	104	93	NR	NR	NR
DBC-F01-MSF003-0-041216 (C0056474 Spk G, 10 ppb Spike)	10	ND	8.48	85	2.09	11.9	98	NR	NR	NR
DBC-F01-MSF003-0-041216 (C0056474 Spk H, 100 ppb Spike)	100	ND	84.0	84	2.09	106	104	NR	NR	NR
DBC-F01-MSF004-0-041216 (C0056475 Spk I, 10 ppb Spike)	10	ND	7.72	77	3.87	12.9	90	NR	NR	NR
DBC-F01-MSF004-0-041216 (C0056475 Spk J, 100 ppb Spike)	100	ND	75.6	76	3.87	98.6	95	NR	NR	NR
DMC-F01-MSF001-0-041216 (C0056476 Spk C, 10 ppb Spike)	10	ND	9.06	91	0.420	9.08	87	NR	NR	NR
DMC-F01-MSF001-0-041216 (C0056476 Spk D, 100 ppb Spike)	100	ND	86.0	86	0.420	102	102	NR	NR	NR
DL1-F01-IPW005-0-041216 (C0056477 Spk I, 10 ppb Spike)	10	0.253	7.40	71	0.644	9.96	93	69.6	83.2	*
DL1-F01-IPW005-0-041216 (C0056477 Spk J, 100 ppb Spike)	100	0.253	72.4	72	0.644	86.8	86	69.6	145	76
DL2-F01-IPW003-0-041201 (C0056478 Spk K, 10 ppb Spike)	10	ND	7.04	70	ND	9.04	90	7.48	13.7	62
DL2-F01-IPW003-0-041201 (C0056478 Spk L, 100 ppb Spike)	100	ND	71.8	72	ND	92.0	92	7.48	84.0	77

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPW005-0-041201 (C8056479 Spk I, 10 ppb Spike)	10	ND	8.16	82	ND	8.96	90	4.68	14.0	91
DL3-F01-IPW005-0-041201 (C8056479 Spk J, 100 ppb Spike)	100	ND	84.8	85	ND	97.2	97	4.68	94.4	90
			Average:	79		Average:	94		Average:	86
			Standard Deviation:	13		Standard Deviation:	12		Standard Deviation:	28

*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	C8 Sulfonate PFOS Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt	Amount Recovered (ng/g) wet wt.	Recovery (%)
DBC-F01-IPW001-0-041021 (C0048151 Spk C, 1000 ppb)	1000	2080	2860	78
DBC-F01-IPW002-0-041021 (C0048152 Spk D, 5000 ppb)	5000	3330	8680	107
DBC-F01-IPW003-0-041021 (C0048153 Spk E, 1000 ppb)	1000	1660	3160	150
DOU-F01-IPF001-0-041021 (C0048154 Spk F, 1000 ppb)	1000	696	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 (C0049450 Spk J, 10,000 ppb)	10000	9480	23200	137
DBC-F01-MSW002-0-041104 (C0049451 Spk K, 10,000 ppb)	10000	7520	16800	91
DBC-F01-MSW003-0-041104 (C0049452 Spk L, 10,000 ppb)	10000	12800	23200	108
DBC-F01-MSW004-0-041104 (C0049453 Spk M, 5000 ppb)	5000	3440	8520	102
DBC-F01-MSW005-0-041104 (C0049454 Spk N, 10,000 ppb)	10000	5240	15200	100
DBC-F01-MSF001-0-041104 (C0049455 Spk C, 10,000 ppb)	10000	5240	14400	92
DBC-F01-MSF002-0-041104 (C0049456 Spk D, 10,000 ppb)	10000	4760	15200	104
DBC-F01-IPF005-0-041104 (C0049458 Spk E, 1000 ppb)	1000	808	1600	99
DBC-F01-IPW004-0-041104 (C0049459 Spk F, 1000 ppb)	1000	1230	2590	136
DBC-F01-IPW005-0-041104 (C0049460 Spk G, 5000 ppb)	5000	2620	6520	78
DOU-F01-IPW003-0-041104 (C0049461 Spk H, 1000 ppb)	1000	2270	2640	37*
DOU-F01-IPW004-0-041104 (C0049462 Spk I, 1000 ppb)	1000	1740	2740	100
DOU-F01-IPW005-0-041104 (C0049463 Spk J, 5000 ppb)	5000	2130	7600	109
DL1-F01-MSW001-0-041104 (C0049465 Spk K, 1000 ppb)	1000	968	1860	89
DL1-F01-MSW002-0-041104 (C0049466 Spk C, 1000 ppb)	1000	708	1780	107
DL1-F01-MSW003-0-041104 (C0049467 Spk D, 1000 ppb)	1000	898	1930	123
DL1-F01-MSW005-0-041104 (C0049469 Spk E, 1000 ppb)	1000	804	1680	108

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

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

		C8 Sulfonate PFOS		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0048471 Spk F, 1000 ppb)	1000	666	1890	112
DL1-F01-MSF003-0-041104 (C0048472 Spk G, 1000 ppb)	1000	432	1670	124
DL1-F01-MSF005-0-041104 (C0048474 Spk H, 1000 ppb)	1000	740	1830	109
DMC-F01-MSW005-0-041105 (C0048486 Spk I, 1000 ppb)	1000	580	1780	122
DMC-F01-MSF002-0-041105 (C0048499 Spk J, 1000 ppb)	1000	620	1500	88
DOU-F01-MSW002-0-041216 (C0056466 Spk K, 10,000 ppb)	10000	28200	32700	45
DOU-F01-MSW003-0-041216 (C0056468 Spk L, 5000 ppb)	5000	4040	10200	123
DOU-F01-MSW001-0-041216 (C0056460 Spk C, 10000 ppb)	10000	7600	25000	174*
DOU-F01-MSW004-0-041216 (C0056461 Spk D, 5000 ppb)	5000	5120	9880	95
DOU-F01-MSW005-0-041216 (C0056462 Spk E, 1000 ppb)	1000	496	1760	126
DOU-F01-MSF005-0-041216 (C0056463 Spk F, 1000 ppb)	1000	720	1820	110
DOU-F01-MSF003-0-041216 (C0056466 Spk G, 1000 ppb)	1000	846	1720	87
DBC-F01-MSF005-1-041216 (C0056467 Spk H, 10000 ppb)	10000	9320	32400	230*
DBC-F01-MSF005-0-041216 (C0056468 Spk I, 10000 ppb)	10000	8960	24100	151
DOU-F01-MSF002-0-041216 (C0056469 Spk J, 1000 ppb)	1000	640	1720	108
DOU-F01-MSF002-1-041216 (C0056471 Spk K, 1000 ppb)	1000	692	1950	126
DOU-F01-MSF004-0-041216 (C0056472 Spk L, 1000 ppb)	1000	1820	2700	88
DOU-F01-MSF001-0-041216 (C0056473 Spk C, 5000 ppb)	5000	2070	7980	118
DBC-F01-MSF003-0-041216 (C0056474 Spk D, 10000 ppb)	10000	4640	15500	109
DBC-F01-MSF004-0-041216 (C0056475 Spk E, 10000 ppb)	10000	9280	24500	152
DMC-F01-MSF001-0-041216 (C0056476 Spk F, 1000 ppb)	1000	840	2040	120
DBC-F01-IPF001-0-041021 (C0048148 Spk G, 1000 ppb)	1000	384	1350	97
DBC-F01-IPF003-0-041021 (C0048150 Spk H, 1000 ppb)	1000	293	1340	105
DOU-F01-IPF003-0-041021 (C0048166 Spk I, 1000 ppb)	1000	359	1400	104

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-I01-CFW001-0-041216 (C0058460 Spk C, 10 ppb)	10	ND	8.98	90	ND	8.18	82	1.82	6.32	45
DL3-I01-CFW001-0-041216 (C0058460 Spk D, 100 ppb)	100	ND	82.4	82	ND	95.6	96	1.82	85.2	83
DMC-I01-CFW001-0-041216 (C0058461 Spk E, 10 ppb)	10	ND	9.04	90	ND	9.44	94	6.16	11.8	51
DMC-I01-CFW001-0-041216 (C0058461 Spk F, 100 ppb)	100	ND	82.8	83	ND	98.8	99	6.16	92.6	87
DL2-I01-CFW001-0-041216 (C0058462 Spk G, 10 ppb)	10	ND	7.26	73	ND	7.84	78	1.81	7.52	57
DL2-I01-CFW001-0-041216 (C0058462 Spk H, 100 ppb)	100	ND	83.6	84	ND	101	101	1.81	88.4	87
DBC-I01-CFW001-0-041216 (C0058463 Spk I, 10 ppb)	10	ND	10.3	103	ND	10.8	108	4.48	13.0	85
DBC-I01-CFW001-0-041215 (C0058463 Spk J, 100 ppb)	100	ND	84.4	84	ND	97.2	97	4.48	86.4	82
DL1-I01-CFW001-0-041216 (C0058464 Spk K, 10 ppb)	10	ND	8.98	90	ND	9.32	93	8.80	16.0	82
DL1-I01-CFW001-0-041216 (C0058464 Spk L, 100 ppb)	100	ND	86.2	86	ND	103	103	8.80	95.8	86
DOU-I01-CFW001-0-041216 (C0058465 Spk M, 10 ppb)	10	ND	8.46	85	0.202	8.32	81	11.4	17.2	58
DOU-I01-CFW001-0-041216 (C0058465 Spk N, 100 ppb)	100	ND	84.4	84	0.202	98.0	98	11.4	98.0	87
			Average:	84		Average:	93		Average:	73
			Standard Deviation:	10		Standard Deviation:	13		Standard Deviation:	16

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

FIGURES

Figure 1. Typical Calibration Curve for PFBS in Methanol

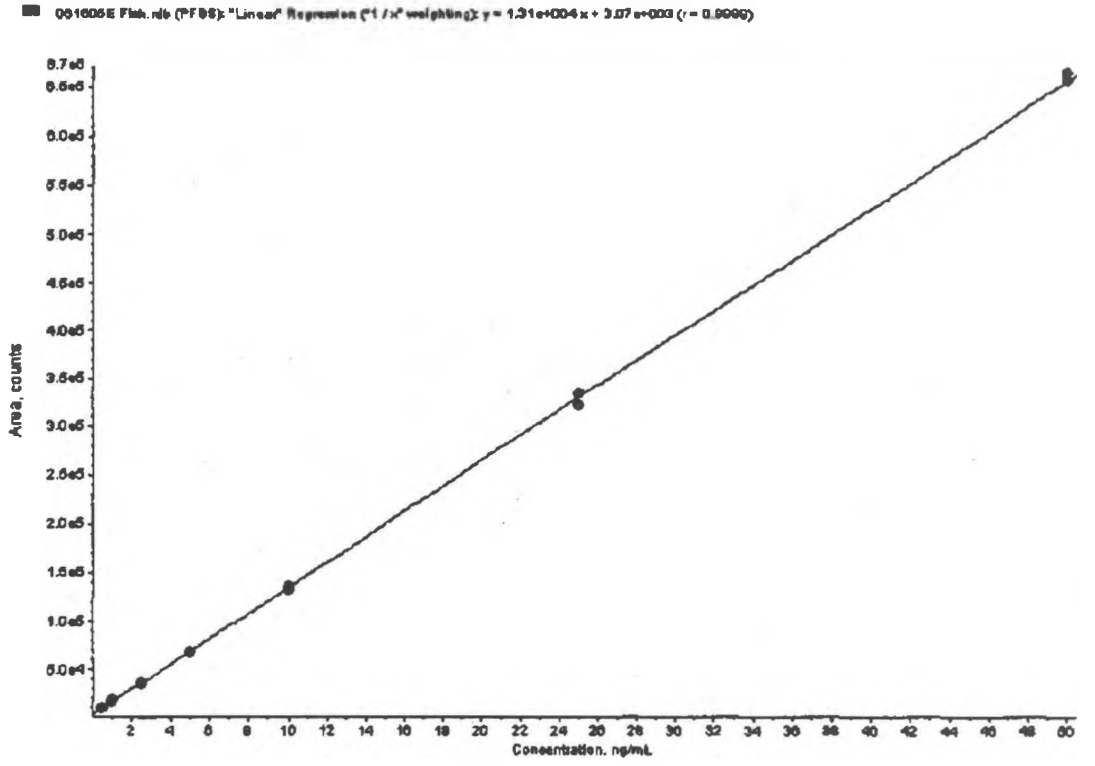


Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

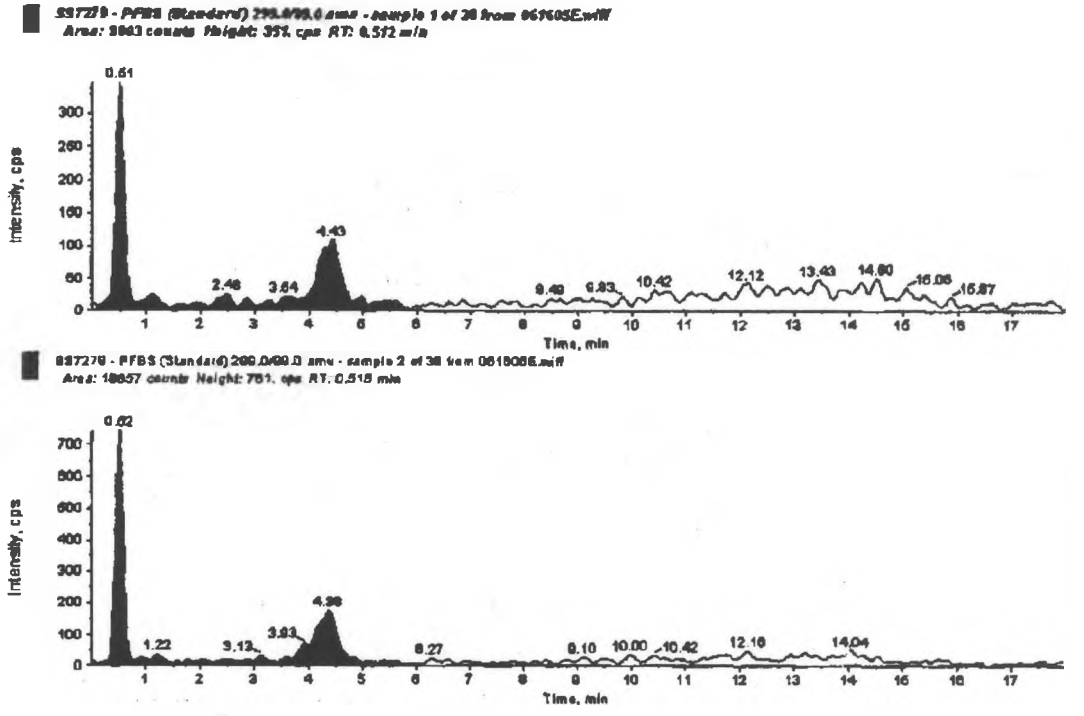


Figure 3. PFBS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

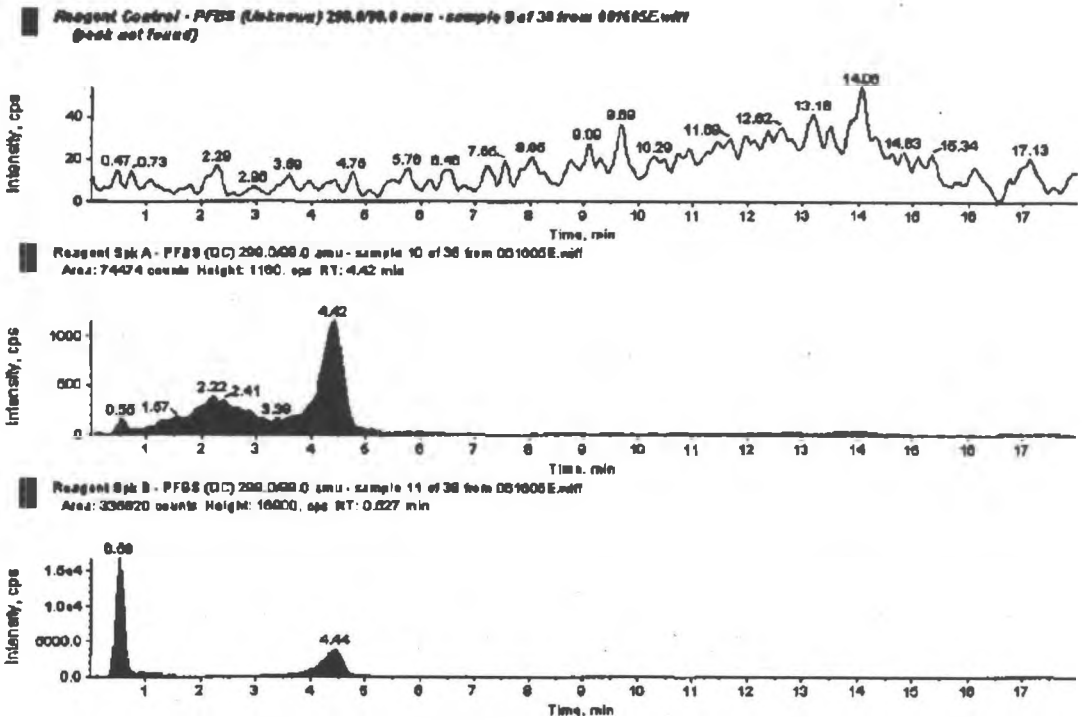


Figure 4. Chromatogram Representing a Fish Sample Analyzed for PFBS (Exygen ID: C0049478, Data Set:061605E)

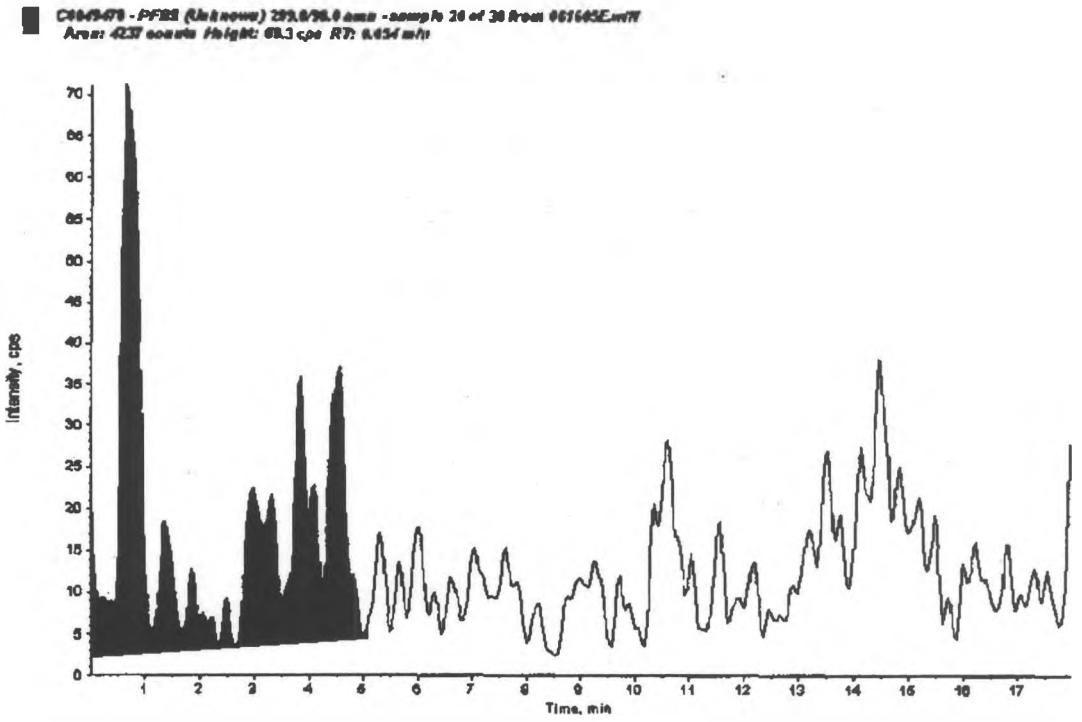


Figure 5. Typical Calibration Curve for PFHS in Methanol

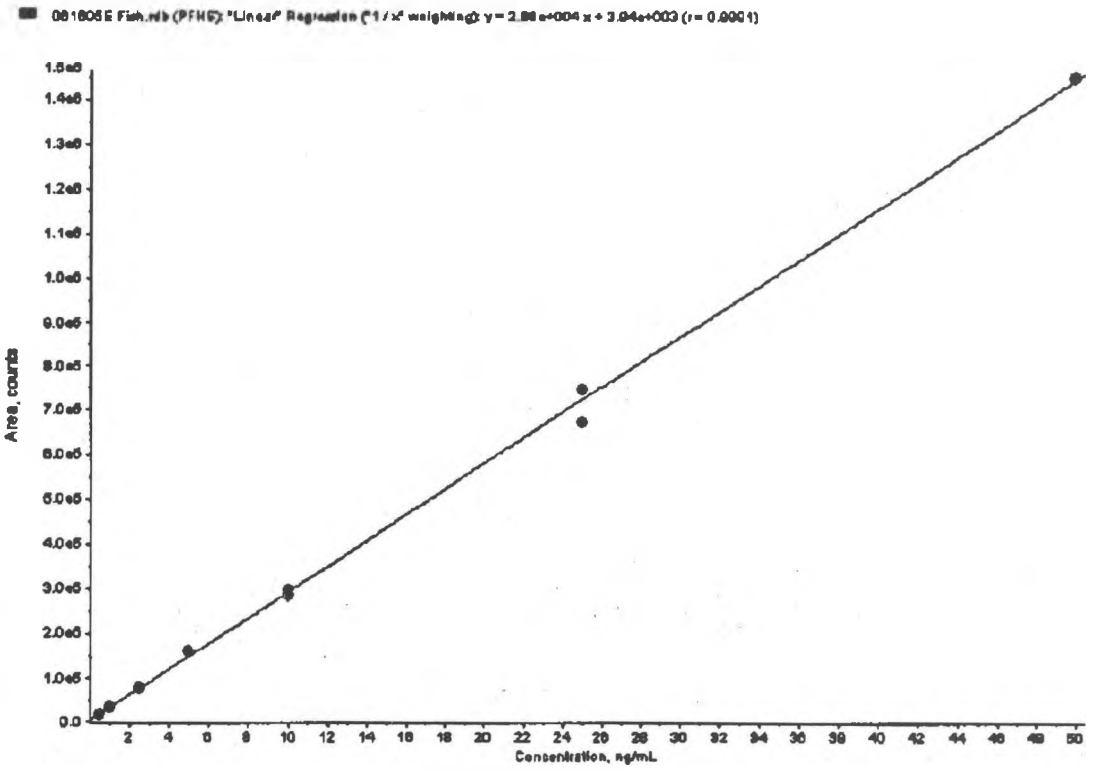


Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

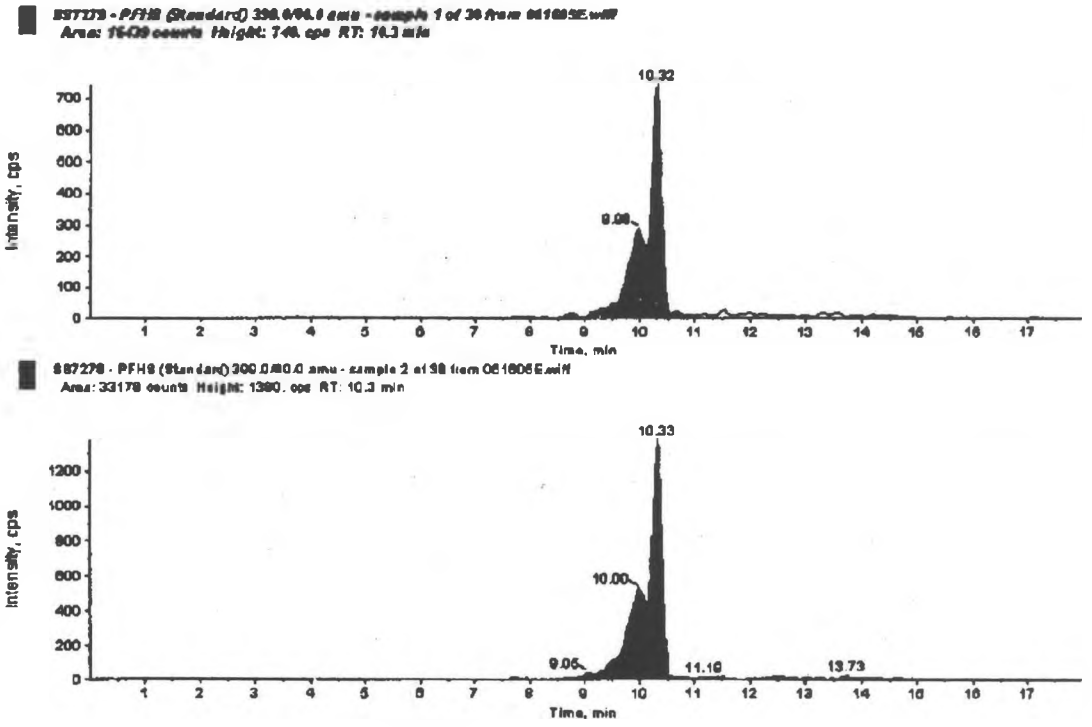


Figure 7. PFHS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

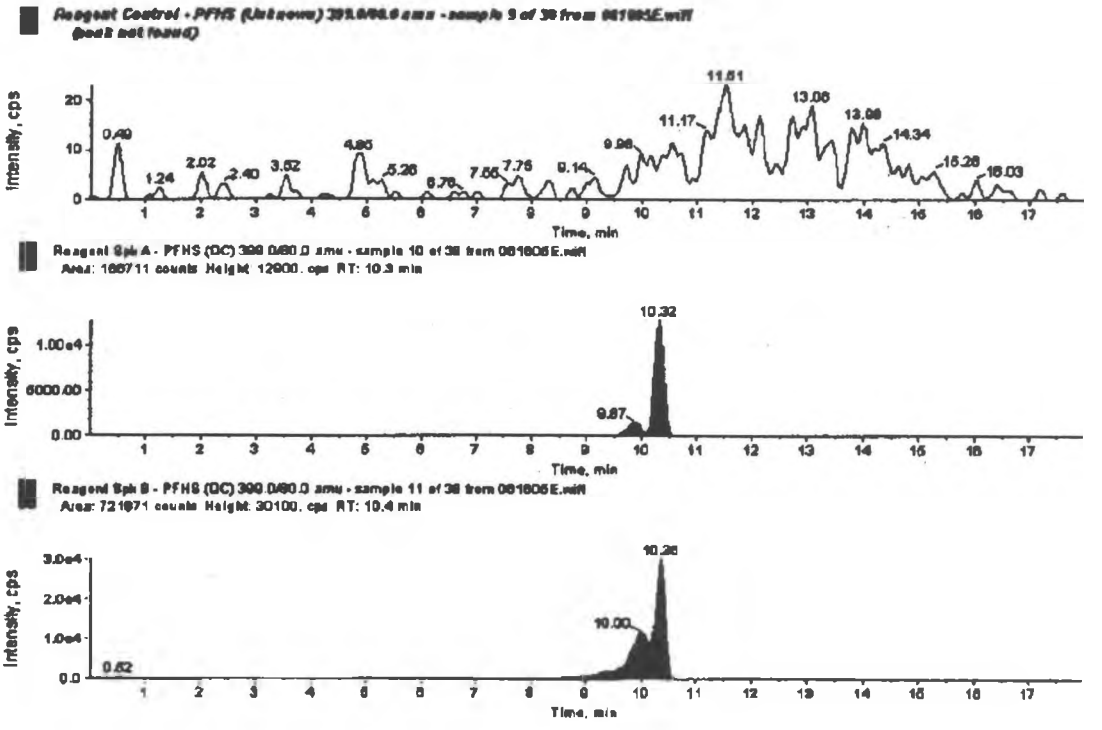


Figure 3. Chromatogram Representing a Fish Sample Analyzed for PFHS (Exygen ID: C0049478, Data Set: 061605E)

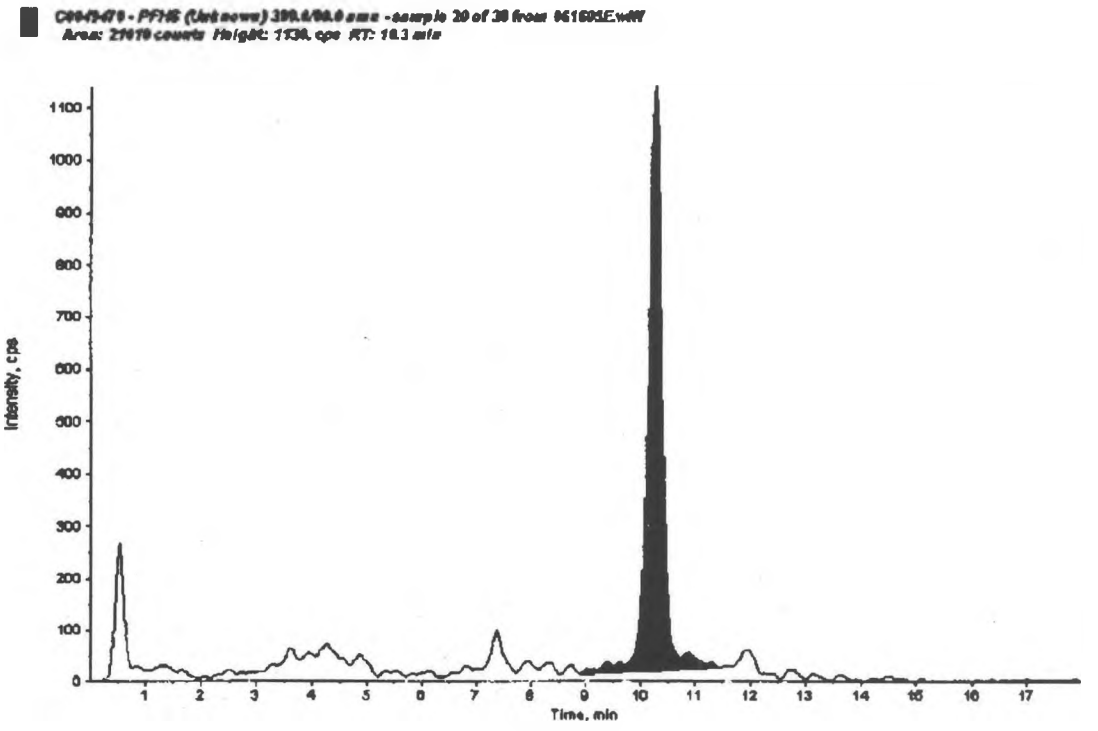


Figure 9. Typical Calibration Curve for PFOS in Methanol

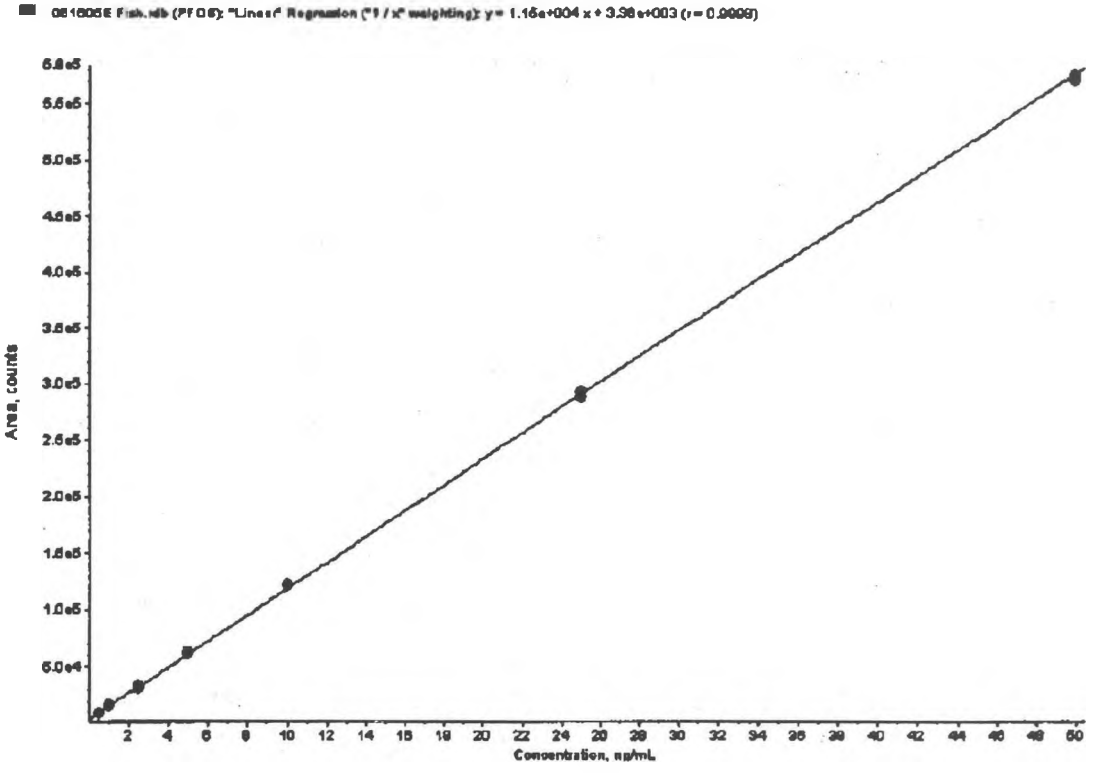


Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.0005 µg/mL and 0.001 µg/mL, Respectively

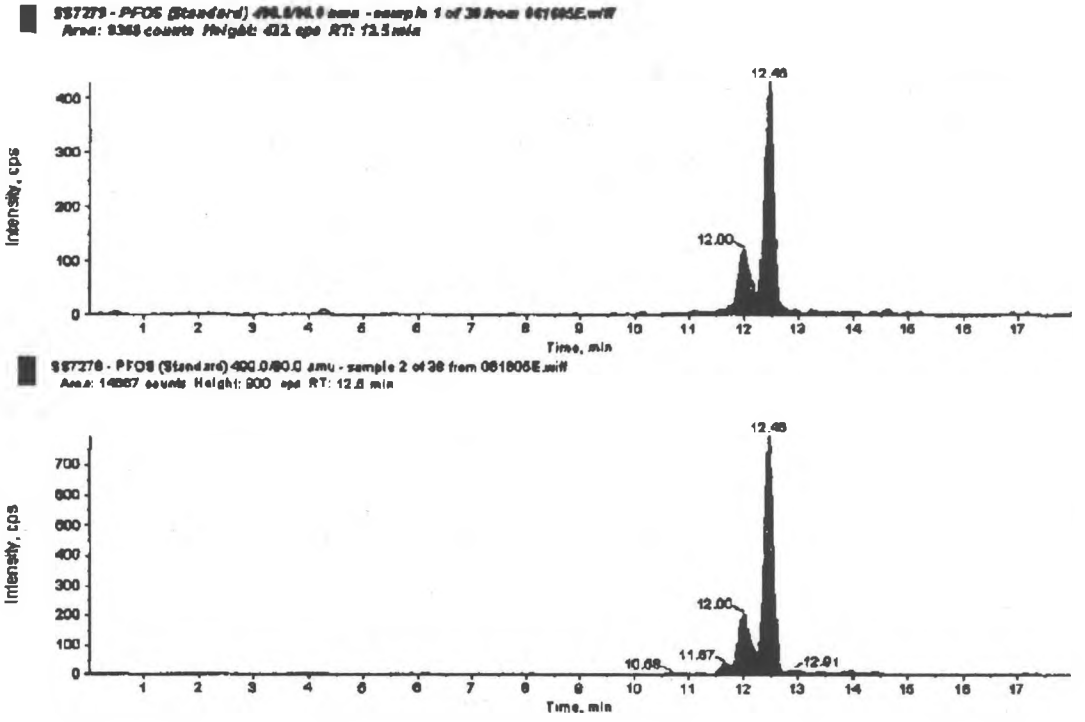


Figure 11. PFOS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

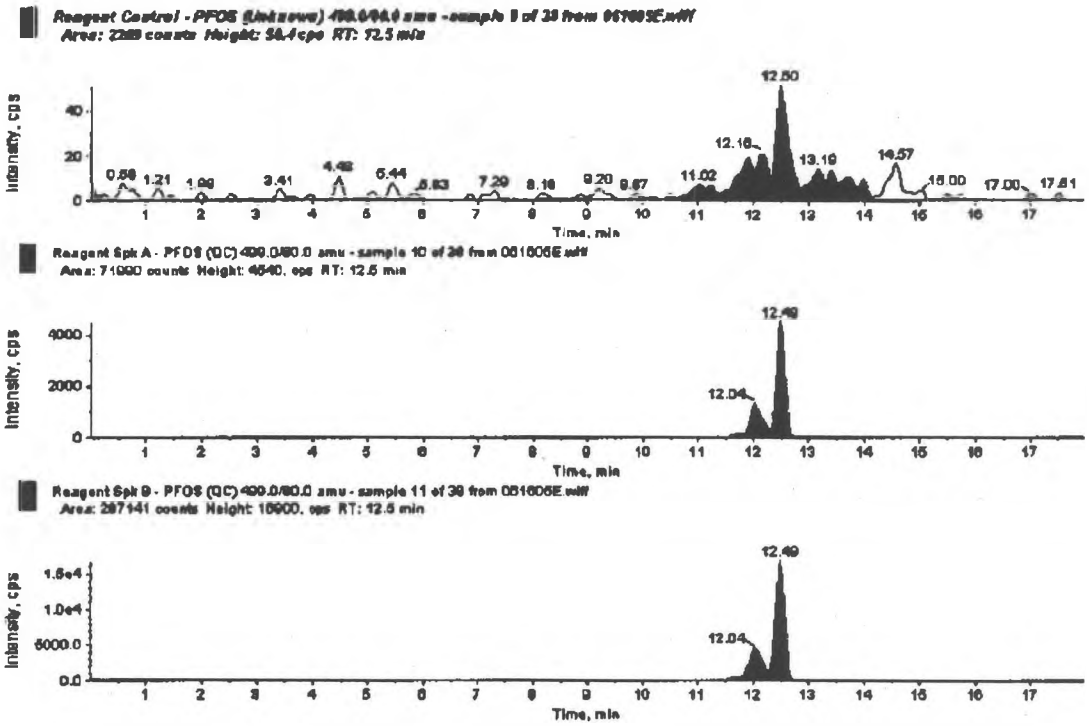
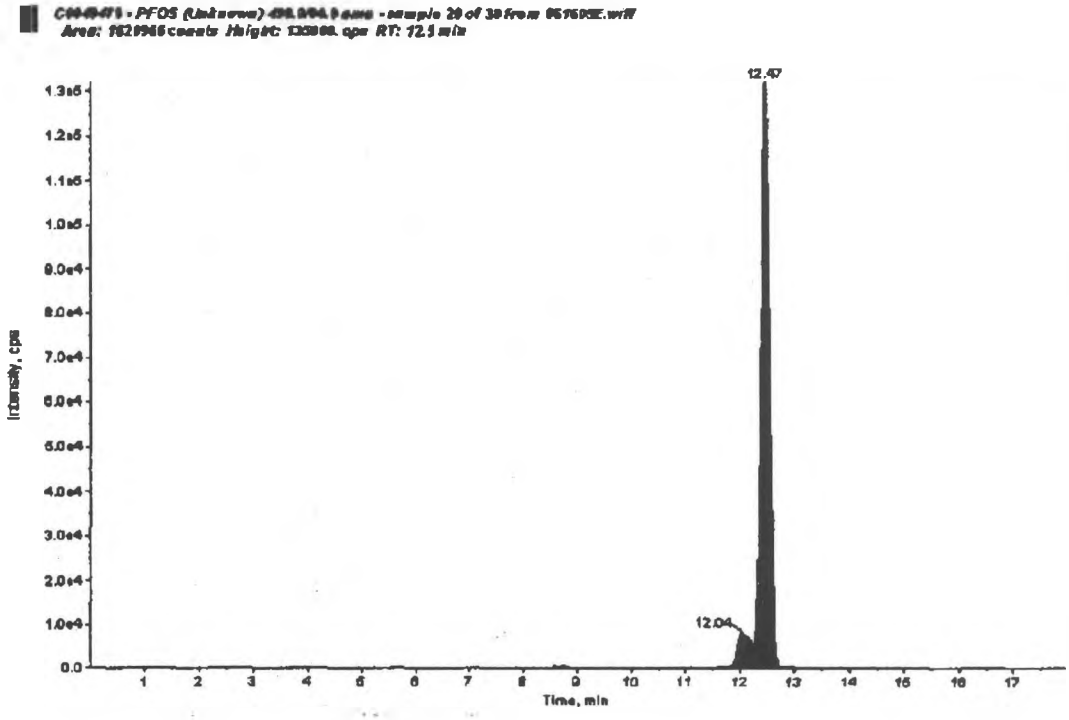


Figure 12. Chromatogram Representing a Fish Sample Analyzed for PFOS (Exygen ID: C0049478, Data Set:061605E)



APPENDIX A

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

STUDY PROTOCOL

Study Title:

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

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

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

DISTRIBUTION:

1) Jaisimha Kesari, Study Director, Weston Solutions

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

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

4) Exygen Research Quality Assurance Unit

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

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

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

John M. Flaherty, Principal Investigator
Exygen Research — 11/29/04 Date

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

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

TABLE OF CONTENTS

Exygen Protocol Number: P0001131

INTRODUCTION

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

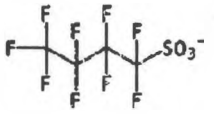
This study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

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

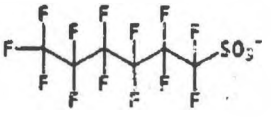
PFBS

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

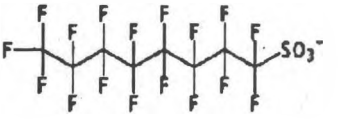


PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SE036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



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

STUDY DIRECTOR

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PRINCIPAL INVESTIGATOR

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john.flaherty@exygen.com

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

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

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

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

The samples will be collected by Weston Solutions. The control samples will be purchased and prepared by the testing facility. Purchase and processing details for the control samples will be included in the final report associated with this study.

The test systems were chosen to access the environmental impact of PFBS, PFHS and PFOS in the Decatur, Alabama area.

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

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

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

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

SAMPLE IDENTIFICATION

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

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

ANALYTICAL PROCEDURE SUMMARY

References:

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

The above methods use analytical conditions capable of separating the isomers of PFBS, PFHS and PFOS. The final report will include the isomers summed into total PFBS, total PFHS, and total PFOS found.

VERIFICATION OF ANALYTICAL PROCEDURE

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

For water sampling, Exygen will supply one bottle per sample collected. The bottles will be 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottles. These bottles have been routinely used for fluorochemical sample

collection at the testing facility and have been shown to be free of PFBS, PFHS and PFOS. Samples will be added to each container to a volumetric fill line at 200 mL. A field duplicate, a low field spike and a high field spike of each sample will be collected. The low and high field spike bottles will contain PFBS, PFHS and PFOS as well as perfluorooctanoic acid (PFOA) and 1,2-13C perfluorooctanoic acid (^{13}C PFOA). PFOA and ^{13}C PFOA are included in the solutions used to spike the samples. The results for PFOA and ^{13}C PFOA will not be reported in this study. Exygen will supply one field blank (control water) and two field blank spikes (control water fortified with PFBS, PFHS and PFOS at a low and high level) for every twenty samples collected. At the testing facility, each water sample (excluding field duplicates and field spikes) will be extracted in duplicate and will also be fortified at a low and high concentration with PFBS, PFHS and PFOS and processed through the described procedure to determine method accuracy and to check for bias.

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

For small mammal liver, Exygen will supply a 50 mL polypropylene centrifuge tube. For small mammal serum, Exygen will supply a collection kit for each sample containing serum separator tubes (red top), vacutainers, needle holders and needles, transfer pipettes, and polypropylene tubes. At the testing facility, each liver and serum sample will be extracted in duplicate and will also be fortified at a known concentration with PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

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

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

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

METHOD FOR CONTROL OF BIAS

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

STATISTICAL METHODS

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

GLP STATEMENT

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

REPORT

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

- The name and address of the Study Director, Sponsor Representative, and of the testing facility.
- A statement of GLP compliance (any related documentation, such as chain-of-custody records, must be in the study records).

- The signed and dated statement by the Exygen Research Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and Management.
- A description of the exact analytical conditions employed in the study. If the subject method was followed exactly, it is necessary to include only a copy of the analytical method. Any modifications to this method will be incorporated into the report. If the method is photo-reduced, the project number and page number must be included on each page.
- Description of the instrumentation used and operating conditions.
- All results from all sets analyzed. Control and fortified samples will be identified and the data table will include sample number and fortification level.
- Representative chromatograms for each analyte in each matrix, including chromatograms of a standard and a control sample, and a chromatogram at a fortification level. The location of the analyte peaks will be clearly identified in all chromatograms.
- All circumstances that may have affected the quality or integrity of the data will be documented in the report.
- Locations where raw data and the final report are to be archived.
- Additions or corrections to the final report shall be in the form of an amendment signed by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and the Sponsor Representative.
- All applicable requirements for reporting of study results as per 40 CFR 792.185.

SAFETY AND HEALTH

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

AMENDMENTS TO PROTOCOL

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

DATA RECORD KEEPING

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

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

Chromatograms- All chromatograms will contain the following:

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

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

QUALITY ASSURANCE

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

RETENTION OF DATA AND ARCHIVING

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

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

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

APPENDIX I

ANALYTICAL METHODS

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001780

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

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

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/24/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001700

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 2 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001750

ANALYTICAL METHOD

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

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

6.0 **Chromatographic System**

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

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

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

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

7.0 **MS/MS System**

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

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

8.0 **Preparation of Solutions**

8.1 **Mobile Phase**

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

Alternate volumes may be prepared.

Page 3 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001716

ANALYTICAL METHOD

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

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

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

Page 4 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001160

ANALYTICAL METHOD

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

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

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

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

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

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

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

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

11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

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

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

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

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

Page 3 of 7

Exygen Protocol Number: P0001131

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

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

13.0 Acceptance Criteria

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

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

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

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

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

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

14.0 Calculations

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

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number: VOB07720

ANALYTICAL METHOD

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

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

Recovery (%) =

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

Page 9 of 9

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001781

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

Analytical Testing Facility: Exygen Research
1050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/22/07
Date

Glen Fisher
Vice President, Operations, Exygen Research

9/24/07
Date

Total Pages: 7

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

1.0 **Scope**

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

2.0 **Safety**

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

3.0 **Sample Requirements**

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

4.0 **Reagents and Standards**

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

5.0 **Instrument and Equipment**

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

Page 2 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

5.14 Wrist-action shaker.

5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.

6.0 Chromatographic System

6.2 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)

6.3 Temperature: 30°C

6.4 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.

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001781

ANALYTICAL METHOD

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

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

* The estimated concentration of the calibration standard is equal to 4x its initial concentration, due to the concentration of the standard during the extraction (SPE).
XC = extracted calibration standard.

Page 4 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

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

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

10.0 **Batch Set Up**

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

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

11.0 **Sample Extraction**

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

11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes

11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.

11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.

11.5 Centrifuge for ~10 minutes at ~3000 rpm.

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

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

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

11.9 Analyze samples using 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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

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

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

13.0 Acceptance Criteria

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

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

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

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

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

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

14.0 Calculations

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

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

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

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

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

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

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

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

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001782

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research 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 Spectrometric Detector (LC/MS/MS) in sediment.

2.0 Safety

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

3.0 Sample Requirement

3.1 At least 10 g of wet sample for extraction.
3.2 No sample processing is needed for sediment samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 1 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001782

ANALYTICAL METHOD

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

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

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001TEI

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 maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

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

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

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

Page 4 of 7

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

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

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

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

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

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

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

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

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

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

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

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

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

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

11.13 Analyze samples using electrospray LC/MS/MS.

Page 3 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

12.0 Chromatography

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

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

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

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

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

13.0 Acceptance Criteria

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

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

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

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

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

Page 5 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

14.0 Calculations

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

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

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

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

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

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

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

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

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

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
3050 Research Drive
State College, PA 16801

Approved By:

Paul Conneelly
Technical Leader, LC-MS, Exygen Research

10/24/06
Date

John Flaherty
Vice President, Operations, Exygen Research

10/24/06
Date

Total Pages: 8

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001783

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 2 of 8

Exygen Protocol Number: P0001131

Exygen Research Method Number V000178

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

6.0 Chromatographic System

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

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

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

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

Page 1 of 1

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001 R1

ANALYTICAL METHOD

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

8.2 Extraction Solutions

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

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

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

Page 4 of 8

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

9.2 Standard Calibration Solutions

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

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

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

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

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (Nalgene or equivalent, replace lid and mix well).

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

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

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

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

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

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

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

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

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

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

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

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

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

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

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

11.17 Transfer the extracts to HPLC vials using disposable pipets.

11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

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

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

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

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

Page 6 of 8

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001PFO

ANALYTICAL METHOD

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

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

13.0 Acceptance Criteria

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

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

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

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

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

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

14.0 Calculations

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

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

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

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

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

Page 7 of 8

Exygen Protocol Number: P0001131

Exygen Research Method Number VB001 03

ANALYTICAL METHOD

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

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

Recovery (%) =

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

Page 8 of 8

Page 44 of 65

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001784

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

Analytical Testing Facility: Exygen Research
3811 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V5001754

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 1 of 7

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 flasks.
5.6 50 mL disposable polypropylene centrifuge tubes.
5.7 15 mL disposable polypropylene centrifuge tubes.
5.8 Disposable micropipets (50-100uL, 100-200uL).
5.9 125-mL LDPE narrow-mouth bottles.
5.10 2 mL clear HPLC vial kit.
5.11 Disposable pipettes.
5.12 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.13 SPE tubes (20mL) (Supelco cat. no. 57177).
5.14 Wrist action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

Page 3 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

8.2 Extraction Solutions

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

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

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

9.2 Standard Calibration Solutions

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

Page 4 of 7

Exygen Research Method Number V0001704

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

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

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

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

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

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

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

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

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 10% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

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

Page 5 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

12.0 Chromatography

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

13.0 Acceptance Criteria

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V080704

ANALYTICAL METHOD

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

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

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

13.4 Any calibration standard found to be a statistical outlier by using the Huge 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 ≥0.995 (R^2 ≥0.9925). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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

14.0 Calculations

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

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

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

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

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 calculate the percent recovery.

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

Page 7 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001785

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

Analytical Testing Facility: Exygen Research
3958 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001745

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 2 of 7

Exygen Protocol Number: P0001131

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 Pak Vac 6 cc (1g) tC18 SPE cartridges
5.12 SPE vacuum manifold.
5.13 Tissuemizer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Phenomex RP (Keystone Scientific), 2.1 mm x 50 mm, 3µ (P/N: 82305-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase: (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

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

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

Page 3 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001705

ANALYTICAL METHOD

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Alternate volumes may be prepared

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

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

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

Page 4 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001703

ANALYTICAL METHOD

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

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

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

11.3 Homogenize sample using a tissuemizer for ~1 minute.

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

11.5 Add 5 mL of acetonitrile and shake for ~30 minutes on a wrist-action shaker.

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

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

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

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

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

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

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

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

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

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

Page 5 of 7

Page 56 of 65

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

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

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

13.0 Acceptance Criteria

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

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

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

13.4 Any calibration standard found to be a statistical outlier by using the Huge Exer 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 ≥0.995 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 6 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

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

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001785

ANALYTICAL METHOD

Method 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 7 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001786

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

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

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

19/3/05
Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001784

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 1 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: M0001?01

ANALYTICAL METHOD

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

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

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V080176

ANALYTICAL METHOD

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

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

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

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

10.0 Batch Set Up

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

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

Page 4 of 5

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001716

ANALYTICAL METHOD

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

11.0 **Sample Extraction**

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

11.2 Add water to the sample for a final volume of 20 mL. Cap tightly

11.3 Vortex for ~1 minute.

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

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

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

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

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

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

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

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 **Chromatography**

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

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

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

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

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

Page 5 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

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

13.0 Acceptance Criteria

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

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

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

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

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.983). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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

14.0 Calculations

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

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

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

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

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

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001706

ANALYTICAL METHOD

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

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

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

Page 7 of 7

Page 65 of 65

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

PROTOCOL AMENDMENT

Amendment Number: 1

Effective Date: 01/19/05

Exygen Study Number: P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

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

AMENDED TO

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

RATIONALE

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

IMPACT ON STUDY

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

Study Director Signature — Date 3/9/05

Principal Investigator Signature — Date 3/2/05

Study Director/Management Signature N/A — Date N/A

Sponsor Signature (If required) for Michael A. Ventura — Date 3/10/05

Exygen QAU Review LJS 03/10/05

LIBRARY ID: VV0001226-6 ADMINISTRATIVE FORM

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

PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 03/07/05
Exygen Study Number P0001131 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

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

AMENDED TO

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

RATIONALE

1) Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timelier manner. manner 03/04/05 JMS
2) The API 4000 LC/MS/MS systems detect the 499 -> 80 PFOS transition with greater sensitivity than the 499 -> 99 transition.

IMPACT ON STUDY

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

Study Director Signature — Date: 3/4/05
Principal Investigator Signature — Date: 3/4/05
MA
Study Director Management Signature — Date:
Exygen Management Signature — Date: 3-MAR-05
Sponsor Signature (if required) — Date: 3/16/05

Exygen QAU Review 1.33 03/04/05

LIBRARY ID: V0001226-8 ADMINISTRATIVE FORM

09-20-2005 02:35pm From-MESTON SOLUTIONS T-384 P.003/004 F-427

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

DEVIATION FORM

Page 1 of 1

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

Exygen Project # : P750/P1131 Deviation # : 2/2

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

Regulatory Driver:
___ GMP
X GLP
___ Other
___ None

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

Sample Description:
Login # : L4264/L4265 Container # : C0056480-86 Lot # : NA

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

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

Impact:
No negative impact on this study.

Corrective Actions:
Deviation issued.

Signatures:

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

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

DEVIATION FORM

Page 1 of 1

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

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

Client Project #: NA Reference #: 05-134 9/20/05

Regulatory Driver:
___ GMP
X GLP
___ Other
___ None

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

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

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

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

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

Corrective Actions:
Deviation issued.

Signatures:

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

INTERIM REPORT #23 Analysis of Sediment Samples

STUDY TITLE

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

DATA REQUIREMENTS

EPA TSCA Good Laboratory Practice Standards 40 CFR 792

STUDY DIRECTOR

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

INTERIM REPORT COMPLETION DATE

May 2, 2006

PERFORMING LABORATORY

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PROJECT

Protocol Number: P0000760
Exygen Study Number: P0000760

Total Pages: 110

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number P0000760, entitled "Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," conducted for 3M Company, is being performed in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by Exygen Research, with one exception. The laboratory control and control spikes for the direct injection and alternate SPE methods and the spiking procedures for the surrogate were not recorded promptly at the time of extraction.

J. M Flaherty 5/2/06

John Flaherty
Principal Investigator
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Date

Jaisimha Kesari 5/3/06

Jaisimha Kesari P.E., DEE
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Date

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Date

QUALITY ASSURANCE STATEMENT

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

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director
31. Final Raw Data Review and Interim Analytical Report Review	5/2/06	5/2/06	5/2/06	5/2/06

Lydia Shaffer
Technical Lead, Quality Assurance Unit

05/02/06
Date

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

CERTIFICATION OF AUTHENTICITY

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

Submitted by: Exygen Research
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5/2/06
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STUDY IDENTIFICATION

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

PROTOCOL NUMBER:	P0000760
EXYGEN STUDY NUMBER:	P0000760
TYPE OF STUDY:	Residue
SAMPLE MATRIX:	Sediment
TEST SUBSTANCE:	Perfluorooctanoic acid (PFOA)
SPONSOR:	3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
STUDY DIRECTOR:	Jaisimha Kesari P.E., DEE Weston Solutions, Inc. 1400 Weston Way West Chester, PA 19380
STUDY MONITOR:	Michael A. Santoro 3M Company 3M Building 0236-01-B-10 St. Paul, MN 55144
PERFORMING LABORATORY:	Exygen Research 3058 Research Drive State College, PA 16801
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 11/05/04 Interim Analytical Start Date: 04/27/06 Interim Analytical Termination Date: 04/30/06 Interim Report Completion Date: 05/02/06

PROJECT PERSONNEL

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

Name	Title
John Flaherty	Vice President
Karen Risha	Scientist
Paul Connolly	Technical Lead-LC/MS
Christine Edwards	Technician
Mark Ammerman	Sample Custodian
Amy Sheehan	Associate Scientist
Brittany Kravets	Technician
Scott Crain	Technician
Frances Crespi	Technician
Devon Ingram	Technician
Christa Gallant	Technician

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Page

1.0 SUMMARY

Exygen Research extracted and analyzed sediment samples for the determination of perfluorooctanoic acid (PFOA) according to Exygen Method V0001782 (**Appendix A**).

The limit of quantitation for PFOA in sediment was 2.0 ng/g.

The standard sediment method (V0001782) was originally used to extract all of the samples. Use of this method yielded poor spiking recoveries for PFOA. Because of this, a direct injection method (**Section 6.2**) was used, which also yielded poor recoveries. Finally, an alternative SPE column extraction (**Section 6.3**) was used to analyze for PFOA and ^{13}C PFOA.

Analytical results for the analysis of PFOA in sediment samples extracted according to the method based on dry weight are summarized in **Table I**. Analytical results for the analysis of PFOA in sediment samples by direct injection, based on dry weight, are summarized in **Table II**. Analytical results for the analysis of PFOA in sediment samples by alternative SPE extraction, based on dry weight, are summarized in **Table III**. The overall average percent recovery ± standard deviation for PFOA in sediment samples based on wet weight was 73 ± 16%. Fortification recoveries for the analysis of PFOA in sediment samples are summarized in **Table IV**. Fortification recoveries for the analysis of PFOA in direct injection sediment samples are summarized in **Table V**. Fortification recoveries for the analysis of PFOA in SPE extracted sediment samples are summarized in **Table VI**. The average percent recovery ± standard deviation for ^{13}C PFOA in sediment samples based on wet weight was 97 ± 27%. Fortification recoveries for ^{13}C PFOA in sediment samples based on wet weight are summarized in **Table VII**. Fortification recoveries for ^{13}C PFOA in direct injection sediment samples based on wet weight are summarized in **Table VIII**. Fortification recoveries for ^{13}C PFOA in SPE extracted sediment samples based on wet weight are summarized in **Table IX**.

The total percent solids for sediment samples are detailed in **Table X**.

2.0 OBJECTIVE

The objective of the analytical part of this study was to determine levels of perfluorooctanoic acid (PFOA) in sediment according to Protocol P0000760 (**Appendix A**).

3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFOA in sediment using the analytical methods entitled, "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS."

The study was initiated on November 05, 2004, when the study director signed protocol number P0000760. The analytical start date for this interim report was April 27, 2006 and the analytical termination date for this interim report was April 30, 2006.

4.0 ANALYTICAL TEST SAMPLES

Three sediment samples (Exygen ID: C0172892–C0172894) were received on wet ice on April 22, 2006 from Tim Frinak at Weston Solutions, Inc. The samples were logged in by Exygen personnel and placed in refrigerated 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

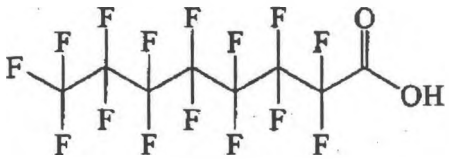
The analytical standard, PFOA, was purchased from Oakwood Products, Inc. and was received at Exygen on March 17, 2005. The surrogate spiking standard, ^{13}C labeled perfluorooctanoic acid (^{13}C PFOA SP0004184), was received at Exygen on April 15, 2004 from the 3M Company.

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

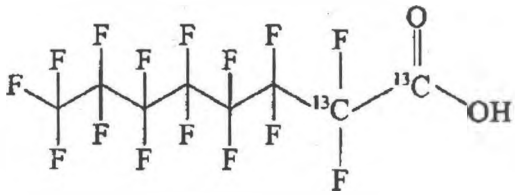
Compound	Exygen Inventory No.	Lot #	Purity (%)	Expiration Date
PFOA	SP0005444	203	99	03/31/07
^{13}C PFOA	SP0004184	3507-195	97	03/29/09

The molecular structure of PFOA is given below:

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



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



6.0 DESCRIPTION OF ANALYTICAL METHOD

The analytical method "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS" was used for the sediment samples in this study.

6.1 Extraction Procedure For Sediment

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

hundred milliliters of water was added to the bottles. The samples were mixed by shaking and loaded onto another C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.2 Direct Inject Extraction Procedure For Sediment

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A one-gram portion of sediment was weighed into a 15-milliliter centrifuge tube for the extraction. Ten milliliters of 1% acetic acid in methanol was added to each sample. The samples were then shaken by hand, vortexed, and sonicated for thirty minutes. The samples were then centrifuged for ~10 minutes at ~3000 rpm. Each sample was analyzed by LC/MS/MS electrospray.

6.3 Additional SPE Extraction Procedure For Sediment

The samples that were prepared in 1% acetic acid for the direct injection method were used for this extraction. Five milliliters of each sample was aliquoted into a 50-mL polypropylene centrifuge tube and the volume was taken to 40 mL with water. The samples were then centrifuged for ~10 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.4 Percent Solids Procedure For Sediment

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

6.5 Preparation of Standards and Fortification Solutions

A mixed stock standard solution of PFOA and ^{13}C PFOA was prepared at a concentration of 1000 µg/mL by dissolving 100 mg of each of the standards (corrected for purity and

salt content) in 100 mL of methanol. From this solution, the following fortification standards were prepared:

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

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

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

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

[1] of ^{13}C PFOA

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

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

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

The standards at final concentrations of 10, 5.0, 2.0, 1.0, 0.5 and 0.2 ng/mL were used for calibration. Extracted calibration standards were prepared following method V0001780. The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation is located in the raw data package associated with this interim report.

6.6 Chromatography

Quantification of PFOA was accomplished by LC/MS/MS electrospray. The retention time of PFOA was ~11.1 min. Peaks above the LOD were not detected in any of the reagent blank samples corresponding to the analyte retention time.

6.7 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.2 ng/mL of PFOA.

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

Instrument: API 4000 Biomolecular Mass Analyzer

Interface: Turbo Ion Spray Liquid Introduction Interface

Computer: DELL OptiPlex GX400

Software: Windows NT, Analyst 1.4.1

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

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

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

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFOA	negative	413 → 369	~11.1 min.
PFOA Confirm Ion	negative	413 → 219	~11.1 min.
13C PFOA	negative	415 → 370	~11.1 min.

6.9 Quantitation and Example Calculation

PFOA concentrations are quantitated in nanogram of PFOA per milliliter of extract and then converted to nanogram per gram. The PFOA concentrations were quantitated in nanogram of PFOA per liter for Set #3 (Set 042806D). 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 seven concentrations of standards. The concentration was determined from the equations below.

Please note that the equations in this section are presented in a different order than the equations in the method (V0001782), but this has no effect on the final calculations.

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

Equation 1:

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

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

For samples fortified with known amounts of PFOA and ^{13}C PFOA prior to extraction, Equation 2 was used to convert the amount of PFOA found in ng/L to ng/g (ppb).

Equation 2:

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/L)} \times \text{SPE volume extracted}]}{[\text{sample aliquot volume} \times (\text{sample weight} / \text{direct inject sample volume})]}$$

Equation 3 was used to calculate the percent recovery.

Equation 3:

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

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

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

Equation 4:

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

An example of a calculation using an actual sample follows:

Sediment sample Exygen ID C0172892 Spk D (Set: 042806D), fortified at 4 ng/mL with PFOA where:

peak area	=	236588
intercept	=	4630
slope	=	420
dilution factor	=	100
ng/g PFOA added (fort level)	=	4000
amt in corresponding sample (ng/g)	=	586
SPE volume extracted (L)	=	0.04
sample aliquot volume (mL)	=	5
sample weight (g)	=	1
direct inject sample volume (mL)	=	10
total solids (%)	=	66.54

From equation 1:

$$\text{Analyte found (ng/L)} = \frac{[236588 - 4630] \times 100}{420}$$

$$= 55200 \text{ ng/L}$$

From equation 3:

$$\text{PFOA found (ppb)} = \frac{(55200 \text{ ng/L} \times 0.04 \text{ L})}{[5 \text{ mL} \times (1 \text{ g} / 10 \text{ mL})]}$$

$$= 4420 \text{ ppb}$$

From equation 3:

$$\text{\% Recovery} = \frac{(4420 \text{ ng/g} - 586 \text{ ng/g})}{4000 \text{ ng/g}} \times 100\%$$

$$= 96\%$$

From equation 4:

PFOA found (ppb) dry weight = 4416 ppb x (100% / 66.54%)

= 6640 ppb

NOTE: This value may be slightly different than that of the raw data due to rounding.

7.0 EXPERIMENTAL DESIGN

^{13}C PFOA was used as a surrogate for all the samples. ^{13}C PFOA was added to the sediment samples in the laboratory after the samples were weighed for extraction. For sediment samples designated as laboratory matrix spikes, PFOA was also added at a known concentration to the samples in the laboratory after the samples were weighed for extraction.

The sediment samples were extracted in one set. The set included one reagent blank and two reagent blanks fortified at known concentrations. The set contained three sediment samples. For each sample, two laboratory matrix spikes and a duplicate were extracted.

8.0 RESULTS

Analytical results for the analysis of PFOA in sediment samples based on dry weight are summarized in **Table I**. Analytical results for the analysis of PFOA in sediment samples by direct injection, based on dry weight, are summarized in **Table II**. Analytical results for the analysis of PFOA in sediment samples by alternative SPE extraction, based on dry weight, are summarized in **Table III**. The overall average percent recovery ± standard deviation for PFOA in sediment samples based on wet weight was 73 ± 16%. Fortification recoveries for the analysis of PFOA in sediment samples are summarized in **Table IV**. Fortification recoveries for the analysis of PFOA in direct injection sediment samples are summarized in **Table V**. Fortification recoveries for the analysis of PFOA in SPE extracted sediment samples are summarized in **Table VI**. The average percent recovery ± standard deviation for ^{13}C PFOA in sediment samples based on wet weight was 97 ± 27%. Fortification recoveries for ^{13}C PFOA in sediment samples based on wet weight are summarized in **Table VII**. Fortification recoveries for ^{13}C PFOA in direct injection sediment samples based on wet weight are summarized in **Table VIII**. Fortification recoveries for ^{13}C PFOA in SPE extracted sediment samples based on wet weight are summarized in **Table IX**.

The total percent solids for sediment samples are detailed in **Table X**.

9.0 CONCLUSIONS

The sediment samples were successfully extracted and analyzed for PFOA using an alternative SPE method, which yielded acceptable QC data.

10.0 RETENTION OF DATA AND SAMPLES

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

TABLES

Table I. Summary of PFOA in Sediment Samples

Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0172892	DAL-SD-BPP01-0-0000	NR	NR
C0172892 Rep	DAL-SD-BPP01-0-0000*	NR	NR
C0172893	DAL-SD-BPP02-0-0000	NR	NR
C0172893 Rep	DAL-SD-BPP02-0-0000*	NR	NR
C0172894	DAL-SD-BPP03-0-0000	NR	NR
C0172894 Rep	DAL-SD-BPP03-0-0000*	NR	NR

*Laboratory Duplicate
NR = Not reported due to quality control result failures. See Table II for re-analysis results.

Table II. Summary of PFOA in Direct Injection Sediment Samples

Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0172892	DAL-SD-BPP01-0-0000	NR	NR
C0172892 Rep	DAL-SD-BPP01-0-0000*	NR	NR
C0172893	DAL-SD-BPP02-0-0000	NR	NR
C0172893 Rep	DAL-SD-BPP02-0-0000*	NR	NR
C0172894	DAL-SD-BPP03-0-0000	NR	NR
C0172894 Rep	DAL-SD-BPP03-0-0000*	NR	NR

*Laboratory Duplicate
NR = Not reported due to quality control result failures. See Table III for re-analysis results.

Table III. Summary of PFOA in SPE Extracted Sediment Samples

Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight C8 Acid PFOA Perfluorooctanoic Acid	Assessed Accuracy (+/- %)
C0172892	DAL-SD-BPP01-0-0000	881	30
C0172892 Rep	DAL-SD-BPP01-0-0000*	945	30
C0172893	DAL-SD-BPP02-0-0000	927	30
C0172893 Rep	DAL-SD-BPP02-0-0000*	823	30
C0172894	DAL-SD-BPP03-0-0000	2390	30
C0172894 Rep	DAL-SD-BPP03-0-0000*	2380	30

*Laboratory Duplicate

Table IV. Matrix Spike Recovery of PFOA in Sediment Samples

		C8 Acid PFOA		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172892 Spk D, 400 ppb Spike)	400	NR	NR	NR
DAL-SD-BPP01-0-0000 (C0172892 Spk E, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP02-0-0000 (C0172893 Spk G, 400 ppb Spike)	400	NR	NR	NR
DAL-SD-BPP02-0-0000 (C0172893 Spk H, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP03-0-0000 (C0172894 Spk J, 400 ppb Spike)	400	NR	NR	NR
DAL-SD-BPP03-0-0000 (C0172894 Spk K, 4000 ppb Spike)	4000	NR	NR	NR
			Average:	**NR**
			Standard Deviation:	**NR**

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

NR = Not reported due to quality control result failures. See Table V for re-analysis results.

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

Table V. Matrix Spike Recovery of PFOA in Direct Injection Sediment Samples

		C8 Acid PFOA		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172892 Spk D, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP01-0-0000 (C0172892 Spk E, 40000 ppb Spike)	40000	NR	NR	NR
DAL-SD-BPP02-0-0000 (C0172893 Spk G, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP02-0-0000 (C0172893 Spk H, 40000 ppb Spike)	40000	NR	NR	NR
DAL-SD-BPP03-0-0000 (C0172894 Spk J, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP03-0-0000 (C0172894 Spk K, 40000 ppb Spike)	40000	NR	NR	NR
			Average:	**NR**
			Standard Deviation:	**NR**

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

NR = Not reported due to quality control result failures. See Table VI for re-analysis results.

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

Table VI. Matrix Spike Recovery of PFOA in SPE Extracted Sediment Samples

		C8 Acid PFOA		
Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172892 Spk D, 4000 ng/g Spike)	4000	586	4420	96
DAL-SD-BPP01-0-0000 (C0172892 Spk E, 40000 ng/g Spike)	40000	586	25600	63
DAL-SD-BPP02-0-0000 (C0172893 Spk G, 4000 ng/g Spike)	4000	504	3880	84
DAL-SD-BPP02-0-0000 (C0172893 Spk H, 40000 ng/g Spike)	40000	504	25100	61
DAL-SD-BPP03-0-0000 (C0172894 Spk J, 4000 ng/g Spike)	4000	1060	4140	77
DAL-SD-BPP03-0-0000 (C0172894 Spk K, 40000 ng/g Spike)	40000	1060	23200	55
			Average:	73
			Standard Deviation:	16

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

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

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/g)	Amount Recovered Wet Weight (ng/g)	Recovery (%)
C0172892	DAL-SD-BPP01-0-0000	4	NR	NR
C0172892 Rep	DAL-SD-BPP01-0-0000	4	NR	NR
C0172892 Spk C	DAL-SD-BPP01-0-0000	4	NR	NR
C0172892 Spk D	DAL-SD-BPP01-0-0000	400	NR	NR
C0172892 Spk E	DAL-SD-BPP01-0-0000	4000	NR	NR
C0172893	DAL-SD-BPP02-0-0000	4	NR	NR
C0172893 Rep	DAL-SD-BPP02-0-0000	4	NR	NR
C0172893 Spk F	DAL-SD-BPP02-0-0000	4	NR	NR
C0172893 Spk G	DAL-SD-BPP02-0-0000	400	NR	NR
C0172893 Spk H	DAL-SD-BPP02-0-0000	4000	NR	NR
C0172894	DAL-SD-BPP03-0-0000	4	NR	NR
C0172894 Rep	DAL-SD-BPP03-0-0000	4	NR	NR
C0172894 Spk I	DAL-SD-BPP03-0-0000	4	NR	NR
C0172894 Spk J	DAL-SD-BPP03-0-0000	400	NR	NR
C0172894 Spk K	DAL-SD-BPP03-0-0000	4000	NR	NR
			Average:	**NR**
			Standard Deviation:	**NR**

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

Table VIII. Surrogate Spike Recovery of ^{13}C PFOA in Direct Injection Sediment Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/g)	Amount Recovered Wet Weight (ng/g)	Recovery (%)
C0172892	DAL-SD-BPP01-0-0000	40	NR	NR
C0172892 Rep	DAL-SD-BPP01-0-0000	40	NR	NR
C0172892 Spk C	DAL-SD-BPP01-0-0000	40	NR	NR
C0172892 Spk D	DAL-SD-BPP01-0-0000	4000	NR	NR
C0172892 Spk E	DAL-SD-BPP01-0-0000	40000	NR	NR
C0172893	DAL-SD-BPP02-0-0000	40	NR	NR
C0172893 Rep	DAL-SD-BPP02-0-0000	40	NR	NR
C0172893 Spk F	DAL-SD-BPP02-0-0000	40	NR	NR
C0172893 Spk G	DAL-SD-BPP02-0-0000	4000	NR	NR
C0172893 Spk H	DAL-SD-BPP02-0-0000	40000	NR	NR
C0172894	DAL-SD-BPP03-0-0000	40	NR	NR
C0172894 Rep	DAL-SD-BPP03-0-0000	40	NR	NR
C0172894 Spk I	DAL-SD-BPP03-0-0000	40	NR	NR
C0172894 Spk J	DAL-SD-BPP03-0-0000	4000	NR	NR
C0172894 Spk K	DAL-SD-BPP03-0-0000	40000	NR	NR
			Average:	NR
			Standard Deviation:	NR

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

Table IX. Surrogate Spike Recovery of ^{13}C PFOA in SPE Extracted Sediment Samples

		^{13}C-PFOA		
Exygen ID	Sample Description	Amount Spiked (ng/g)	Amount Recovered Wet Weight (ng/g)	Recovery (%)
C0172892	DAL-SD-BPP01-0-0000	40	52.0	130
C0172892 Rep	DAL-SD-BPP01-0-0000	40	51.0	128
C0172892 Spk D	DAL-SD-BPP01-0-0000	4000	3750	94
C0172892 Spk E	DAL-SD-BPP01-0-0000	40000	26800	65
C0172893	DAL-SD-BPP02-0-0000	40	47.8	120
C0172893 Rep	DAL-SD-BPP02-0-0000	40	51.6	129
C0172893 Spk G	DAL-SD-BPP02-0-0000	4000	3500	88
C0172893 Spk H	DAL-SD-BPP02-0-0000	40000	25200	63
C0172894	DAL-SD-BPP03-0-0000	40	45.2	113
C0172894 Rep	DAL-SD-BPP03-0-0000	40	40.3	101
C0172894 Spk J	DAL-SD-BPP03-0-0000	4000	3060	77
C0172894 Spk K	DAL-SD-BPP03-0-0000	40000	22100	55
			Average:	**97**
			Standard Deviation:	**27**

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

Table X. Total Percent Solids in Sediment Samples

Exygen ID	Sample Description	Total Solids (%)
C0172892	DAL-SD-BPP01-0-0000	66.54
C0172893	DAL-SD-BPP02-0-0000	54.37
C0172894	DAL-SD-BPP03-0-0000	44.46

FIGURES

Figure 1. Typical Calibration Curve for PFOA in Methanol

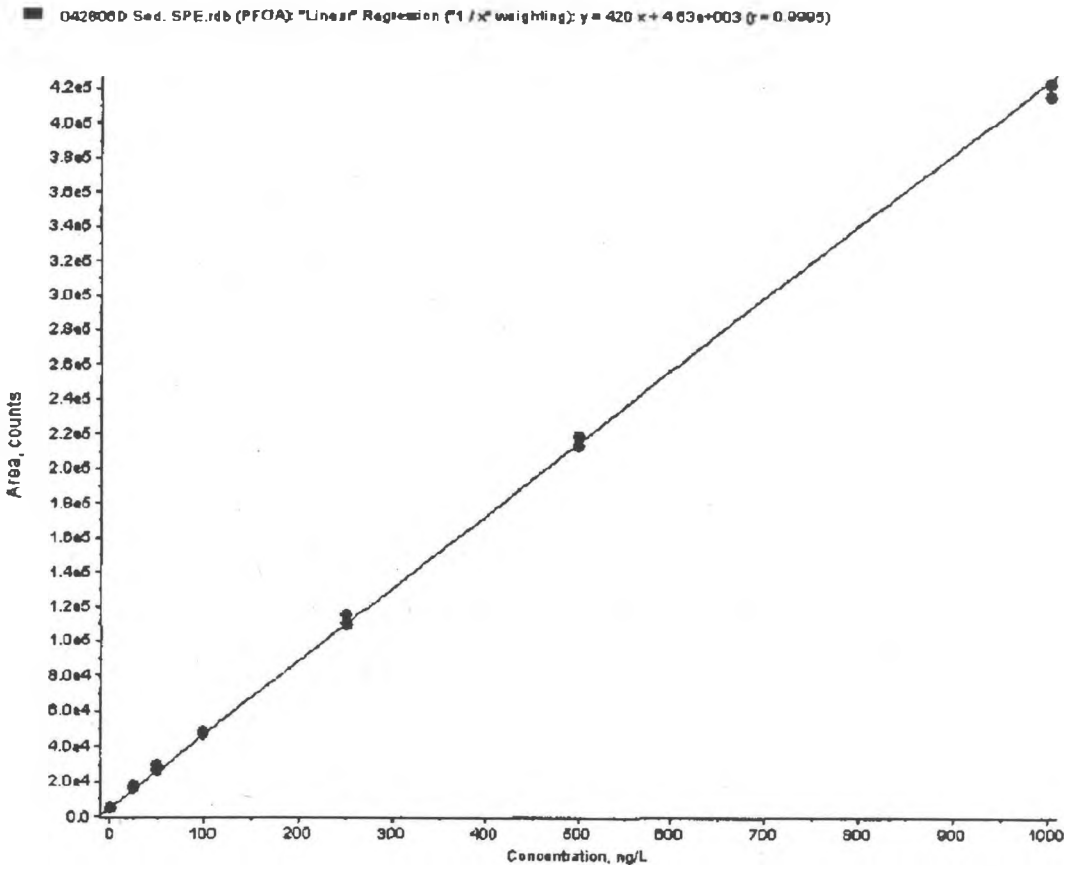


Figure 2. Extracted Standards of PFOA in Methanol, 25 ng/L and 50 ng/L, Respectively

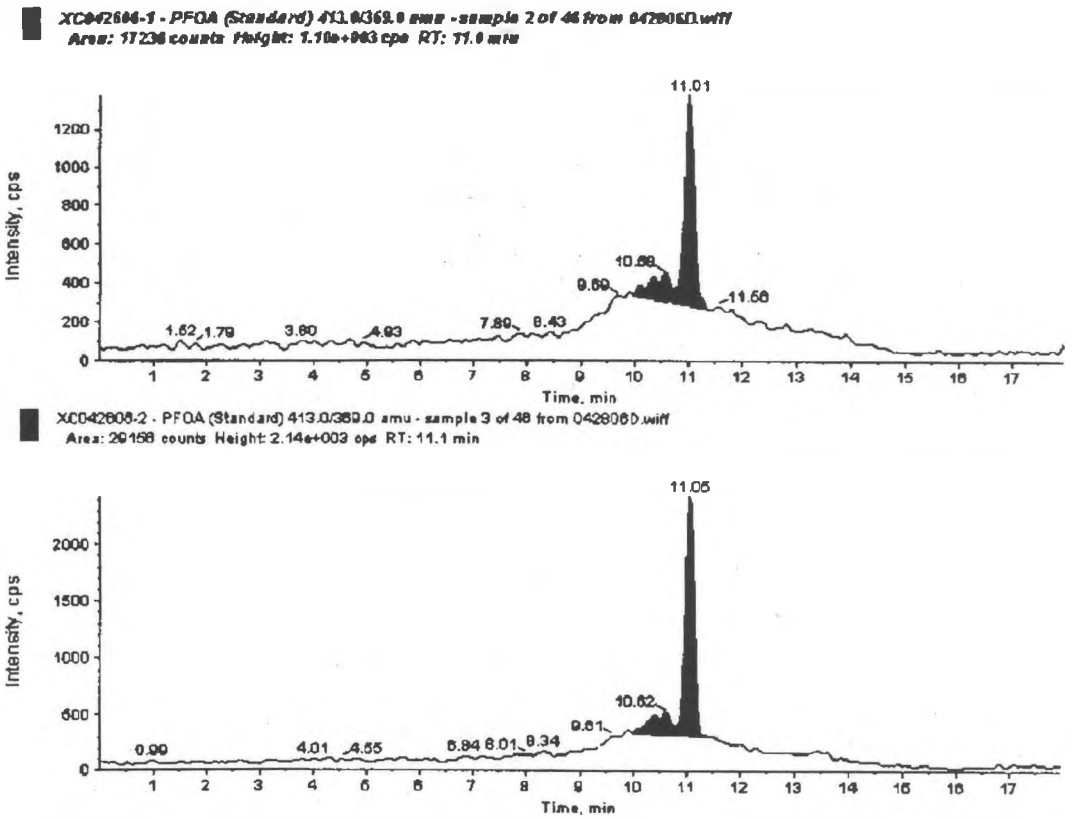


Figure 3. PFOA in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

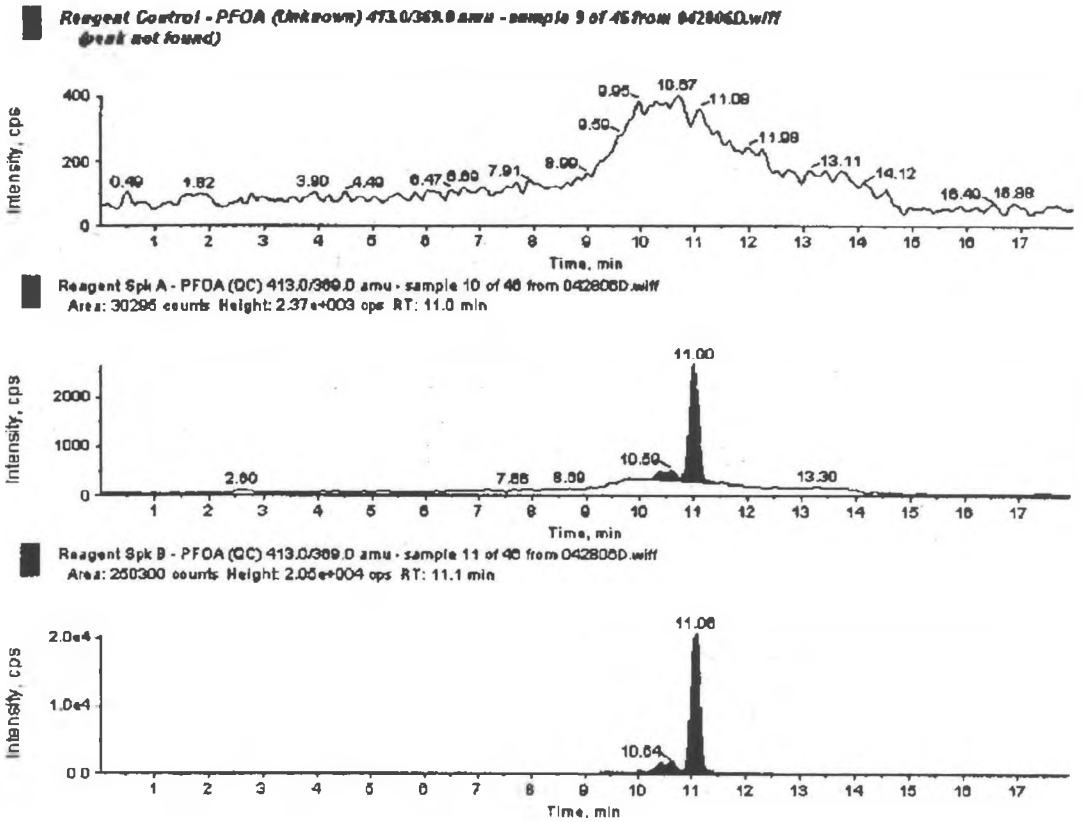


Figure 4. Chromatogram Representing a Sediment Sample Analyzed for PFOA (Exygen ID: C0172892, Data Set: 042806D)

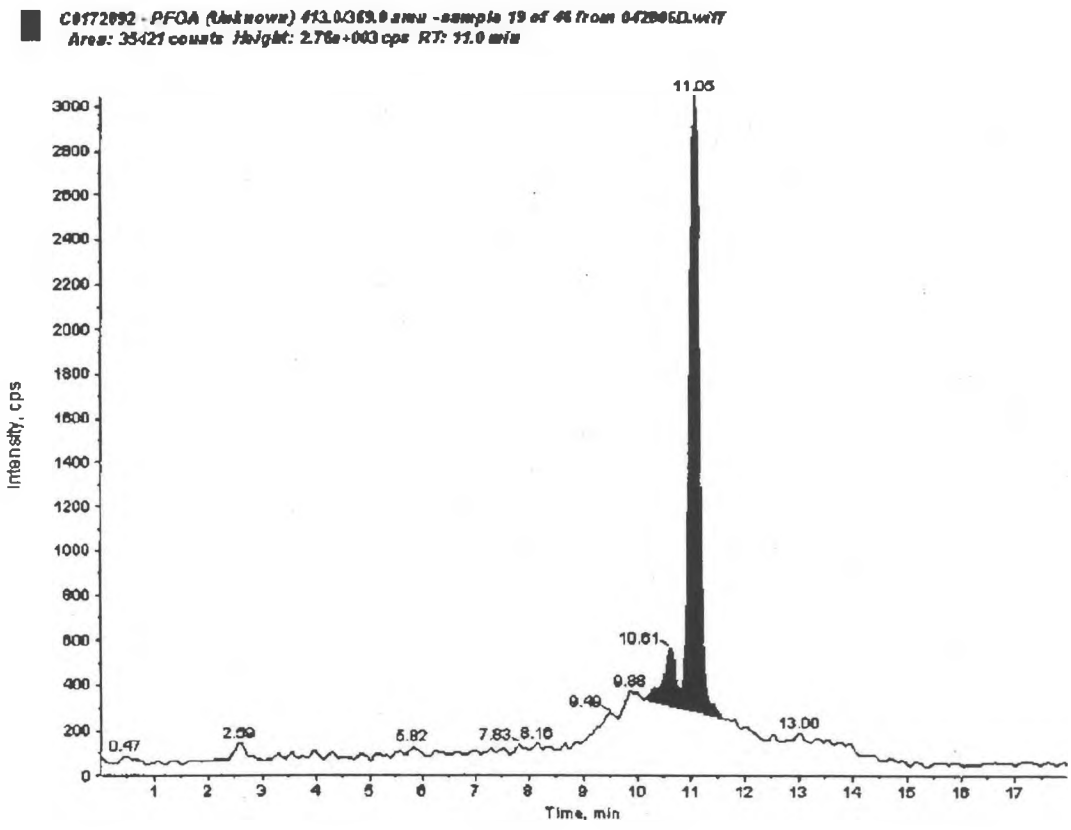


Figure 5. Typical Calibration Curve for ^{13}C PFOA in Methanol

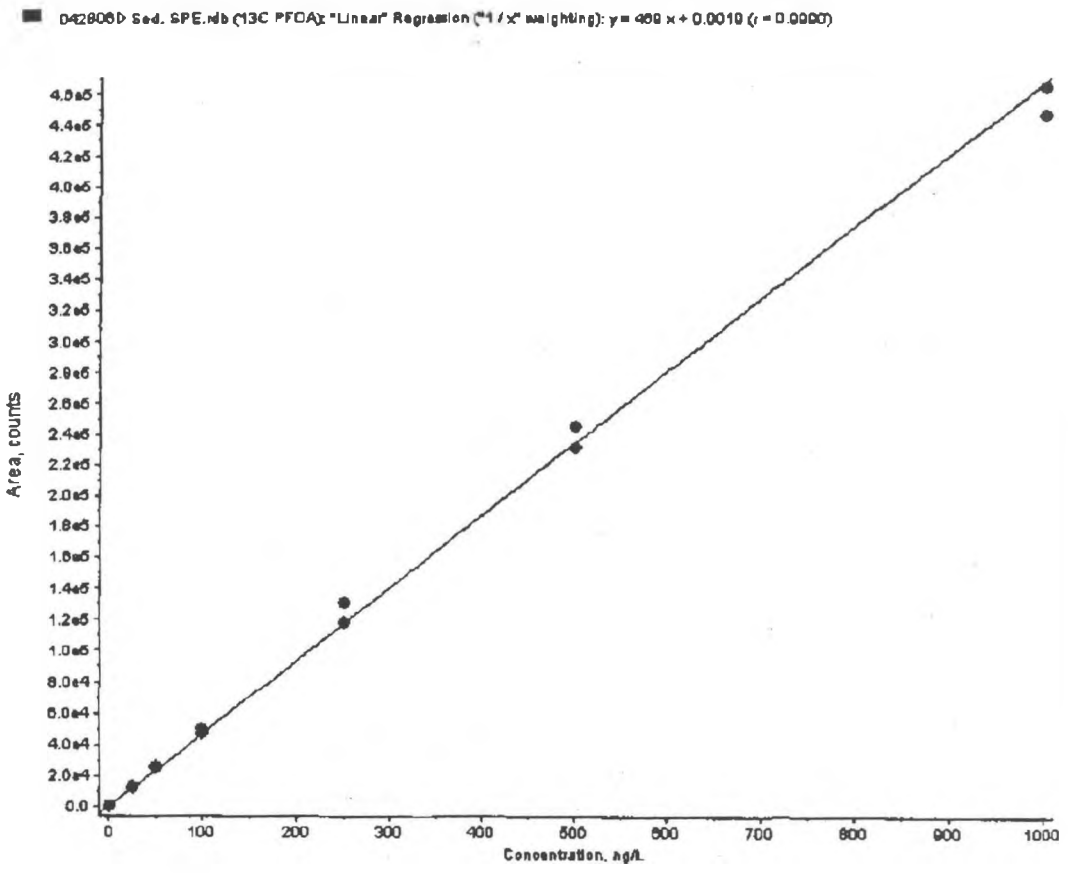


Figure 6. Extracted Standards of ^{13}C PFOA in Methanol, 25 ng/L and 50 ng/L, Respectively

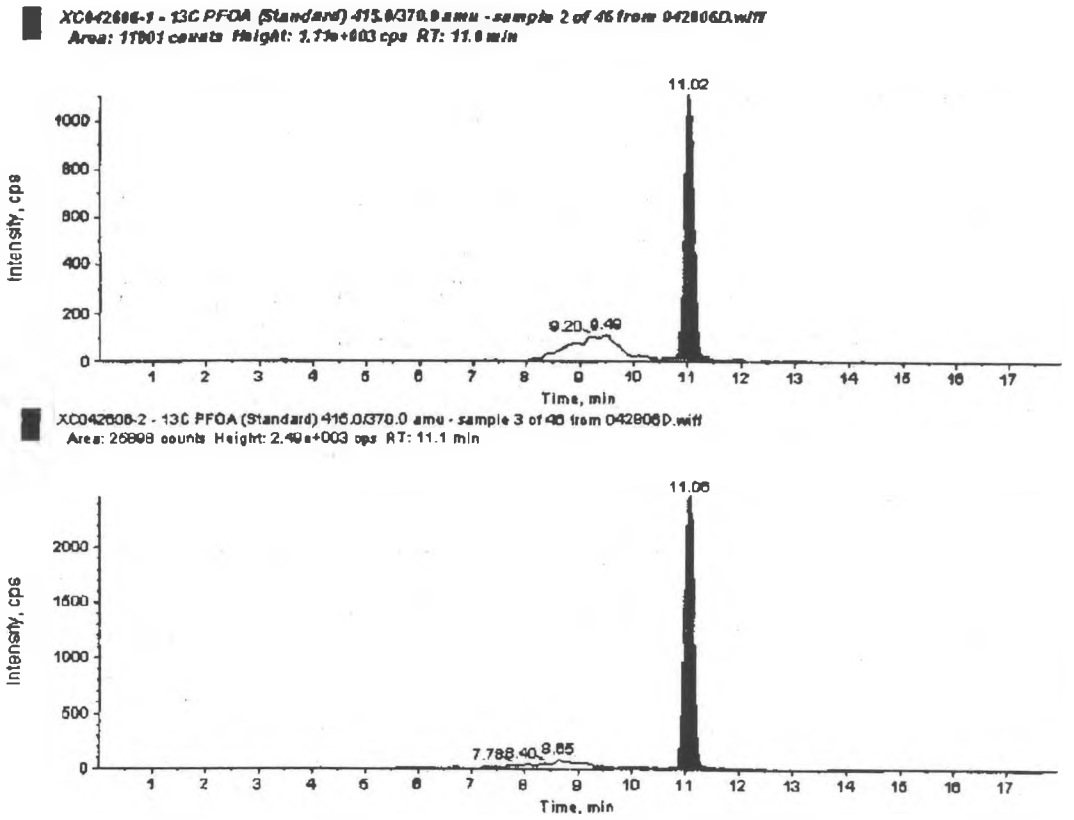


Figure 7. ^{13}C PFOA in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

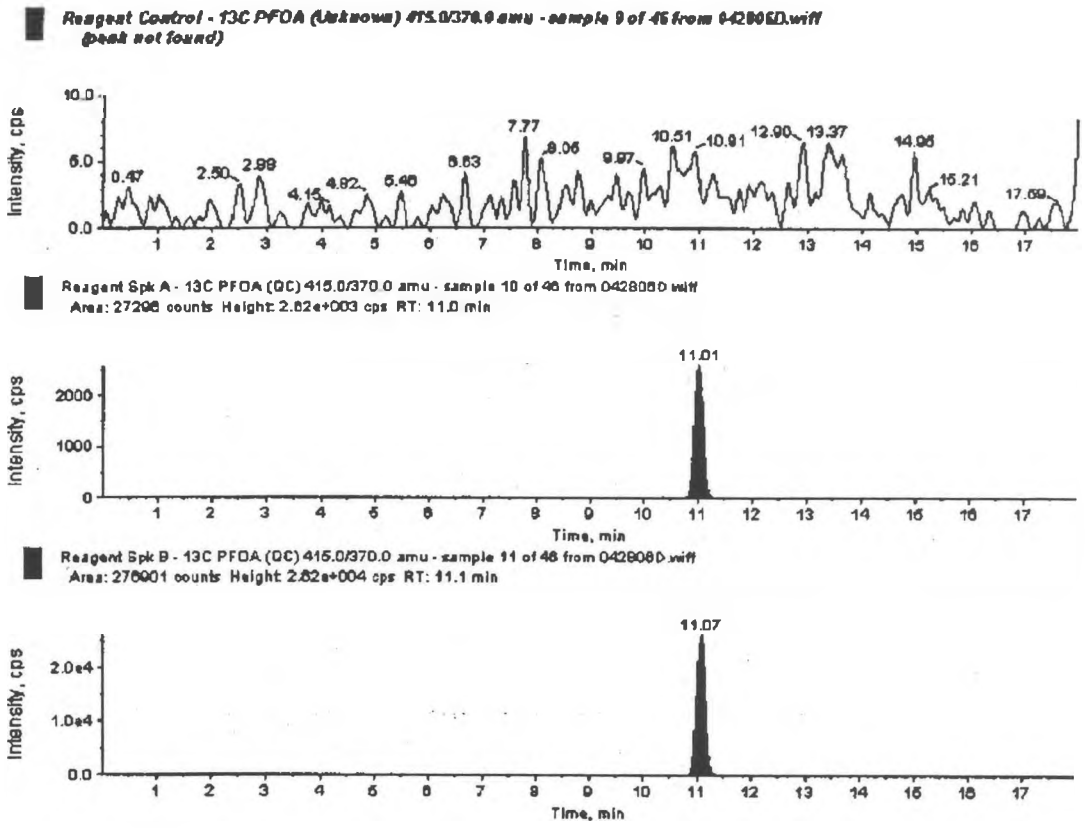
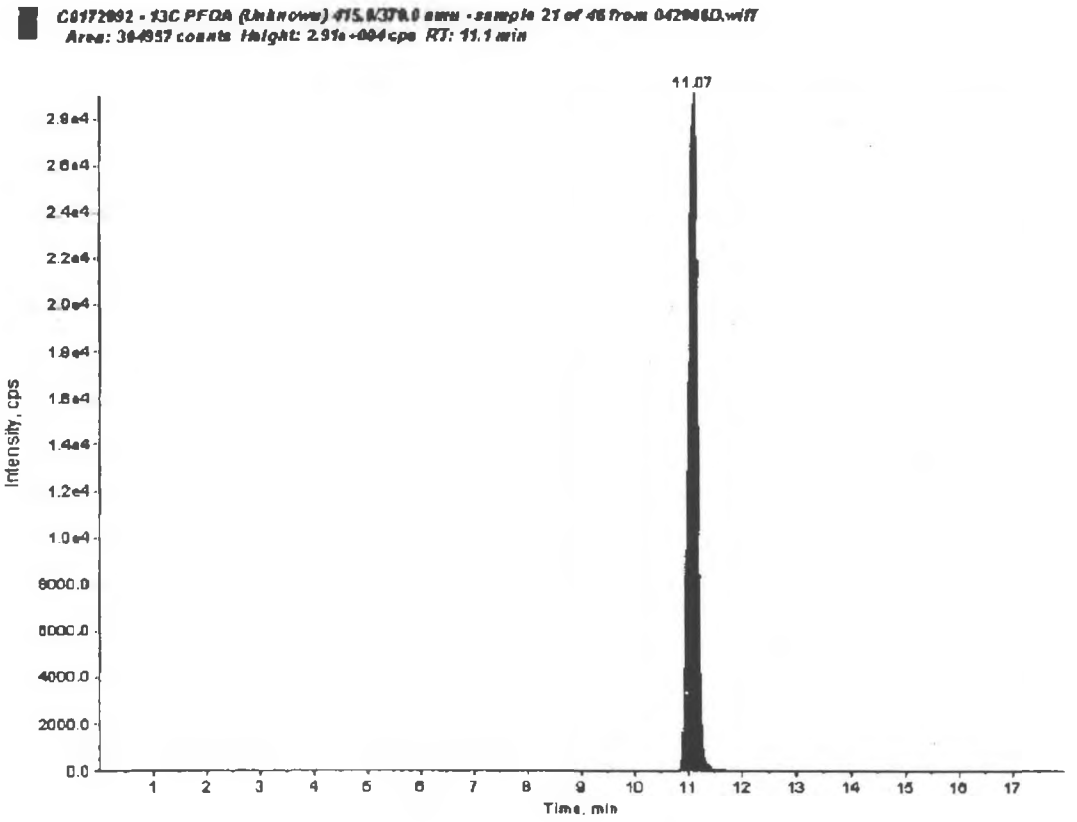


Figure 8. Chromatogram Representing a Sediment Sample Analyzed for ^{13}C PFOA (Exygen ID: C0172892, Data Set: 042806D)



APPENDIX A

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

STUDY PROTOCOL

Study Title:

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

Exygen Protocol Number: P0000760

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

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

DISTRIBUTION:

1) Jaisimha Kesari, Study Director, Weston Solutions

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

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

4) Exygen Research Quality Assurance Unit

PROTOCOL APPROVAL

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

Exygen Protocol Number: P0000760

APPROVALS

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

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

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

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

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

TABLE OF CONTENTS

Exygen Protocol Number: P0000760

INTRODUCTION

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

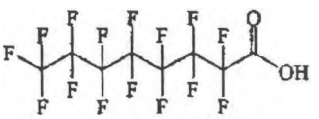
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIAL

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

PFOA

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



SURROGATE FIELD SPIKE COMPOUND

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

^{13}C PFOA

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

OBJECTIVE

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

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

TESTING FACILITY

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

STUDY DIRECTOR

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SPONSOR REPRESENTATIVE

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PRINCIPAL INVESTIGATOR

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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.

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

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

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

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

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

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

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

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

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

SAMPLE IDENTIFICATION

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

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

ANALYTICAL PROCEDURE SUMMARY

References:

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

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

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

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

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

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

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

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

VERIFICATION OF ANALYTICAL PROCEDURE

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

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

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

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

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

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

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

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

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

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

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

METHOD FOR CONTROL OF BIAS

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

STATISTICAL METHODS

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

GLP STATEMENT

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

REPORT

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

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

SAFETY AND HEALTH

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

AMENDMENTS TO PROTOCOL

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

DATA RECORD KEEPING

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

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

Chromatograms- All chromatograms will contain the following:

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

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

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

QUALITY ASSURANCE

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

RETENTION OF DATA AND ARCHIVING

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

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

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

APPENDIX I

ANALYTICAL METHODS

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

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001780

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

Analytical Testing Facility: Exygen Research
3050 Research Drive
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Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001780

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges

Page 3 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

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

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

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Alternate volumes may be prepared.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001710

ANALYTICAL METHOD

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

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

* The extracted concentration of the calibration standard is equal to 5x its initial concentration, due to the concentration of the standard during the extraction (SPE).
XC = extracted calibration standard.

Page 4 of 7

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

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

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

10.0 *Batch Set Up*

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

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

11.0 Sample Extraction

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

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

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

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

11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

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

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

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

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

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

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

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

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

13.0 Acceptance Criteria

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

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

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

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

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

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

14.0 Calculations

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

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

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

Exygen Protocol Number: P0000760

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.

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

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001781

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

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Exygen Protocol Number: P0000760

Exygen Research Method Number V0001711

ANALYTICAL METHOD

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

1.0 **Scope**

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

2.0 **Safety**

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

3.0 **Sample Requirement**

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

4.0 **Reagents and Standards**

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

5.0 Instrument and Equipment

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

Page 2 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

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

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

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Alternate volumes may be prepared.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001781

ANALYTICAL METHOD

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

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

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

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

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

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

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

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

9.2 Standard Calibration Solutions

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

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

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

* The extracted concentration of the calibration standard is equal to 2x its initial concentration, due to the concentration of the standard during the extraction (SPE).
XC = extracted calibration standard.

Page 4 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

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

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

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

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

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

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

11.0 Sample Extraction

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

11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes.

11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.

11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.

11.5 Centrifuge for ~10 minutes at ~3000 rpm.

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

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

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

11.9 Analyze samples using 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 3 of 5

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

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

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

13.0 Acceptance Criteria

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

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

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

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

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

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

Exygen Protocol Number: P0000760

14.0 Calculations

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

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

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

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

Recovery (%) =

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

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

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

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

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

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001712

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

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Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number: VG001782

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

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

Page 3 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

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

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

6.0 Chromatographic System

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

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

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Exygen Protocol Number: P0000760

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 maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

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

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

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

ANALYTICAL METHOD

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

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

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

10.0 Batch Set Up

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

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

11.0 Sample Extraction

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

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

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

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

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

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

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

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

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

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

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

11.12 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.13 Analyze samples using electrospray LC/MS/MS.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

DF = factor by which the final volume was diluted, if necessary.

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (3 g)}}$$

14.4 Use the following equation (if necessary) to calculate the amount of PFOA found in ppb based on dry weight.

$$\text{PFOA found (ppb) dry weight} = \text{PFOA found (ppb)} \times [100\% / \text{total solids(\%)}]$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001783

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

12/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

12/26/04
Date

Total Pages: 5

Exygen Protocol Number P0000760

Exygen Research Method Number V0001713

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in fish and clams.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in freezer storage overnight to allow the carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedure will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Supelclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

Page 1 of 8

Exygen Protocol Number: P0000760

5.4 Rotary evaporator.
5.5 Thermolyne.
5.6 125 mL pear-shaped flasks.
5.7 50 mL disposable polypropylene centrifuge tubes.
5.8 15 mL disposable polypropylene centrifuge tubes.
5.9 Disposable micropipets (50-100uL, 100-200uL).
5.10 125-mL LDPE narrow-mouth bottles.
5.11 2 mL clear HPLC vial kit.
5.12 Disposable pipettes.
5.13 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.14 SPE tubes (20mL) (Supelco cat. no. N057177).
5.15 Wrist action shaker.
5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program.

Time (min)	%A	%B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve
6.8 Run Time: ~ 23 minutes

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

7.0 **MS/MS System**

7.1 **Mode:** Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 **Preparation of Solutions**

8.1 **Mobile Phase**

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 **Extraction Solutions**

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 **Standard Preparation**

9.1 **Standard Stock/Fortification Solution**

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.003	10	100	0.0003

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Place the tubes in a freezer for ~1 hour.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

11.7 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.8 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat phase using a tissumizer for ~30 seconds and rinse the tissumizer with ~10 mL of acetonitrile into the tube.

11.10 Shake the sample again for ~10 minutes on a wrist-action shaker.

11.11 Place the tubes in a freezer for ~ 1 hour more.

11.12 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~40 minutes.

11.13 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluant from the initial extraction.

11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.

11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).

11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.

11.17 Transfer the extracts to HPLC vials using disposable pipets.

11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.5 (or equivalent) software system.

Page 1 of 8

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.995 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}}$$

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

Exygen Protocol Number: P0000760

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{\left[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}\right]}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001784

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

Analytical Testing Facility:

Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number: V0901 7B0

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

1.0 **Scope**

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in vegetation.

2.0 **Safety**

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 **Sample Requirement**

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 **Reagents and Standards**

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Supercleam LC-NH_2 – Reagent grade
4.8 1-Octanol - HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid - Sigma-Aldrich

5.0 **Instrument and Equipment**

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS)
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

Page 1 of 7

Exygen Protocol Number: P0000760

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 flasks.
5.6 50 mL disposable polypropylene centrifuge tubes.
5.7 15 mL disposable polypropylene centrifuge tubes.
5.8 Disposable micropipets (50-100uL, 100-200uL).
5.9 125-mL LDPE narrow-mouth bottles.
5.10 2 mL clear HPLC vial kit.
5.11 Disposable pipettes.
5.12 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.13 SPE tubes (20mL) (Supelco cat. no. N057177).
5.14 Wrist action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm

6.0 Chromatographic System

6.1 Analytical Column: Phenyhexe RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

Exygen Protocol Number: P0000760

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 MSMS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethyldichlorosilane in toluene is prepared by bringing 3 mL of dimethyldichlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

Exygen Protocol Number: P0000760

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the columns to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

11.8 Add 20 mL of acetonitrile to the sample in the 50 mL centrifuge tube.
11.9 Shake the sample again for ~10 minutes on a wrist-action shaker.
11.10 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~5 minutes.
11.11 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.
11.12 Repeat steps 11.8 through 11.11 again.
11.13 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).
11.14 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear shaped flask and swirl to mix/dissolve.
11.15 Transfer the extracts to HPLC vials using disposable pipets.
11.16 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

Page 6 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

14.2 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)} \times \text{DF}]}{\text{sample weight (g)}}$$

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 calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}]}{\text{analyte added (ng/g)}} \times 100$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001785

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001781

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal liver.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 5 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave, open in freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternately, if there is an insufficient amount of sample (~less than 5 g), then no processing is necessary and the sample can be used as supplied.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.

Page 3 of 7

Exygen Protocol Number: P0000760

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 Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Tissuemizer.
5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm

6.0 **Chromatographic System**

6.1 Analytical Column: Phenylhexe RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL)
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimise 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.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

8.0 **Preparation of Solutions**

8.1 **Mobile Phase**

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

9.0 **Standard Preparation**

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.3.4 Alternate volumes and concentrations of standards may be prepared as needed.

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

10.0 **Batch Set Up**

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 **Sample Extraction**

11.1 Weigh 1 g of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate weights of liver may be measured depending on the sample size available for use.

11.2 Add water to the sample for a final volume of 10 mL.

11.3 Homogenize sample using a tissuemizer for ~1 minute.

11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.

11.5 Add 3 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.

11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.

11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry.

11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 **Chromatography**

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/g, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the MassLynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{Intercept})}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 10

Exygen Protocol Number: P0000760

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample weight (g)}}$$

Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001786

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/26/01
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/01
Date

Total Pages: 7

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001746

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal serum.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 1 mL of test sample for extraction.
3.2 No sample processing is needed for serum samples. However, frozen serum samples must be allowed to completely thaw to room temperature before use.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL)
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Vortexer.

Page 1 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

5.14 Wrist-action shaker.
5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program.

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

Exygen Protocol Number: P0000760

Exygen Research Method Number V0001706

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

Page 4 of 7

Exygen Protocol Number: P0000760

Exygen Research Method Number: V0001786

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

11.0 Sample Extraction

11.1 Measure 1 mL of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate volumes of serum may be measured depending on the sample size available for use.
11.2 Add water to the sample for a final volume of 20 mL. Cap tightly
11.3 Vortex for ~1 minute.
11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.
11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.
11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry
11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.
11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).
11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system
12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

Page 5 of 7

Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 20

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

Page 6 of 7

Exygen Protocol Number: P0000760

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ppb.

$$\text{PFOA found (ppb)} = \frac{\text{PFOA found (ng/mL)} \times \text{final volume (mL)}}{\text{sample volume (mL)}}$$

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PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 01/19/05
Exygen Study Number: 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 9.1.8, Alternate weights of standards may be used to prepare alternate concentrations of stock solutions as necessary. Alternate levels of fortification solutions may also be prepared.
2) Low and high spiking levels of the analytes for each matrix may be altered depending on sample size available for extraction and/or to cover analyte concentrations expected in the samples.

RATIONALE

1) Higher concentrations of standards need to be prepared in order to spike the sample bottles at higher levels.
2) The sample size available for small mammal liver and serum was smaller than expected. Spiking at the pre-determined levels in the protocol puts the spiked concentration lower than the detection limit. Also, the analyte levels in the ground water samples are expected to greatly exceed the pre-determined spiking levels listed in the protocol. When the levels in the samples greatly exceed the spiking levels, an accurate recovery value cannot be calculated for the QC sample. Higher spiking levels in the bottles will cover the analyte concentrations expected in the water samples.

IMPACT ON STUDY

The LOQ is 100 ng/g for a 0.1 g sample of small mammal liver and is 1000 ng/mL for a 0.01 mL sample of small mammal serum.
Higher levels of spiking for the water samples will ensure that more QC recovery data can be used.

Study Director Signature — Date 3/9/05
Principal Investigator Signature — Date 3/2/05
Study Director/Management Signature N/A — Date N/A
Sponsor Signature (if required) Mary Heberstein for Michael A. Santoro — Date 3/8/05

Exygen QAU Review LJS 02/01/05

LIBRARY ID: VV0001226-6 ADMINISTRATIVE FORM

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PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 03/07/05
Exygen Study Number P0001131 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

1) Report, page 11 of 85
2) Test Materials, page 6 of 85: PFOS transition monitored 499 -> 99.

AMENDED TO

1) Instead of one final report, interim reports will be issued.
2) PFOS transition monitored may also be 499 -> 80.

RATIONALE

1) Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timelier manner. manner 03/01/05 RJS
2) The API 4000 LC/MS/MS systems detect the 499 -> 80 PFOS transition with greater sensitivity than the 499 -> 99 transition.

IMPACT ON STUDY

1) The client will be able to receive and review the data more quickly.
2) The 499 -> 80 transition can be detected with greater sensitivity; therefore, giving better chromatography.

Study Director Signature	Date 3/9/05
Principal Investigator Signature	Date 3/4/05
Study Director Management Signature MA	Date
Exygen Management Signature	Date 8-MAR-05
Sponsor Signature (if required)	Date 3/10/05

Exygen QAU Review LJJ 03/08/05

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DEVIATION FORM

General:

__X__ Project Specific Deviation ____ Facility Deviation Date of Occurrence: 04/26/06

Exygen Project # : P760/P1131 Deviation # : 5

Client Project # : NA Reference # : 06-076

Regulatory Driver:

____ GMP
__X__ GLP
____ Other
____ None

Deviation Type: *(Include V# for methods and SOPs)*

__X__ Protocol ____ Method ____ SOP

V#: NA

Notebook reference: NA

Sample Description:

Login # : L0008191 Container # : NA Lot # : NA

Summary of Deviation:

The three sediment samples in L8191 (C0172892 – C0172894) were originally extracted using the sediment method V0001782. Poor recoveries were obtained for PFOS, PFOA and ^{13}C PFOA. Because of this, the study sponsor requested the use of an alternative extraction for these compounds, as follows:

Direct Injection Method: Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A one-gram portion of sediment was weighed into a 15-milliliter centrifuge tube for the extraction. Ten milliliters of 1% acetic acid in methanol was added to each sample. The samples were then shaken by hand, vortexed, and sonicated for thirty minutes. The samples were then centrifuged for ~10 minutes at ~3000 rpm. Each sample was analyzed by LC/MS/MS electrospray.

Using this method acceptable data was obtained for PFOS, but the recoveries for PFOA and ^{13}C PFOA were still poor. Another alternative method was then used for PFOA and ^{13}C PFOA, as follows:

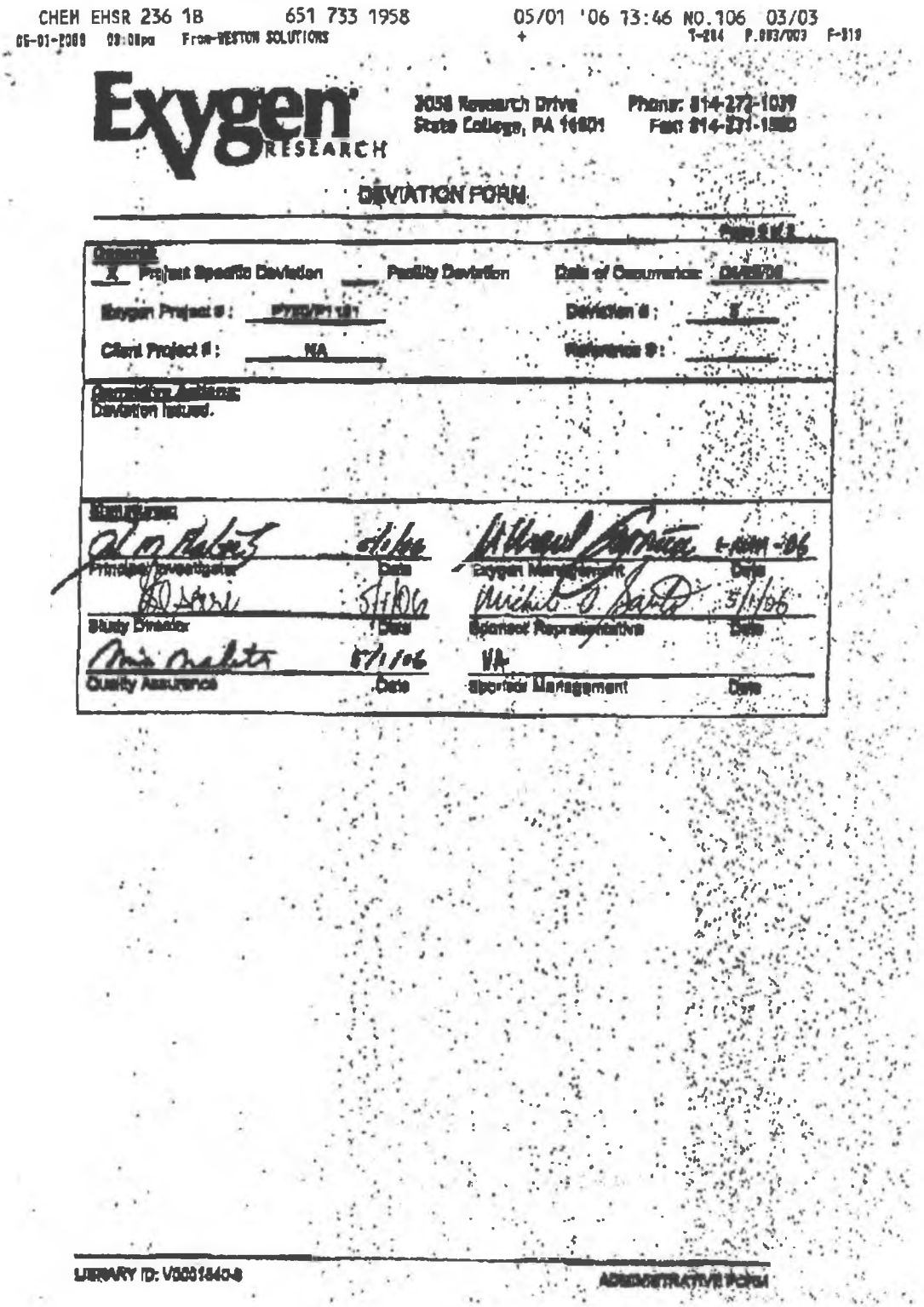
Alternative SPE Method: The samples that were prepared in 1% acetic acid for the direct injection method were used for this extraction. Five milliliters of each sample was aliquoted into a 50-mL polypropylene centrifuge tube and the volume was taken to 40 mL with water. The samples were then centrifuged for ~10 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

Cause: ____ Preparation ____ Analysis ____ Instrument ____ Client Request __X__ Other

Impact:

More usable data was obtained.

LIBRARY ID: V0001640-6 ADMINISTRATIVE FORM



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Exygen RESEARCH

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DEVIATION FORM

Page 1 of 2

Deviation
X Project Specific Deviation ___ Facility Deviation Date of Occurrence: 04/28/06

Exygen Project #: P7000/P1101 Deviation #: 5

Client Project #: NA Reference #:

Description/Justification:
Deviation Issued.

Signatures:

Principal Investigator — Date: 5/1/06

Exygen Management — Date: 5-May-06

Study Director — Date: 5/1/06

Sponsor Representative — Date: 5/1/06

Quality Assurance — Date: 5/1/06

Sponsor Management — Date: NA

LIBRARY ID: V0001540-6 ADMINISTRATIVE Form

INTERIM REPORT # 23 - Analysis of Sediment 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 Kesari P.E., DEE
Weston Solutions, Inc.
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Phone: 610-701-3761

INTERIM REPORT COMPLETION DATE

May 2, 2006

PERFORMING LABORATORY

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STUDY SPONSOR

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3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: 651-733-6374

PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 110

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, with one exception. The laboratory control and control spikes for the direct injection method were not recorded promptly at the time of extraction.

John Flaherty
Principal Investigator
Exygen Research

5/2/06
Date

Jaisimha Kesari P.E., DEE
Study Director
Weston Solutions, Inc.

5/3/06
Date

Michael A. Santoro
Sponsor Representative
3M Company

5/4/06
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number P0001131, entitled, "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program". All reviewed phases[1] were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

Phase	Date Inspected	Date Reported to Principal Investigator	Date Reported to Exygen Management	Date Reported to Study Director
25. Final Raw Data Review and Interim Analytical Report Review	05/02/06	05/02/06	05/02/06	05/02/06

Lydia Shaffer
Technical Lead, Quality Assurance Unit

05/02/06
Date

[1]Note: All in-lab inspections will be documented in the QA statement for the final analytical report at the conclusion of the study. This QA statement involves only the review of the interim report and associated raw data.

CERTIFICATION OF AUTHENTICITY

This interim report, for Exygen Study Number P0001131, is a true and complete representation of the raw data.

Submitted by: Exygen Research
3058 Research Drive
State College, PA 16801
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Principal Investigator, Exygen:

J. L. M. Flaherty 5/2/06

John Flaherty
Vice President
Exygen Research

Date

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FOR Richard Grazzini 5/2/06

Richard A. Grazzini
President
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Date

Study Director, Weston Solutions, Inc.

5/3/06

Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.

Date

Sponsor Representative, 3M Company:

5/4/06

Michael A. Santoro
Director of Regulatory Affairs

Date

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:	Sediment
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: 04/27/06 Interim Analytical Termination Date: 04/30/06 Interim Report Completion Date: 05/02/06

PROJECT PERSONNEL

The Study Director for this project is Jaisimha Kesari at Weston Solutions, Inc. The following personnel from Exygen Research were associated with various phases of this interim portion of the study:

Name	Title
John Flaherty	Vice President
Karen Risha	Scientist
Paul Connolly	Technical Lead-LC/MS
Christine Edwards	Technician
Mark Ammerman	Sample Custodian
Amy Sheehan	Associate Scientist
Brittany Kravets	Technician
Scott Crain	Technician
Frances Crespi	Technician
Devon Ingram	Technician
Christa Gallant	Technician

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

Page

1.0 SUMMARY

Exygen Research extracted and analyzed sediment samples for the determination of perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS), and perfluorooctanesulfonate (PFOS) according to Exygen Method V0001782 (**Appendix A**).

The limit of quantitation for PFBS and PFHS in sediment was 0.2 ng/g. The limit of quantitation for PFOS in sediment was 2.0 ng/g.

The standard sediment method (V0001782) was originally used to extract all of the samples. Use of this method yielded poor recoveries for PFOS. Because of this, a direct injection method (**Section 6.2**) was used to analyze for PFOS.

Analytical results for the analysis of PFBS, PFHS and PFOS found in sediment samples extracted with the original method are summarized in **Table I.** Analytical results for the analysis of PFOS found in sediment samples by direct injection are summarized in **Table II.**

The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in sediment samples were 85 ± 19%, 99 ± 29%, and 93 ± 6%, respectively. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in sediment samples extracted with the original method are summarized in **Table III**. Fortification recoveries for the analysis of PFOS in sediment samples by direct injection are summarized in **Table IV**.

The total percent solids for sediment samples are detailed in **Table V**.

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 sediment 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 sediment using the analytical methods entitled, "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS."

The study was initiated on November 05, 2004, when the study director signed protocol number P0001131. The analytical start date for this interim report was April 27, 2006 and the analytical termination date for this interim report was April 30, 2006.

4.0 ANALYTICAL TEST SAMPLES

Three sediment samples (Exygen ID: C0172892–C0172894) were received on wet ice on April 22, 2006 from Tim Frinak at Weston Solutions, Inc. The samples were logged in by Exygen personnel and placed in refrigerated storage.

Sample log-in and chain of custody information is located in the raw data package associated with this interim report. Storage records will be kept at Exygen Research.

5.0 REFERENCE MATERIAL

The analytical standards, PFBS and PFHS, were supplied by 3M. PFBS was received from 3M at Exygen on May, 13, 2005. PFHS was received from 3M at Exygen on January, 20, 2003. PFOS was purchased from Fluka Corporation and was received at Exygen on April, 23, 2003.

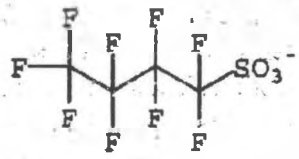
The available information for the reference materials is listed below. PFBS and PFHS were stored frozen and PFOS was stored refrigerated.

Compound	Exygen Inventory No.	Lot #	Purity (%)	Expiration Date
PFBS	SP0005726	101	96.7	12/04/06
PFHS	SP0002401	SE036	98.6	10/18/06
PFOS	SP0002694	430180-1	101.2	10/31/07

The molecular structures of PFBS, PFHS and PFOS are given below:

PFBS

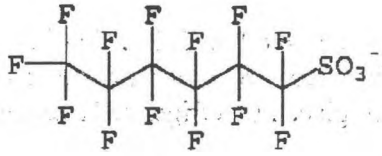
Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Transitions Monitored: 299 → 99
Structure:



PFHS

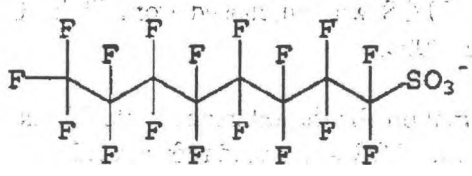
Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Transitions Monitored: 399 → 80

Structure:



PFOS

Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Transitions Monitored: 499 → 80
Structure:



6.0 DESCRIPTION OF ANALYTICAL METHOD

The analytical method "V0001782: Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS" was used for the analysis of PFBS and PFHS in sediment samples. A direct injection method, detailed in **Section 6.2**, was used for the analysis of PFOS in sediment samples.

6.1. Extraction Procedure For Sediment

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A 5 gram portion of sediment was weighed into a fifty milliliter centrifuge tube for the extraction. After fortification of appropriate samples, 35 mL of 1% acetic acid in water was added to the samples. The samples were vortexed and allowed to shake on a wrist action shaker for ~60 minutes. The samples were centrifuged for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 20 mL of water. The eluate was discarded. Twenty milliliters of methanol was added to the sediment samples left in the centrifuge tube. The samples were vortexed and allowed to shake on a wrist action shaker for another 30 minutes. The samples were centrifuged again for ~20 minutes at ~3000 rpm. The supernatant was then loaded onto the same C_{18} SPE cartridge. The eluate was collected into a 500 mL Nalgene Bottle. The column was washed with 4 mL of methanol. The wash was collected in the same bottle as the eluate. Approximately two hundred milliliters of water was added to the bottles. The samples were mixed by shaking and loaded onto another C_{18} SPE cartridge conditioned with 10 mL of methanol

and 20 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

6.2 Direct Inject Extraction Procedure For Sediment

Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A one-gram portion of sediment was weighed into a 15-milliliter centrifuge tube for the extraction. Ten milliliters of 1% acetic acid in methanol was added to each sample. The samples were then shaken by hand, vortexed, and sonicated for thirty minutes. The samples were then centrifuged for ~10 minutes at ~3000 rpm. Each sample was analyzed by LC/MS/MS electrospray.

6.3 Percent Solids Procedure For Sediment

Percent solids were determined using the procedure indicated in Exygen method V0000427. Approximately 20 grams of sample was weighed into a pan. The weight of the sample plus the pan was recorded. The samples were then dried in an oven overnight at 104 ± 2 °C. Then the samples were transferred to a dessicator and allowed to cool for ~15 minutes. Each sample was then weighed again, including the weight of the pan. The percent solid for each sample was then calculated. See **Table V** for percent solids results.

6.4 Preparation of Standards and Fortification Solutions

A mixed stock standard solution of PFBS, PFHS, and PFOS was prepared at a concentration of 1000 µg/mL by dissolving 100 mg of each of the standards (corrected for purity and salt content) in 100 mL of methanol. From this solution, the following fortification standards were prepared:

Conc. of Stock or Fort. Solution (µg/mL)[1]	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Fortification Std. (µg/mL)
1000	10	100	100
100	10	100	10
10	10	100	1.0
1.0	10	100	0.1
0.1	10	100	0.01

[1] of PFBS, PFHS, and PFOS

A set of non-extracted calibration standards containing PFBS, PFHS, and PFOS was prepared in methanol, as specified in Exygen method V0001782. The following concentrations were prepared:

Conc. of Fort or Calibration Solution (ng/mL)[1]	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Calibration Std. (ng/mL)
100	1.0	10	10
100	0.5	10	5.0
100	0.2	10	2.0
10	1.0	10	1.0
5.0	1.0	10	0.5
2.0	1.0	10	0.2

[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.

6.5 Chromatography

Quantification of PFBS, PFHS and PFOS was accomplished by LC/MS/MS electrospray. The retention times of PFBS, PFHS and PFOS were ~0.56mins, ~9.4 mins, and ~11.4 mins, respectively. Peaks above the LOD were not detected in any of the reagent blank samples corresponding to the analyte retention time.

6.6 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.2 ng/mL of PFBS, PFHS and PFOS.

6.7 Description of LC/MS/MS Instrument and Operating Conditions

Instrument:	API 4000 Biomolecular Mass Analyzer
Interface:	Turbo Ion Spray Liquid Introduction Interface
Computer:	DELL OptiPlex GX400
Software:	Windows NT, Analyst 1.4.1
HPLC:	Hewlett Packard (HP) Series 1100 HP Quat Pump

HP Vacuum Degasser
HP Autosampler
HP Column Oven

HPLC Column: Thermo Fluophase RP, 50 mm x 2 1 mm
Column Temp.: ~30° C
Injection Vol.: 15 µL
Mobile Phase (A): 2 mM Ammonium Acetate in water
Mobile Phase (B): Methanol

Time (min)	% A	% B
0.0	65	35
1.0	65	35
8.0	25	75
10.0	25	75
11.0	65	35
18.0	65	35

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

Analyte	Mode	Transition Monitored	Approximate Retention Time (min)
PFBS	negative	299 → 99	~0.56 min.
PFHS	negative	399 → 80	~9.4 min.
PFOS	negative	499 → 80	~11.4 min.

6.8 Quantitation and Example Calculations

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.

Please note that the equations in this section are presented in a different order than the equations in the method (V0001782), but this has no effect on the final calculations.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{(\text{Peak area - intercept}) \times \text{DF}}{\text{Slope}}$$

Where: DF = Dilution Factor, factor by which the final volume was diluted, if necessary.

For samples fortified with known amounts of PFBS and PFHS prior to extraction, Equation 2 was used to convert the amount of PFBS and PFHS found in ng/mL to ng/g (ppb).

Equation 2:

$$\text{Analyte found (ppb)} = \frac{[\text{Analyte found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (5 g)}}$$

Equation 3 was used to calculate the percent recovery:

Equation 3:

$$\text{Recovery (\%)} = \frac{(\text{analyte found (ppb) - analyte in control (ppb)})}{\text{amount added (ppb)}} \times 100\%$$

Equation 4 was then used to calculate the amount of PFBS and PFHS found in ppb based on dry weight.

Equation 4:

Analyte found (ppb) dry weight = Analyte found (ppb) x [100% / total solids(%)]

An example of a calculation using an actual sample follows (calculation is for PFBS only):

Sediment sample Exygen ID: C0172893 Spk F (Set: 042606F), fortified at 4 ng/mL with PFBS where:

peak area	=	253819
intercept	=	-592
slope	=	42300
dilution factor	=	1
ng/g PFBS added (fort level)	=	4
amt in corresponding sample (ng/g)	=	2.41
final volume (mL)	=	5
sample weight (g)	=	5
total solids (%)	=	54.37

From equation 1:

$$\text{Analyte found (ng/mL)} = \frac{[253819 - (-592)]}{42300} \times 1$$

$$= 6.01 \text{ ng/mL}$$

From equation 2:

$$\text{Analyte found (ppb)} = \frac{(6.01 \text{ ng/mL} \times 5 \text{ mL})}{5 \text{ g}}$$

$$= 6.01 \text{ ppb}$$

From equation 3:

$$\text{\% Recovery} = \frac{(6.01 \text{ ppb} - 2.41 \text{ ppb})}{4 \text{ ppb}} \times 100\%$$

$$= 90\%$$

From equation 4:

Analyte found (ppb) dry weight = 6.01 ppb x (100% / 54.37%)

= 11.1 ppb

NOTE: This value may be slightly different than that of the raw data due to rounding.

A slightly different set of calculations was used for the direct injection samples analyzed for PFOS.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{(\text{Peak area - intercept})}{\text{Slope}} \times \text{DF}$$

Where: DF = Dilution Factor, factor by which the final volume was diluted, if necessary.

For samples fortified with known amounts of PFOS prior to extraction,
Equation 2 was used to convert the amount of PFOS found in ng/mL to ng/g (ppb).

Equation 2:

$$\text{Analyte found (ppb)} = \frac{[\text{Analyte found (ng/mL)} \times \text{volume extracted (10 mL)}]}{\text{sample weight (1 g)}}$$

Equation 3 was used to calculate the percent recovery.

Equation 3:

Recovery (%) =

$$\frac{\text{(analyte found (ng/g) - analyte in control (ng/g))}}{\text{amount added (ng/g)}} \times 100\%$$

Equation 4 was then used to calculate the amount of PFOS found in ppb based on dry weight.

Equation 4:

Analyte found (ppb) dry weight = Analyte found (ppb) x [100% / total solids(%)]

An example of a calculation using an actual sample follows:

Sediment sample Exygen ID: C0172892 Spk D (Set: 042806B), fortified at 400 ng/mL with PFOS where:

peak area	=	152371
intercept	=	3720
slope	=	22700
dilution factor	=	100
ng/g PFOS added (fort level)	=	4000
amt in corresponding sample (ng/g)	=	2360
volume extracted (mL)	=	10
sample weight (g)	=	1
total solids (%)	=	66.54

From equation 1:

$$\text{Analyte found (ng/L)} = \frac{[152371 - 3720] \times 100}{22700}$$

$$= 654 \text{ ng/mL}$$

From equation 2:

$$\text{Analyte found (ppb)} = \frac{(654 \text{ ng/mL} \times 10 \text{ mL})}{1 \text{ g}}$$

$$= 6540 \text{ ppb}$$

From equation 3:

$$\text{\% Recovery} = \frac{(6540 \text{ ng/g} - 2360 \text{ ng/g})}{4000 \text{ ng/g}} \times 100\%$$

$$= 105\%$$

From equation 4:

Analyte found (ppb) dry weight = 6540 ppb x (100% / 66.54%)

$$= 9830 \text{ ppb}$$

NOTE: This value may be slightly different than that of the raw data due to rounding.

7.0 EXPERIMENTAL DESIGN

For sediment 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 sediment samples were extracted in one set. The set included one reagent blank and two reagent blanks fortified at known concentrations. The set contained three sediment samples. For each sample, two laboratory matrix spikes and a duplicate were extracted. Due to poor recoveries, the three sediment samples were extracted and analyzed using a direct injection method for PFOS. This method is detailed in **Section 6.2**.

8.0 RESULTS

Analytical results for the analysis of PFBS, PFHS and PFOS found in sediment samples extracted with the original method are summarized in **Table I.** Analytical results for the analysis of PFOS found in sediment samples by direct injection are summarized in **Table II.**

The average percent recoveries ± standard deviations for PFBS, PFHS, PFOS in sediment samples were 85 ± 19%, 99 ± 29%, and 93 ± 6%, respectively. Fortification recoveries for the analysis of PFBS, PFHS, and PFOS in sediment samples extracted with the original method are summarized in **Table III**. Fortification recoveries for the analysis of PFOS in sediment samples by direct injection are summarized in **Table IV**.

The total percent solids for sediment samples are detailed in **Table V**.

9.0 CONCLUSIONS

The sediment samples were successfully extracted and analyzed for PFBS and PFHS according to analytical method V0001782. The sediment samples were successfully extracted and analyzed for PFOS using an alternative method, which yielded acceptable QC data.

10.0 RETENTION OF DATA AND SAMPLES

When the final analytical report is complete, all original paper data generated by Exygen Research will be shipped to the study director. This does not include facility-specific raw data such as instrument or temperature logs. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Exygen Research archives for the period of time specified in EPA TSCA Good Laboratory Practice Standards 40 CFR 792.

TABLES

Table I. Summary of PFBS, PFHS and PFOS in Sediment Samples

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate Analyte Found (ppb, ng/g) Dry Weight	C4 Sulfonate PFBS Assessed Accuracy (+/- %)	C6 Sulfonate PFHS Perfluorohexanesulfonate Analyte Found (ppb, ng/g) Dry Weight	C6 Sulfonate PFHS Assessed Accuracy (+/- %)	C8 Sulfonate PFOS Analyte Found (ppb, ng/g) Dry Weight	C8 Sulfonate PFOS Assessed Accuracy (+/- %)
C0172892	DAL-SD-BPP01-0-0000	3.14	40	84.3	30	NR	NR
C0172892 Rep	DAL-SD-BPP01-0-0000*	3.10	40	86.1	30	NR	NR
C0172893	DAL-SD-BPP02-0-0000	4.43	30	74.5	30	NR	NR
C0172893 Rep	DAL-SD-BPP02-0-0000*	4.84	30	64.7	30	NR	NR
C0172894	DAL-SD-BPP03-0-0000	9.87	30	139	50	NR	NR
C0172894 Rep	DAL-SD-BPP03-0-0000*	8.73	30	123	50	NR	NR

*Laboratory Duplicate
NR = Not reported due to quality control result failures. See Table II for re-analysis results.

Table II. Summary of PFOS in Direct Injection Sediment Samples

		C8 Sulfonate PFOS Perfluorooctanesulfonate	
Exygen ID	Client Sample ID	Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)
C0172892	DAL-SD-BPP01-0-0000	3550	30
C0172892 Rep	DAL-SD-BPP01-0-0000*	3520	30
C0172893	DAL-SD-BPP02-0-0000	4160	30
C0172893 Rep	DAL-SD-BPP02-0-0000*	3840	30
C0172894	DAL-SD-BPP03-0-0000	11900	30
C0172894 Rep	DAL-SD-BPP03-0-0000*	12000	30

*Laboratory Duplicate

Table III. Matrix Spike Recovery of PFBS, PFHS and PFOS in Sediment Samples

Sample Description	C4 Sulfonate PFBS Wet Weight				C6 Sulfonate PFHS Wet Weight			C8 Sulfonate PFOS Wet Weight		
	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 (%)
DAL-SD-BPP01-0-0080 (C9172883 Spk C, 4 ppb Spike)	4	2.09	4.47	60	-	-	-	-	-	-
DAL-SD-BPP01-0-0080 (C9172882 Spk D, 400 ppb Spike)	400	2.09	282	66	56.1	454	99	-	-	-
DAL-SD-BPP01-0-0080 (C9172882 Spk E, 4000 ppb Spike)	4000	2.09	2670	67	56.1	5190	129	NR	NR	NR
DAL-SD-BPP02-0-0080 (C9172883 Spk F, 4 ppb Spike)	4	2.41	8.02	90	-	-	-	-	-	-
DAL-SD-BPP02-0-0080 (C9172883 Spk G, 400 ppb Spike)	400	2.41	374	93	48.5	345	76	-	-	-
DAL-SD-BPP02-0-0080 (C9172883 Spk H, 4000 ppb Spike)	4000	2.41	3920	98	48.5	5090	126	NR	NR	NR
DAL-SD-BPP03-0-0080 (C9172884 Spk I, 4 ppb Spike)	4	4.39	7.71	83	-	-	-	-	-	-
DAL-SD-BPP03-0-0080 (C9172884 Spk J, 400 ppb Spike)	400	4.39	359	89	61.8	283	55	-	-	-
DAL-SD-BPP03-0-0080 (C9172884 Spk K, 4000 ppb Spike)	4000	4.39	4840	121	61.8	4500	111	NR	NR	NR
			Average:	86		Average:	88		Average:	NR
			Standard Deviation:	19		Standard Deviation:	29		Standard Deviation:	NR

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 0.2 ng/g (wet weight)
NR = Not reported due to quality control result failures. See Table IV for re-analysis results.
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 of PFOS in Direct Injection Sediment Samples

		C8 Sulfonate PFOS Wet Weight		
Sample Description	Amount Spiked (ppb)	Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172892 Spk D, 4000 ppb Spike)	4000	2360	6540	105
DAL-SD-BPP01-0-0000 (C0172892 Spk E, 40000 ppb Spike)	40000	2360	40000	94
DAL-SD-BPP02-0-0000 (C0172893 Spk G, 4000 ppb Spike)	4000	2260	5950	92
DAL-SD-BPP02-0-0000 (C0172893 Spk H, 40000 ppb Spike)	40000	2260	38000	89
DAL-SD-BPP03-0-0000 (C0172894 Spk J, 4000 ppb Spike)	4000	5280	8770	87
DAL-SD-BPP03-0-0000 (C0172894 Spk K, 40000 ppb Spike)	40000	5280	41200	90
			Average:	93
			Standard Deviation:	6

*Sample residue exceeds the spiking level significantly (3x spiking level); therefore, an accurate recovery value cannot be calculated
ND = Not detected at or above the Limit of Quantitation (LOQ) of 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.

Table V. Total Percent Solids in Sediment Samples

Exygen ID	Sample Description	Total Solids (%)
C0172892	DAL-SD-BPP01-0-0000	66.54
C0172893	DAL-SD-BPP02-0-0000	54.37
C0172894	DAL-SD-BPP03-0-0000	44.46

FIGURES

Figure 1. Typical Calibration Curve for PFBS in Methanol

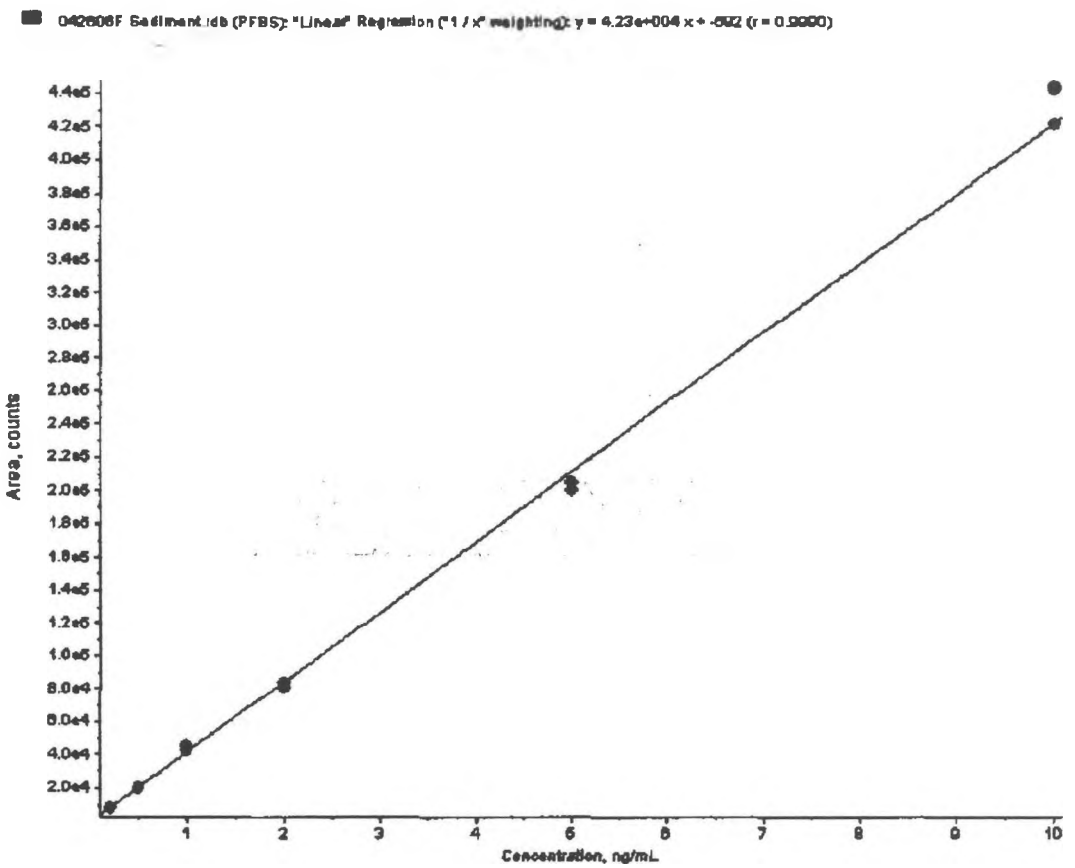


Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

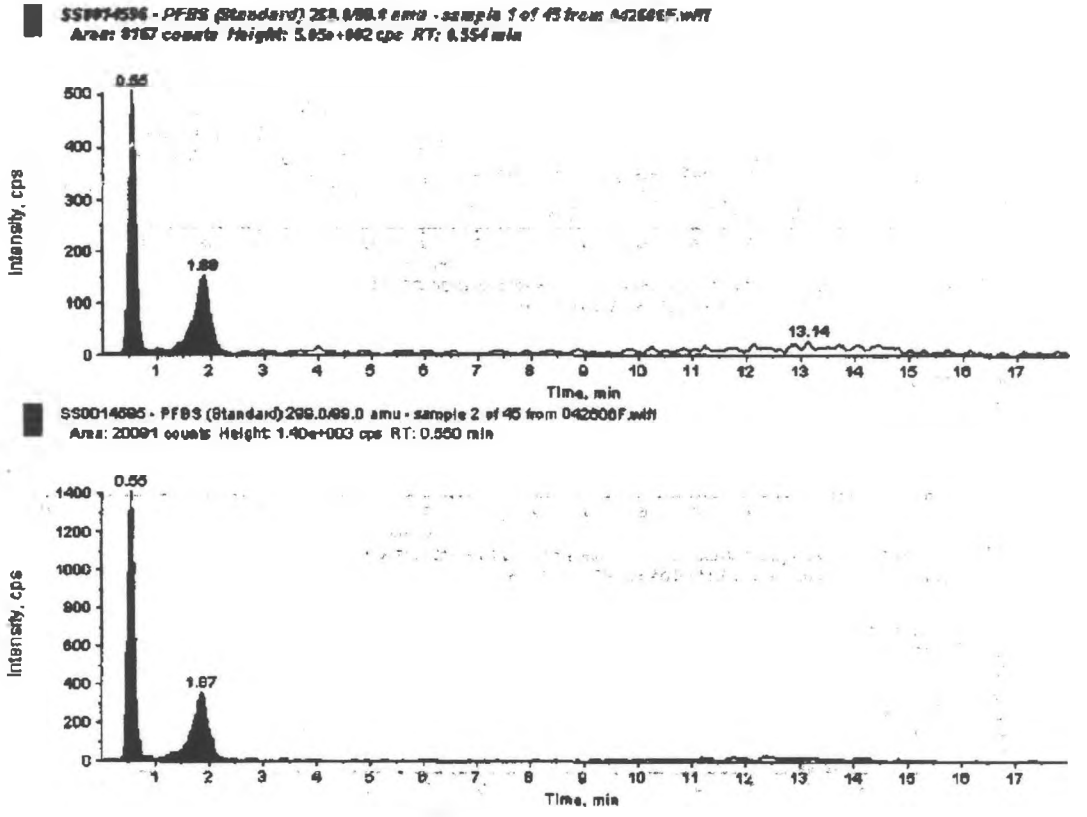


Figure 3. PFBS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

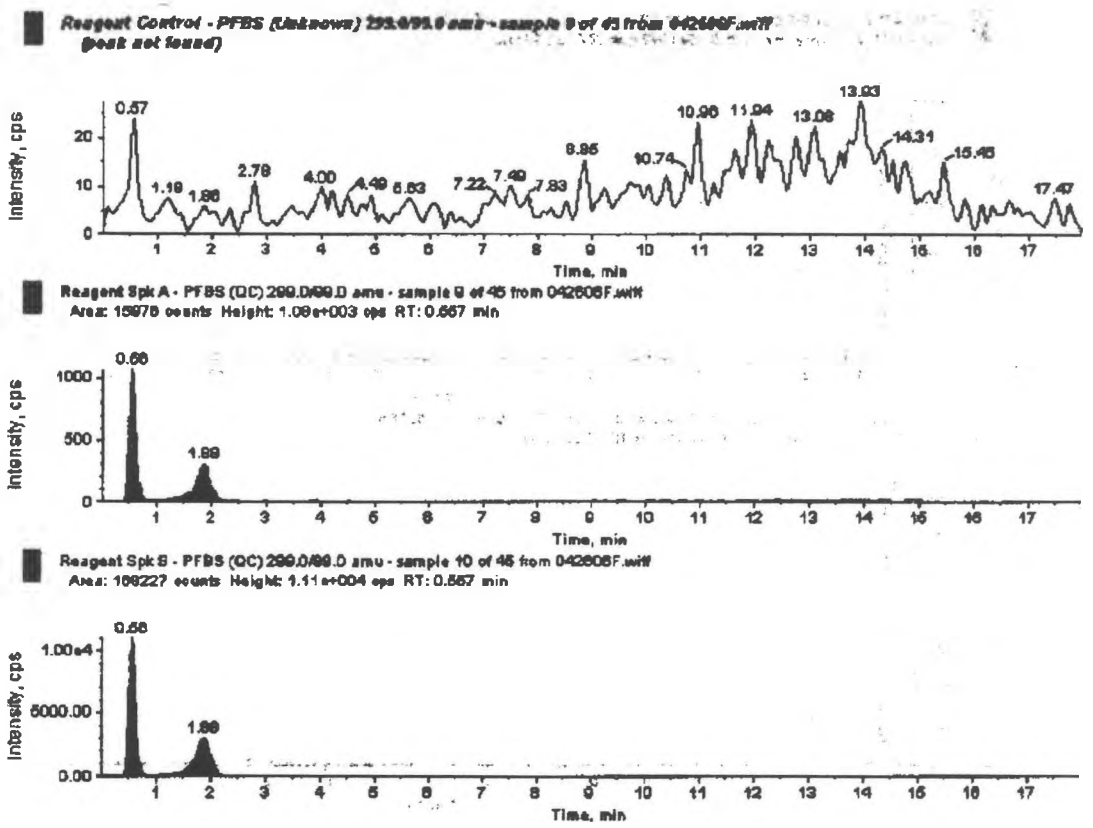


Figure 4. Chromatogram Representing a Sediment Sample Analyzed for PFBS (Exygen ID: C0172892, Data Set: 042606F)

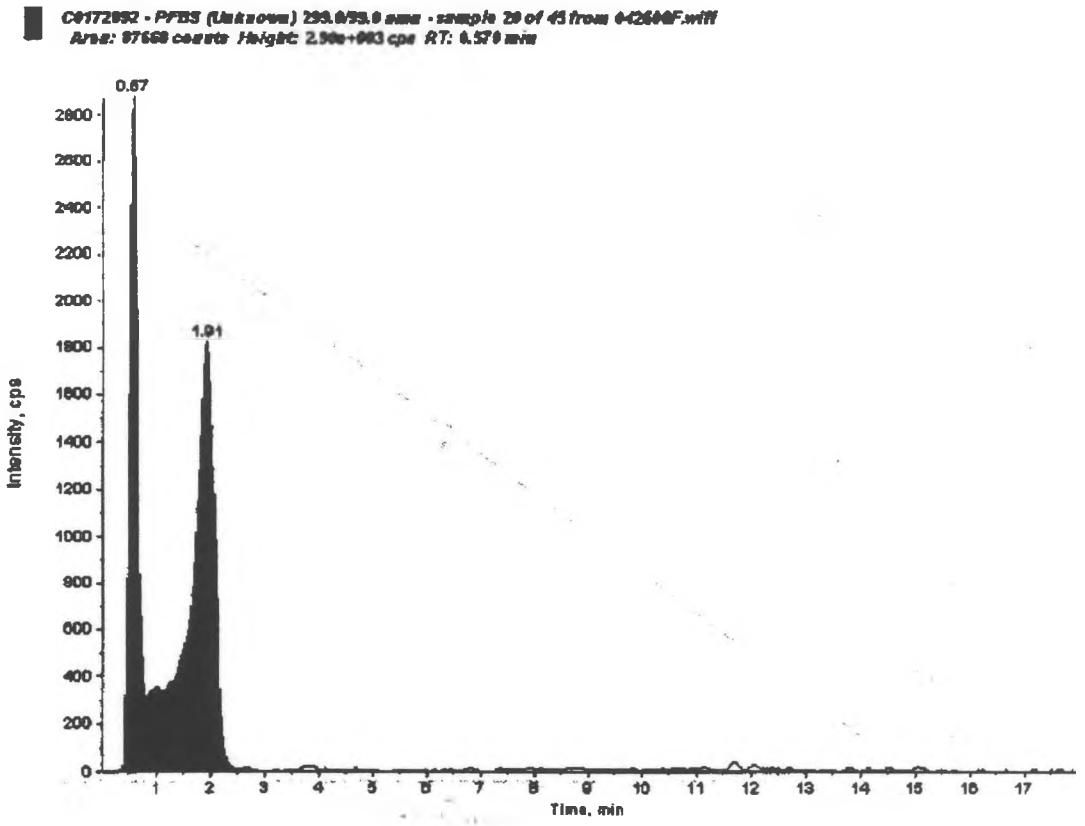


Figure 5. Typical Calibration Curve for PFHS in Methanol

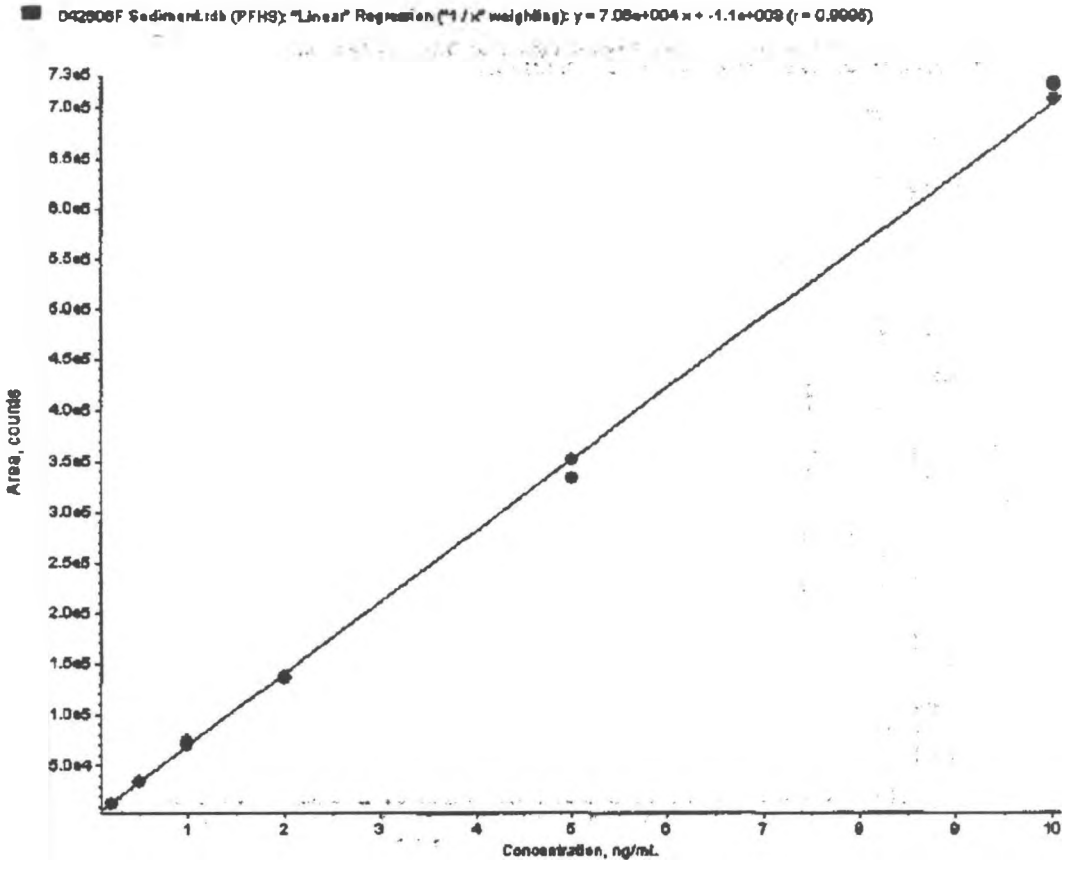


Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

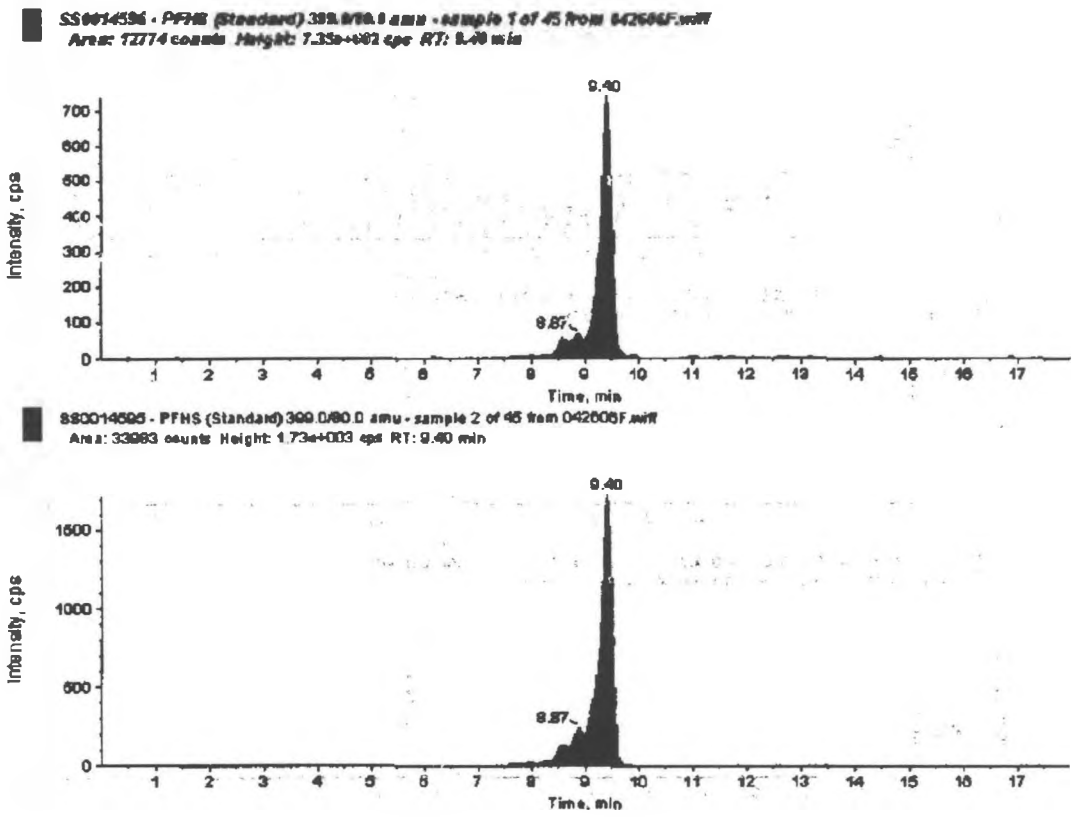


Figure 7. PFHS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

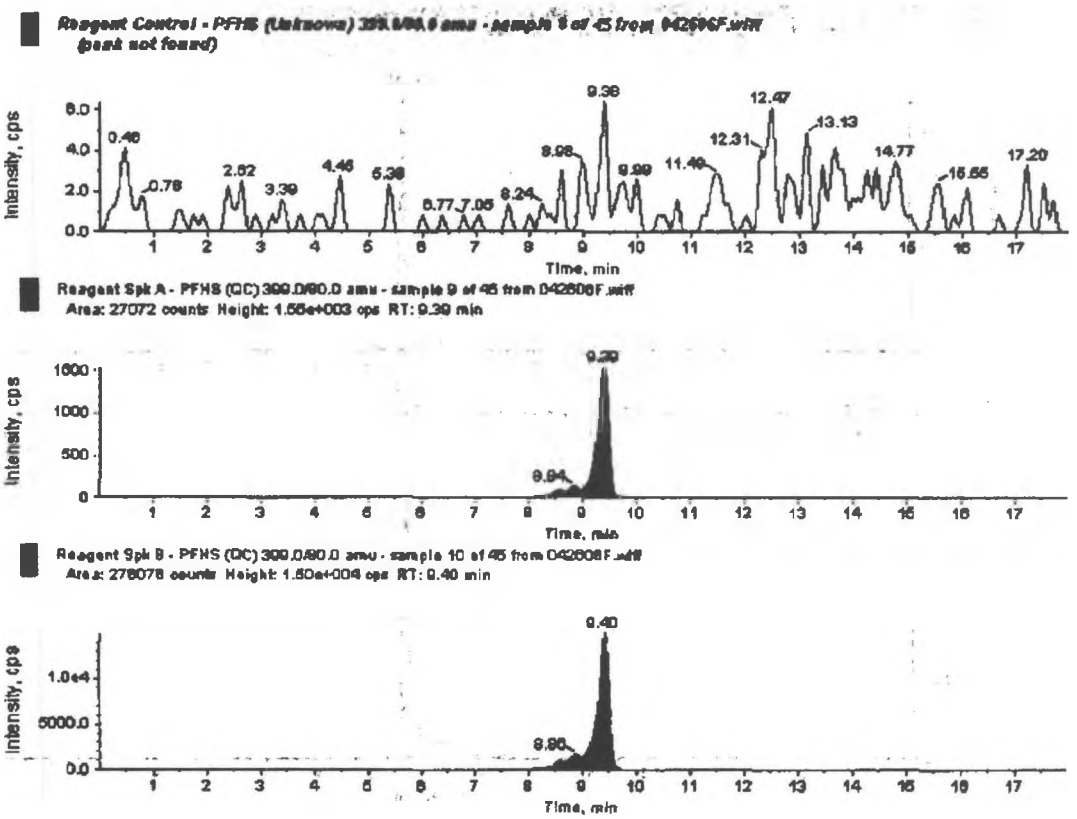


Figure 8. Chromatogram Representing a Sediment Sample Analyzed for PFHS (Exygen ID: C0172892, Data Set: 042606F)

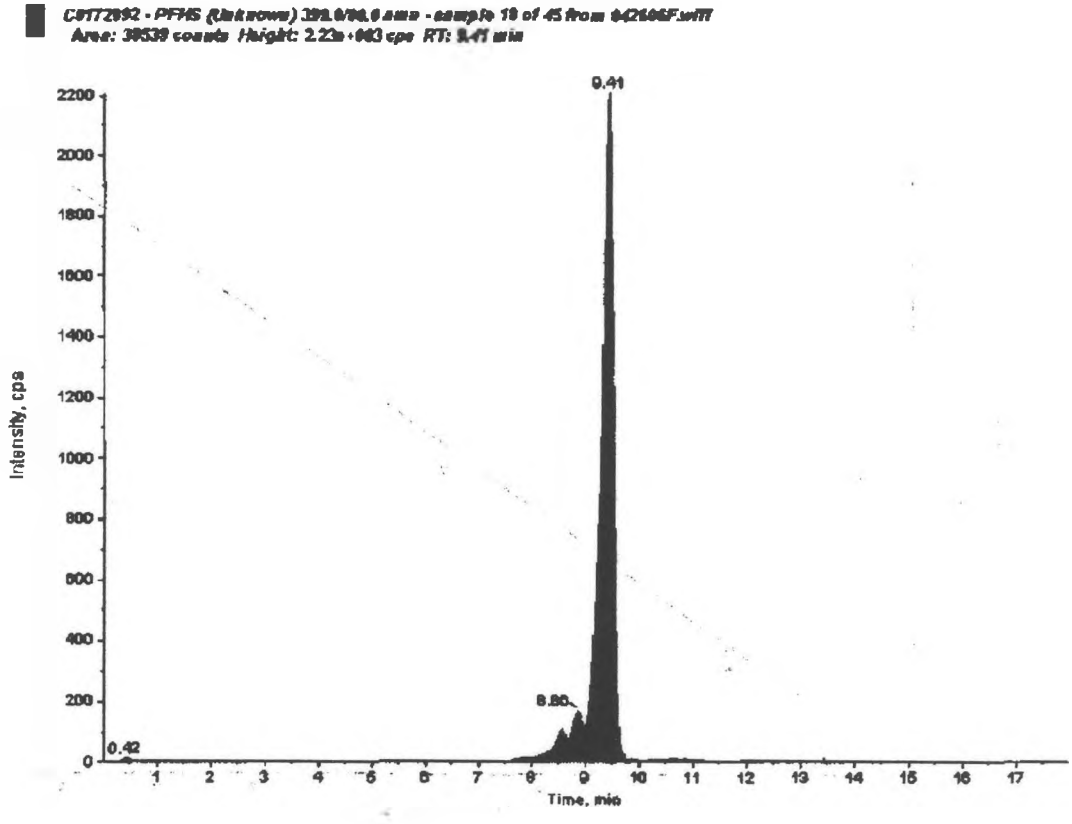


Figure 9. Typical Calibration Curve for PFOS in Methanol

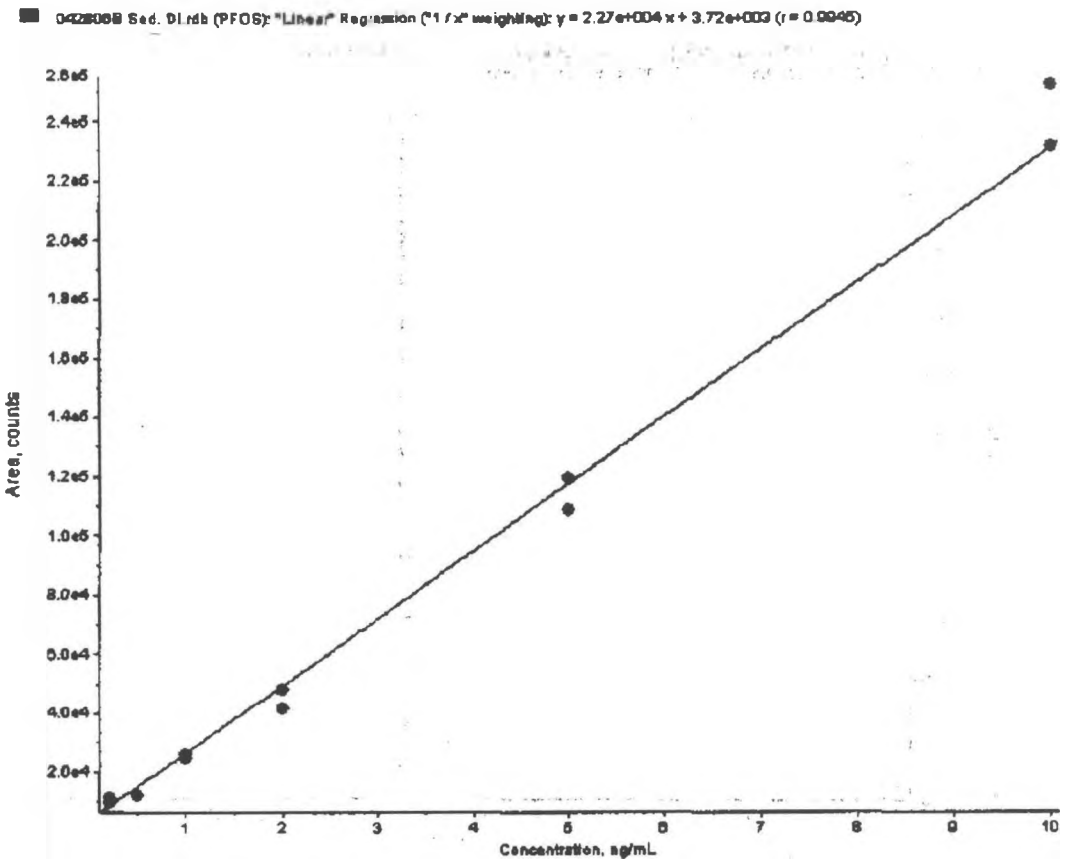


Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

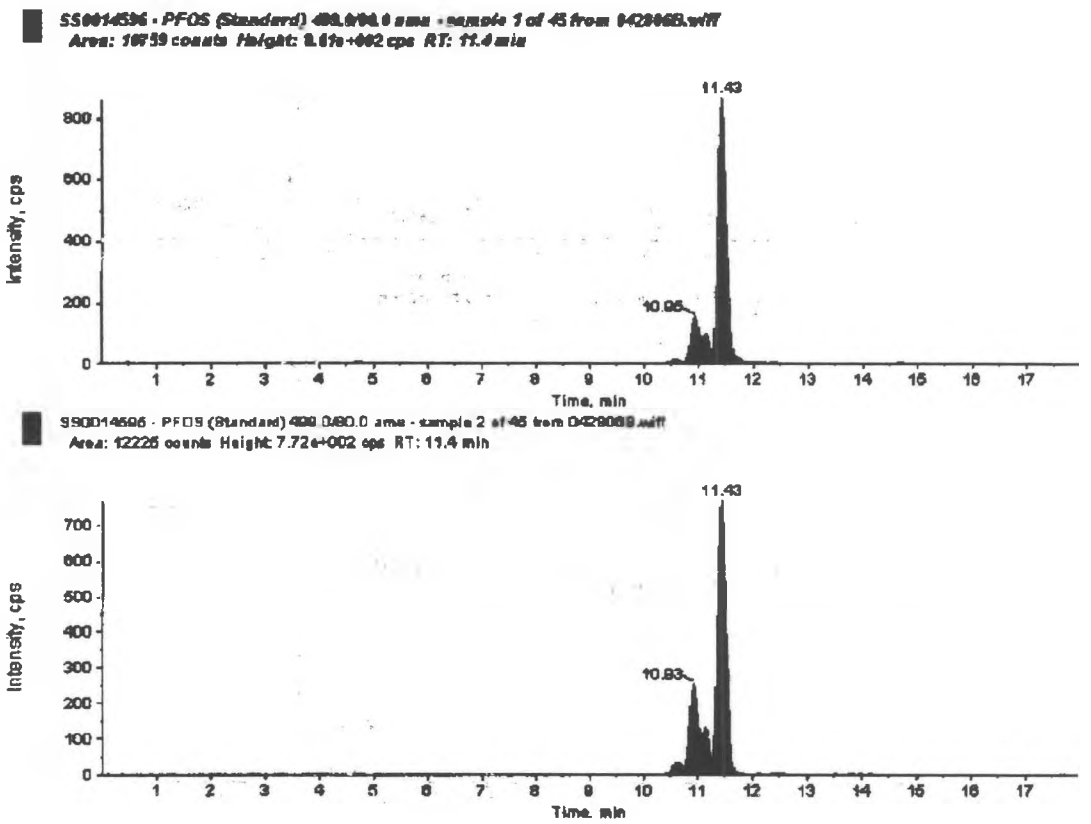


Figure 11. PFOS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

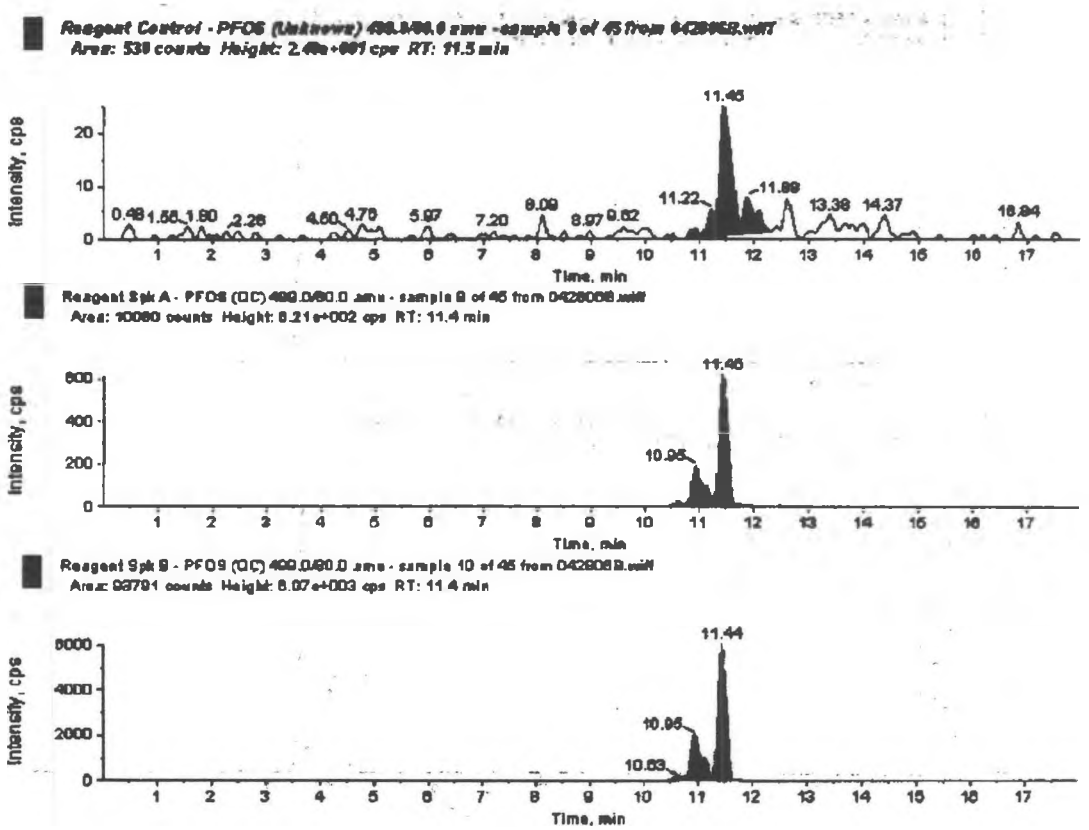
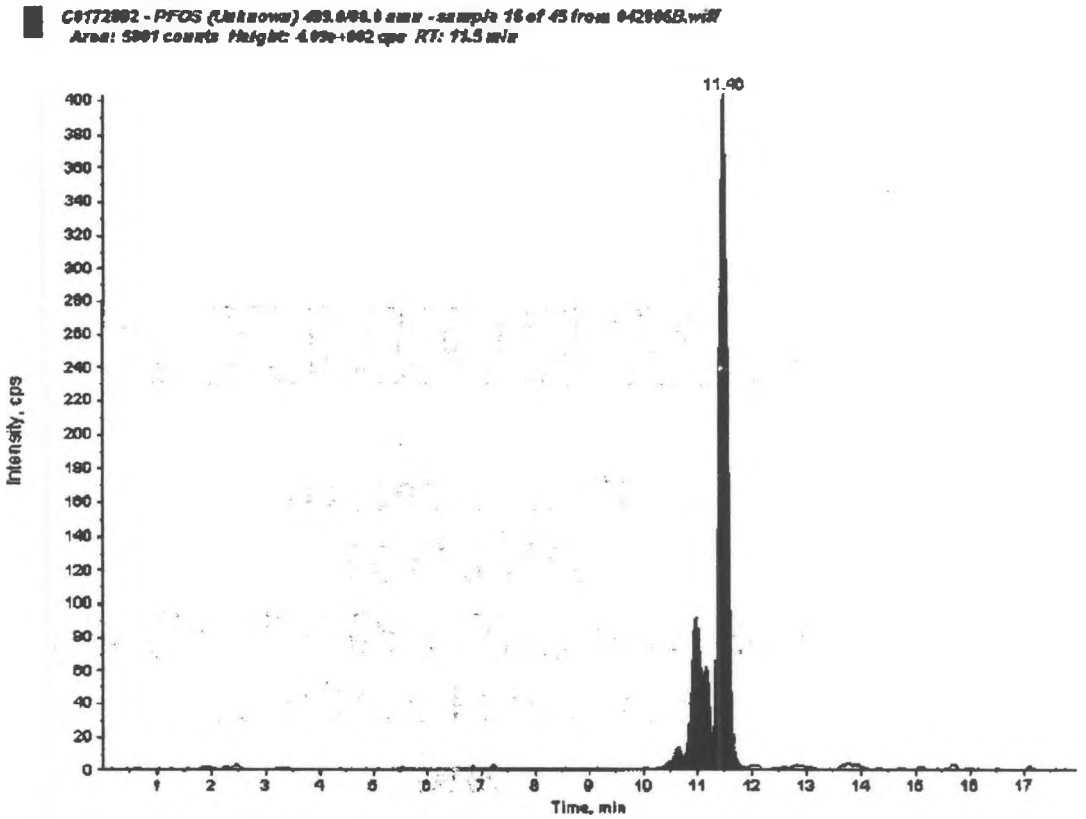


Figure 12. Chromatogram Representing a Sediment Sample Analyzed for PFOS (Exygen ID: C0172892, Data Set:042806B)



APPENDIX A

Study Protocol
P0001131
(Exygen Study No. P0001131) with
Analytical Methods
and
Protocol Amendments

STUDY PROTOCOL

Study Title:

Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

Performing Laboratory:
Exygen Research
3058 Research Drive
State College, PA 16801
Phone: (814) 272-1039

Sponsor Representative:
Michael A. Santoro
Director of Regulatory Affairs
3M Building 0236-01-B-10
St. Paul, MN 55144
Phone: (651) 733-6374

DISTRIBUTION:

1) Jaisimha Kesari, Study Director, Weston Solutions

2) John M. Flaherty, Principal Investigator, Exygen Research

3) Michael A. Santoro, Sponsor Representative, 3M Company

4) Exygen Research Quality Assurance Unit

PROTOCOL APPROVAL

Study Title: Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Livers and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

APPROVALS

Jaisimha Kesari, Study Director
Weston Solutions
11/5/04
Date

Michael A. Santoro, Sponsor Representative
3M Company
11/11/04
Date

John M. Flaherty, Principal Investigator
Exygen Research
11/29/04
Date

Richard A. Grazzini, President, Facility Management
Exygen Research
29-OCT-2004
Date

Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research
11/29/04
Date

TABLE OF CONTENTS

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.

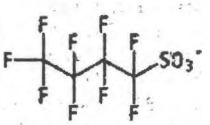
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

The test materials are perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) and are all supplied by 3M.

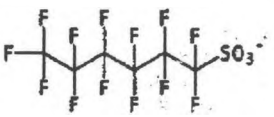
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^-K^+$)
Lot Number: 101
Purity: 96.7%
Transitions Monitored: 299 → 99
Structure:

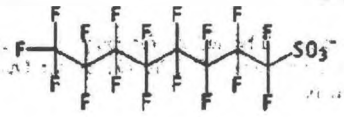


PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3^-K^+$)
Lot Number: SB036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:



PFOS
Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 538 supplied as the potassium salt ($C_8F_{17}SO_3^-K^+$)
Lot Number: 217
Purity: 86.9%
Transitions Monitored: 499 → 99
Structure:



OBJECTIVE

The purpose of this study is to perform analysis for perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHS) and perfluorooctanesulfonate (PFOS) in water, soil, sediment, fish, clams, vegetation, small mammal livers and small mammal serum for the 3M Decatur Monitoring Program using the current versions of the following Exygen analytical methods:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"
V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"
V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"
V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"
V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"
V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"
V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

TESTING FACILITY

Exygen Research
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PRINCIPAL INVESTIGATOR

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Phone: (814) 272-1039
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PROPOSED EXPERIMENTAL START AND TERMINATION DATES

It is proposed that the analytical portion of this study be conducted from October 01, 2004 to December 31, 2005. The actual experimental start and termination dates will be included in the final report.

IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

- Water (groundwater and surface water)
- Soil
- Sediment
- Fish
- Clams
- Vegetation
- Small Mammal Liver
- Small Mammal Serum

The samples will be collected by Weston Solutions. The control samples will be purchased and prepared by the testing facility. Purchase and processing details for the control samples will be included in the final report associated with this study.

The test systems were chosen to access the environmental impact of PFBS, PFHS and PFOS in the Decatur, Alabama area.

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

Water, soil, sediment, fish, clam, vegetation, small mammal liver and small mammal serum samples will be received at Exygen directly from Weston Solutions. The details of sample procurement for this study are outlined in the 3M work plan entitled "Phase 2 Work Plan for Sampling Environmental Media." The number and types of samples collected will vary depending availability in the field. The total number of samples received and analyzed for each matrix will be documented in the final report associated with this study.

Water, soil, and sediment samples will be used as received without further processing at Exygen. These samples will be stored refrigerated at 2°C-8°C. Fish, clam, vegetation and small mammal liver samples will be processed according to the appropriate analytical method (see Appendix I). These samples will be stored frozen at ≤ -10°C. Small mammal whole blood samples will be centrifuged in the field at the time of collection and the serum fraction will be used for the study. Small mammal serum will be stored frozen at ≤ -10°C.

The receipt and processing of the samples will be documented in the final report and raw data associated with the study.

SAMPLE IDENTIFICATION

Prior to analysis, each sample will be assigned a laboratory sample reference number. The reference number will be unique and will distinguish each laboratory sample that is processed throughout the analytical procedure. Chromatographic data will be identified by the laboratory sample reference number.

Sample storage conditions and locations will be documented throughout the study.

ANALYTICAL PROCEDURE SUMMARY

References:

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"

V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"

V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"

V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"

V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"

V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"

V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

The above methods use analytical conditions capable of separating the isomers of PFBS, PFHS and PFOS. The final report will include the isomers summed into total PFBS, total PFHS, and total PFOS found.

VERIFICATION OF ANALYTICAL PROCEDURE

A laboratory control sample will be used for the preparation of fortified control samples. The test substance will be made into solutions as per the method, and added to the matrices via a micropipette.

For water sampling, Exygen will supply one bottle per sample collected. The bottles will be 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottles. These bottles have been routinely used for fluorochemical sample

collection at the testing facility and have been shown to be free of PFBS, PFHS and PFOS. Samples will be added to each container to a volumetric fill line at 200 mL. A field duplicate, a low field spike and a high field spike of each sample will be collected. The low and high field spike bottles will contain PFBS, PFHS and PFOS as well as perfluorooctanoic acid (PFOA) and 1,2-13C perfluorooctanoic acid (^{13}C PFOA). PFOA and ^{13}C PFOA are included in the solutions used to spike the samples. The results for PFOA and ^{13}C PFOA will not be reported in this study. Exygen will supply one field blank (control water) and two field blank spikes (control water fortified with PFBS, PFHS and PFOS at a low and high level) for every twenty samples collected. At the testing facility, each water sample (excluding field duplicates and field spikes) will be extracted in duplicate and will also be fortified at a low and high concentration with PFBS, PFHS and PFOS and processed through the described procedure to determine method accuracy and to check for bias.

For soil, sediment, clams, and vegetation, Exygen will supply one 500 mL precleaned Sci/Spec Premier wide mouth HDPE bottle per sample collected or a zip-seal bag. All containers/bags used for sample collection will be shipped to the sample location. Samples will be added to each container or bag in the field. At the testing facility, each sample will be extracted in duplicate and will also be fortified at a known concentration with PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

For small mammal liver, Exygen will supply a 50 mL polypropylene centrifuge tube. For small mammal serum, Exygen will supply a collection kit for each sample containing serum separator tubes (red top), vacutainers, needle holders and needles, transfer pipettes, and polypropylene tubes. At the testing facility, each liver and serum sample will be extracted in duplicate and will also be fortified at a known concentration with PFBS, PFHS and PFOS at both a low and high level and processed through the described procedure to determine method accuracy and to check for bias.

Low and high spiking levels for each matrix are defined below:

Matrix	Low Spiking Level	High Spiking Level
Water	500 ng/L	5000 ng/L
Soil	4 ng/g	40 ng/g
Sediment	4 ng/g	40 ng/g
Fish	10 ng/g	100 ng/g
Clams	10 ng/g	100 ng/g
Vegetation	10 ng/g	100 ng/g
Small Mammal Liver	10 ng/g	100 ng/g
Small Mammal Serum	10 ng/mL	100 ng/mL

Recoveries are anticipated to be between 70% and 130% of the fortified levels; however, the exact precision and accuracy will be determined by the analysis of the quality control samples described above. A statement of accuracy will be included in the final report.

METHOD FOR CONTROL OF BIAS

Control of bias will be addressed by taking representative sub-samples from a homogeneous mixture of each matrix from untreated control samples, and by analyzing at least two levels of fortifications.

STATISTICAL METHODS

Statistics will be limited to those specified in the subject methods and to the calculation of average recoveries, as applicable.

GLP STATEMENT

All aspects of this study shall be performed and reported in compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792. The final report or data package (supplied to the Sponsor) shall contain a statement that the study was conducted in compliance with current and applicable GLP standards and will outline any deviations in the study from those standards. This statement will be signed by the Study Director and Sponsor Representative.

REPORT

A final report will be prepared by the principal investigator or their designee at the conclusion of the study. The report will include, but will not be limited to, the following:

- The name and address of the Study Director, Sponsor Representative, and of the testing facility.
- A statement of GLP compliance (any related documentation, such as chain-of-custody records, must be in the study records).

- The signed and dated statement by the Exygen Research Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and Management.
- A description of the exact analytical conditions employed in the study. If the subject method was followed exactly, it is necessary to include only a copy of the analytical method. Any modifications to this method will be incorporated into the report. If the method is photo-reduced, the project number and page number must be included on each page.
- Description of the instrumentation used and operating conditions.
- All results from all sets analyzed. Control and fortified samples will be identified and the data table will include sample number and fortification level.
- Representative chromatograms for each analyte in each matrix, including chromatograms of a standard and a control sample, and a chromatogram at a fortification level. The location of the analyte peaks will be clearly identified in all chromatograms.
- All circumstances that may have affected the quality or integrity of the data will be documented in the report.
- Locations where raw data and the final report are to be archived.
- Additions or corrections to the final report shall be in the form of an amendment signed by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and the Sponsor Representative.
- All applicable requirements for reporting of study results as per 40 CFR 792.185.

SAFETY AND HEALTH

- Laboratory personnel will practice good sanitation and health habits.
- Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test or reference substance(s).

AMENDMENTS TO PROTOCOL

All significant changes to the analytical protocol outlined here will be expressed in writing, signed and dated by the Study Director and Sponsor Representative. Amendments usually will be issued prior to initiation of study plan change. However, when a change is required without sufficient time for the issue of a written amendment, that change may be effected verbally with supporting documentation signed and dated by the Study Director and followed with a written amendment as soon as possible. In this case, the effective date of the written amendment will be the date of the documented change. Copies of the signed amendments will be appended to all distributed study plan copies. The original amendment will be maintained with the original study plan. Any deviations from the study plan or from the analytical method as provided will be documented and reported promptly to the Sponsor Representative.

DATA RECORD KEEPING

Records to be maintained include the following (as appropriate):

- Sample tracking sheet(s)
- Sample receipt records, storage history, and chains of custody
- History and preparation of standards (stock, fortification, calibration)
- Description of any modifications to the method
- Instrument run sheets, bench-sheets or logs
- Analytical data tables
- All chromatographic and instrumental conditions
- Sample extraction and analysis dates
- A complete listing of study personnel, signatures and initials
- Chronological presentation of all study correspondence
- Any other documentation necessary for the reconstruction of the study

Chromatograms- All chromatograms will contain the following:

- Sample identification, injection date, arrow or other indication of the area of interest, and injection number corresponding to the run.
- Additionally, fortifications will include the amount of analyte added and the sample number of the sample that was fortified.
- Analytical standard chromatograms will additionally include the concentration (e.g., µg/mL).

- As part of the documentation the following sheets will be included in each analytical set: a run sheet listing the samples to be run in the set, and an instrument conditions sheet describing the instrument type and operating conditions.

QUALITY ASSURANCE

The QA Unit of Exygen Research will inspect the study at intervals adequate to assure compliance with GLP's, and will report the findings of audits to the Study Director, Exygen Management, and the Sponsor Representative.

RETENTION OF DATA AND ARCHIVING

All hard copy raw data, including, but not limited to, the original chromatograms, worksheets, correspondence, and results shall be included with the data package submitted to the Study Director. These will be archived with the original study plan, amendments, final report, and all pertinent information from the Sponsor.

The testing facility shall keep all electronic raw data and any instrument, equipment, and storage logs for the period of time specified in 40 CFR 792.195. An exact copy of the materials submitted to the study director will also be kept at Exygen Research.

Exygen will obtain permission from the study director before discarding or returning samples.

Exygen Protocol Number: P0001131

APPENDIX I

ANALYTICAL METHODS

V0001780: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS"
V0001781: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS"
V0001782: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS"
V0001783: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS"
V0001784: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS"
V0001785: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS"
V0001786: "Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS"

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001700

Method of Analysis for the Determination of PerfluorooctanoicAcid (PFOA) in Water by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research
Date: 10/24/04

John Flaherty
Vice President, Operations, Exygen Research
Date: 10/24/04

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001720

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

1.0 **Scope**

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in water.

2.0 **Safety**

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 **Sample Requirement**

3.1 At least 40 mL of test sample for extraction.
3.2 No sample processing is needed for water samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Any samples containing particles should be centrifuged at ~3000 rpm for ~5 minutes and the supernatant used for the extraction.
3.6 Sample collection procedures will be specified in the sampling plan for this project.

4.0 **Reagents and Standards**

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Ammonium Acetate – A.C.S. Reagent Grade
4.4 Perfluoroctanoic Acid – Sigma-Aldrich

5.0 **Instrument and Equipment**

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.

Page 2 of 7

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 SPE vacuum manifold.
5.13 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Phenomenex RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82305-052150)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	%A	%B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternative solutions may be prepared.

Page 3 of 7

Page 18 of 65

Exygen Protocol Number: P0001131

Exygen Research Method Number: VQ201709

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure, identical to samples.

9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Fortification Volume (mL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ng/L)*	Calibration Standard ID (example)
0	0	40	0	XCmmddyy-0
10	100	40	25	XCmmddyy-1
20	200	40	50	XCmmddyy-2
40	400	40	100	XCmmddyy-3
100	100	40	250	XCmmddyy-4
200	200	40	500	XCmmddyy-5
400	400	40	1000	XCmmddyy-6

* The extracted concentration of the calibration standard is equal to 2x its initial concentration, due to the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

Page 4 of 7

Exygen Research Method Number: VM001T80

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

9.2.3 A zero standard solution (reagent blank) must be processed with each set of standards extracted.

9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using HPLC water) and two reagent controls fortified at known concentrations (lab control spiked to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Measure 40 mL of sample or a portion of sample diluted to 40 mL with water into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 droplets). Do not let column run dry

11.3 Load sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.4 Rinse with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

Page 3 of 7

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

versus calibration standard concentration using MassLynx 1.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the MassLynx 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.

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001720

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

Recovery (%) =

$$\frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

Page 7 of 7

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
3858 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/22/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/22/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: M0001*01

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

1.0 **Scope**

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in soil.

2.0 **Safety**

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 **Sample Requirement**

3.1 At least 15 g of wet 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 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 polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100mL, 100-200mL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Ultrasonic bath.

Page 2 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001781

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

5.14 Wrist-action shaker.

5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Phenomenex RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82565-052130)

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
6.0	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

Exygen Protocol Number: P0001131

Exygen Research Method Number V0301751

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in HPLC water. The calibration standards are processed through the extraction procedure, identical to samples.

9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ppb)	Fortification Volume (µL)	Volume of Purified Control Sample (mL)	Final Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XCnnnddyy-0
10	100	40	25	XCnnnddyy-1
10	200	40	50	XCnnnddyy-2
10	400	40	100	XCnnnddyy-3
100	100	40	250	XCnnnddyy-4
100	200	40	500	XCnnnddyy-5
100	400	40	1000	XCnnnddyy-6

* The estimated concentration of the calibration standard is equal to 5x its initial concentration, due to the concentration of the standard during the extraction (SPE)
XC = extracted conversion standard.

Page x of 7

Page 26 of 63

Exygen Protocol Number: P0001131

Exygen Research Method Number: V000171

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

9.2.3 A zero standard solution (reagent blank) must be prepared with each set of standards extracted.

9.2.4 Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.

9.2.5 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 5 mL of methanol and shake on a wrist action shaker for ~15 minutes.

11.3 Transfer the tubes to an ultrasonic bath and sonicate for ~15 minutes.

11.4 Bring the volume up to 40 mL with water in the 50 mL polypropylene centrifuge tube.

11.5 Centrifuge for ~10 minutes at ~3000 rpm.

11.6 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry.

11.7 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.

11.8 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.9 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample onto 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

Exygen Protocol Number: P0001131

extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Rosner Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/L, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/L)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF}$$

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.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/L)} - \text{analyte found in control (ng/L)}]}{\text{analyte added (ng/L)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/L to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/L)} \times \text{volume extracted (0.04 L)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation to calculate the amount of PFOA found in ppb based on dry weight.

$$\text{PFOA found (ppb) dry weight} = \text{PFOA found (ppb)} \times [100\% / \text{total solids(\%)}]$$

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0081702

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

Analytical Testing Facility: Exygen Research
3521 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V8001752

ANALYTICAL METHOD

Method or Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by: High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in sediment.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 30 g of test sample for extraction.
3.2 No sample processing is needed for sediment samples.
3.3 Samples stored refrigerated should be allowed to equilibrate to room temperature.
3.4 All samples must be thoroughly mixed before being sampled for extraction.
3.5 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetic Acid – Reagent grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL, connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-180uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.

Page 3 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

5.13 Vortexer.

5.14 Wrist-action shaker.

5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 3800 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82105-052130)

6.2 Temperature: 30°C

6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water

6.4 Mobile Phase (B): Methanol

6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).

6.7 Quantitation: Peak Area – external standard calibration curve.

6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Exygen Protocol Number: P0001131

Exygen Research Method Number: VM01782

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 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 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	10	100	10.0
100	5	100	5.0
100	2	100	2.0
10	10	100	1.0
5	10	100	0.5
2	10	100	0.2

Page 4 of 7

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

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 unspiked control and two extracted controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (Jortify as needed, replace lid and mix well).

11.2 Add 35 mL of 1% acetic acid, cap, vortex and shake on a wrist action shaker for ~60 minutes.

11.3 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.4 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drops/sec). Do not let column run dry.

11.5 Load (decant) the sample on the conditioned C_{18} SPE cartridge. Discard eluate.

11.6 Add 20 mL of methanol to the sediment left in the bottom of the 50 mL centrifuge tube. Cap, vortex and shake on a wrist action shaker for ~30 minutes.

11.7 Centrifuge the tubes at ~3000 rpm for ~20 minutes.

11.8 Decant the methanol onto the same SPE cartridge. Collect the eluate.

11.9 Wash the extract with 4 mL of methanol. Collect the eluate and add it to the eluate collected in step 11.8.

11.10 Condition a second C_{18} SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~ 2 drops/sec). Do not let column run dry.

11.11 Add the methanol to ~200 mL of water and load on the second conditioned SPE cartridge.

11.12 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.13 Analyze samples using electrospray LC/MS/MS.

Page 5 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001752

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analytical samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Hugo Ehrer Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

Page 6 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001722

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 ±2.4 % 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 MassLynx 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.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb).

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (5 mL)}]}{\text{sample weight (5 g)}}$$

14.4 Use the following equation (if necessary) to calculate the amount of PFOA found in ppb based on dry weight.

$$\text{PFOA found (ppb) dry weight} = \text{PFOA found (ppb)} \times [100\% / \text{total solids(\%)}]$$

Page 7 of 7

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
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

19/3/2004
Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 8

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in fish and clams.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 20 g of wet sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer storage until time of analysis.
3.3 Sample collection procedure will be specified in the sampling plan for this project.

4.0 Reagents and Standards

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Supelclean LC-NH_2 – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 Instrument and Equipment

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

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 Thermolyne.
5.6 15 mL pear-shaped flasks.
5.7 50 mL disposable polypropylene centrifuge tubes.
5.8 15 mL disposable polypropylene centrifuge tubes.
5.9 Disposable transpipets (50-100uL, 100-200uL).
5.10 175-mL LDPE narrow-mouth bottles.
5.11 2 mL clear HPLC vial kit.
5.12 Disposable pipettes.
5.13 Autopipettes (125-1000 µL and 10-100 µL), with disposable tips.
5.14 SPE tubes (2GmL) (Supelco cat. no. N857177).
5.15 Wrist action shaker.
5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Fluophase RP (Keystone Scientific). 2.1 mm x 50 mm, 5µ (P/N: 82585-852130)
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.

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethylsilichexolane in toluene is prepared by bringing 3 mL of dimethylsilichexolane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001783

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

9.2 **Standard Calibration Solutions**

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.025	10	100	0.0025

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 unfortified control and two unfortified controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 **Sample Extraction**

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (family as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a wrist action shaker for ~15 minutes.

11.3 Place the tubes in a freezer for ~1 hour.

11.4 Pack and condition the SPE tubes and silanize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH_2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the columns to dry.

11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

Page 5 of 6

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

11.7 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.8 Decant the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shape flask.

11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat plug using a tissuemizer for ~30 seconds and rinse the tissuemizer with ~10 mL of acetonitrile into the tube.

11.10 Shake the sample again for ~10 minutes on a wrist-action shaker.

11.11 Place the tubes in a freezer for ~ 1 hour more.

11.12 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.13 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluent from the initial extraction.

11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.

11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).

11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.

11.17 Transfer the extracts to HPLC vials using disposable pipets.

11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards) (in either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

Page 6 of 7

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 Chromatograms must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Exner Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}}$$

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.

Exygen Protocol Number: P0001131

Exygen Research Method Number: V080178

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS

14.3 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}]}{\text{analyte added (ng/g)}} \times 100$$

Page 8 of 8

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001784

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Technical Leader, LC-MS, Exygen Research

Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Denmark Method Number: VE04194

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

1.0 **Scope**

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in vegetation.

2.0 **Safety**

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 **Sample Requirement**

3.1 At least 20 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer storage until time of analysis.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 **Reagents and Standards**

4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Supelclean LC-NH$_2$ – Reagent grade
4.8 1-Octanol – HPLC grade
4.9 L-Ascorbic acid – Reagent grade
4.10 Dimethyldichlorosilane – Reagent grade
4.11 Toluene – Reagent grade
4.12 Ammonium Acetate – A.C.S. Reagent Grade
4.13 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 **Instrument and Equipment**

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL, connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

Page 2 of 7

Page 46 of 65

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001781

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 flasks.
5.6 50 mL disposable polypropylene centrifuge tubes.
5.7 15 mL disposable polypropylene centrifuge tubes.
5.8 Disposable micropipets (50-100uL, 100-200uL).
5.9 125-mL LDPE narrow-mouth bottles.
5.10 2 mL clear HPLC vial kit.
5.11 Disposable pipettes.
5.12 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.13 TPH tubes (20mL) (Bapalon cat. no. N057177).
5.14 Wrist action shaker.
5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm

6.0 Chromatographic System

6.1 Analytical Column: Phenyhexe RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82500-052100)
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.

Page 3 of 7

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 MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

8.2 Extraction Solutions

8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.

8.2.2 30% Dimethylchloroamine in toluene is prepared by bringing 3 mL of dimethylchloroamine to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 1.0 µg/mL fortification solution.

Page 4 of

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
1.0	5.0	100	0.05
1.0	2.5	100	0.025
1.0	1.0	100	0.01
0.05	10	100	0.005
0.025	10	100	0.0025
0.1	10	100	0.001
0.005	10	100	0.0005

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 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 extracted (typically 20 or less) must include at least one unfortified control and two fortified controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).

11.2 Add 30 mL of acetonitrile and shake on a twist action shaker for ~15 minutes.

11.3 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.

11.4 Pack and condition the SPE tubes and siliconize the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH2. Condition the columns with 20 mL of methanol, then 20 mL of acetonitrile. Discard all washes. Do not allow the column to dry.

11.6 Siliconize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

11.7 Elongate the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL siliconized pear-shape flask.

Exygen Protocol Number: P0001131

Exygen Research Method Number: V20011701

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

11.8 Add 20 mL of acetonitrile to the sample in the 50 mL centrifuge tube.

11.9 Shake the sample again for ~10 minutes on a wrist-action shaker.

11.10 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~5 minutes.

11.11 Decant the extract onto the mated SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluant 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% acetatic acid in methanol to the pear-shaped flask and swirl to solubilize.

11.15 Transfer the extracts to HPLC vials using disposable pipets.

11.16 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatograms must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

Page 6 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001731

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Rizer Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations:

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}}$$

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 calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/g)} - \text{analyte found in control (ng/g)}]}{\text{analyte added (ng/g)}} \times 100$$

Page 1 of 1

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001783

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

Analytical Testing Facility: Exygen Research
3668 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC/MS, Exygen Research

10/26/04
Date

John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

1.0 Scope

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal liver.

2.0 Safety

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 Sample Requirement

3.1 At least 2 g of test sample for extraction.
3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in freezer storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in freezer storage until time of analysis. Alternately, if there is an insufficient amount of sample (~less than 5 g), then no processing is necessary and the sample can be used as supplied.
3.3 Sample collection procedures will be specified in the sampling plan for the 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 1-200 µL combined 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 micropipettes (50-100mL, 100-200mL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.

Page 2 of 7

Exygen Protocol Number: P0001131

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 Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Transmitter.
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: Phenyhexe RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82585-652130)
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.

Exygen Protocol Number: P0001131

Exygen Research Method Number: VM001705

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

9.0 Standard Preparation

9.1 Standard Stock/Fortification Solution

9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.

9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.

9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.

9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (ng/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 4°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

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001785

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

10.0 Blank Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one unspiked control and two untreated controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

11.1 Weigh 1 g of sample into a 50 mL polypropylene centrifuge tube (*fortify as needed*, replace lid and mix well). Note that alternate weights of liver may be used depending on the sample size available for use.

11.2 Add water to the sample for a final volume of 10 mL.

11.3 Homogenize sample using a tissuemizer for ~1 minute.

11.4 Triturate 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.

11.5 Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.

11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.

11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

11.8 Condition the C_{18} SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drops/sec). Do not let cartridge run dry.

11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.

11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).

11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.

12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.

12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.

12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

Page 5 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS

versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

12.3 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatograms must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/g, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Rator Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{Intercept}) \times \text{DF} \times \text{aliquot factor}}{\text{slope}}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 10

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001705

ANALYTICAL METHOD

Method 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.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ng/g (ppb):

$$\text{PFOA found (ppb)} = \frac{\text{PFOA found (ng/mL)} \times \text{final volume (mL)}}{\text{sample weight (g)}}$$

Page 7 of 7

Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001786

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

Analytical Testing Facility: Exygen Research
3050 Research Drive
State College, PA 16801

Approved By:

Paul Connelly
Technical Leader, LC MS, Exygen Research

13/2/2004
Date

John Flaherty
Vice President, Operations, Exygen Research

Date

Total Pages: 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001706

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

1.0 **Scope**

This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometric Detector (LC/MS/MS) in small mammal serum.

2.0 **Safety**

2.1 Always observe safe laboratory practices.
2.2 Consult the appropriate MSDS before handling any chemical for proper safety precautions.

3.0 **Sample Requirement**

3.1 At least 1 mL of test sample for extraction.
3.2 No sample processing is needed for serum samples. However, frozen serum samples must to allowed to completely thaw to room temperature before use.
3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 **Reagents and Standards**

4.1 Water – HPLC grade
4.2 Methanol – HPLC grade
4.3 Acetonitrile – HPLC grade
4.4 Ammonium Acetate – A.C.S. Reagent Grade
4.5 Perfluorooctanoic Acid – Sigma-Aldrich

5.0 **Instrument and Equipment**

5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.
5.4 50 mL disposable polypropylene centrifuge tubes.
5.5 15 mL disposable polypropylene centrifuge tubes.
5.6 Disposable micropipets (50-100uL, 100-200uL).
5.7 125-mL LDPE narrow-mouth bottles.
5.8 2 mL clear HPLC vial kit.
5.9 Disposable pipettes.
5.10 Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11 Waters Sep Pak Vac 6 cc (1g) tC18 SPE cartridges.
5.12 SPE vacuum manifold.
5.13 Vortexer.

Page 3 of 7

Exygen Protocol Number: P0001131

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: Phenyhexe RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82565-052130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
6.7 Quantitation: Peak Area – external standard calibration curve.
6.8 Run Time: ~ 23 minutes.

The above conditions are intended as a guide and may be changed in order to optimize the HPLC system.

7.0 MS/MS System

7.1 Mode: Electrospray Negative MRM mode, monitoring 413 → 369 m/z for PFOA.

The above conditions are intended as a guide and may be changed in order to optimize the MS/MS system.

8.0 Preparation of Solutions

8.1 Mobile Phase

8.1.1 2 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

Exygen Protocol Number: P0001131

Exygen Research Method Number V000178a

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 viable 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	5.0	100	5.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 6°C, up to six months.

9.2.4 Alternate volumes and concentrations of standards may be prepared as needed.

10.0 Batch Set Up

10.1 Each batch of samples extracted (typically 20 or less) must include at least one unspiked control and two unspiked controls fortified at known concentrations (lab control spike) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

Page 4 of 7

Page 62 of 63

Exygen Protocol Number: P0001131

Exygen Research Method Number V0001706

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

11.0 Sample Extraction

11.1. Measure 1 mL of sample into a 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well). Note that alternate volumes of serum may be measured depending on the sample size available for use.
11.2 Add water to the sample for a final volume of 20 mL. Cap tightly
11.3 Vortex for ~1 minute.
11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.
11.5. Add 5 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
11.6. Centrifuge the tubes at ~3000 rpm for ~5 minutes.
11.7. Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
11.8 Condition the C_{18} SPE cartridges (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~ 2 drop/sec). Do not let column run dry
11.9 Load the sample on conditioned C_{18} SPE cartridge. Discard eluate.
11.10 Elute with ~2 mL of methanol. Collect 2 mL of eluate into a graduated 15 mL polypropylene centrifuge tube (final volume = 2 mL).
11.11 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a run followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

Page 5 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: VOS01730

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 Chromatograms must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.

13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

13.4 Any calibration standard found to be a statistical outlier by using the Huge Hiter Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

13.5 The correlation coefficient (R) for calibration curves generated must be ≥0.992 (R^2 ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

13.6 Retention times between standards and samples must not drift more than ± 4 % within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

14.1 Use the following equation to calculate the amount of PFOA found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Mass Lynx software program:

$$\text{PFOA found (ng/mL)} = \frac{(\text{Peak area} - \text{intercept})}{\text{slope}} \times \text{DF} \times \text{aliquot factor}$$

DF = factor by which the final volume was diluted, if necessary.
Aliquot factor = 20

14.2 For samples fortified with known amounts of PFOA prior to extraction, use the following equation to calculate the percent recovery.

$$\text{Recovery (\%)} = \frac{[\text{total analyte found (ng/mL)} - \text{analyte found in control (ng/mL)}]}{\text{analyte added (ng/mL)}} \times 100$$

Page 4 of 7

Exygen Protocol Number: P0001131

Exygen Research Method Number: V0001704

ANALYTICAL METHOD

Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS

14.3 Use the following equation to convert the amount of PFOA found in ng/mL to ppb:

$$\text{PFOA found (ppb)} = \frac{[\text{PFOA found (ng/mL)} \times \text{final volume (mL)}]}{\text{sample volume (mL)}}$$

Page 7 of 7

Exygen RESEARCH
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PROTOCOL AMENDMENT

Amendment Number: 1
Effective Date: 01/19/05
Exygen Study Number: P0001131 Client Study Number: None
Page 1 of 1

DESCRIPTION OF AMENDED SECTION

1) Analytical Procedure Summary V0001780:Section 9.1
2) Verification of Analytical Procedure

AMENDED TO

1) Add to Section 9.1: Section 9.1.6, Alternate weights of standards may be used to prepare alternate concentrations of stock solutions as necessary. Alternate levels of fortification solutions may also be prepared.
2) Low and high spiking levels of the analytes for each matrix may be altered depending on sample size available for extraction and/or to cover analyte concentrations expected in the samples.

RATIONALE

1) Higher concentrations of standards need to be prepared in order to spike the sample bottles at higher levels.
2) The sample size available for small mammal liver and serum was smaller than expected. Spiking at the pre-determined levels in the protocol puts the spiked concentration lower than the detection limit. Also, the analyte levels in the ground water samples are expected to greatly exceed the pre-determined spiking levels listed in the protocol. When the levels in the samples greatly exceed the spiking levels, an accurate recovery value cannot be calculated for the QC sample. Higher spiking levels in the bottles will cover the analyte concentrations expected in the water samples.

IMPACT ON STUDY

The LOQ is 100 ng/g for a 0.1 g sample of small mammal liver and is 1000 ng/mL for a 0.01 mL sample of small mammal serum.
Higher levels of spiking for the water samples will ensure that more QC recovery data can be used.

Study Director Signature — Date 3/9/05
Principal Investigator Signature — Date 3/2/05
Safety Director/Management Signature — Date N/A
Sponsor Signature (if required) — Date 3/9/05

Exygen QAU Review 1/25 02/1/05

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Exygen RESEARCH
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039
Fax: 814-231-1580

PROTOCOL AMENDMENT

Amendment Number: 2
Effective Date: 03/07/05
Exygen Study Number P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

1) Report, page 11 of 85
2) Test Materials, page 6 of 85: PFOS transition monitored 499 -> 99.

AMENDED TO

1) Instead of one final report, interim reports will be issued.
2) PFOS transition monitored may also be 499 -> 80.

RATIONALE

1) Due to the excessive sizes of the data sets, interim reports will be issued to allow the client to receive data in a timelier manner.
2) The API 4000 LC/MS/MS systems detect the 499 -> 80 PFOS transition with greater sensitivity than the 499 -> 99 transition.

IMPACT ON STUDY

1) The client will be able to receive and review the data more quickly.
2) The 499 -> 80 transition can be detected with greater sensitivity; therefore, giving better chromatography.

Study Director Signature	Date 3/9/05
Principal Investigator Signature	Date 3/4/05
Study Director Management Signature	Date
Exygen Management Signature	Date 8-MAR-05
Sponsor Signature (if required)	Date 3/10/05

Exygen QAU Review LJJ 03/08/05

LIBRARY ID: V0001228-8 ADMINISTRATIVE FORM

Exygen RESEARCH
3058 Research Drive Phone: 814-272-1039
State College, PA 16801 Fax: 814-231-1580

DEVIATION FORM

General:
X Project Specific Deviation ___ Facility Deviation Date of Occurrence: 04/26/06

Exygen Project # : P760/P1131 Deviation # : 5

Client Project # : NA Reference # : 08-076

Regulatory Driver:
___ GMP
X GLP
___ Other
___ None

Deviation Type: *(Include V# for methods and SOPs)*
X Protocol ___ Method ___ SOP
V#: NA
Notebook reference: NA

Sample Description:
Login # : L0008191 Container # : NA Lot # : NA

Summary of Deviation:
The three sediment samples in L8191 (C0172892 – C0172894) were originally extracted using the sediment method V00D1782. Poor recoveries were obtained for PFOS, PFOA and ^{13}C PFOA. Because of this, the study sponsor requested the use of an alternative extraction for these compounds, as follows:

Direct Injection Method: Before the samples were weighed for the extraction, they were mixed thoroughly by vigorously shaking the container. A one-gram portion of sediment was weighed into a 15-milliliter centrifuge tube for the extraction. Ten milliliters of 1% acetic acid in methanol was added to each sample. The samples were then shaken by hand, vortexed, and sonicated for thirty minutes. The samples were then centrifuged for ~10 minutes at ~3000 rpm. Each sample was analyzed by LC/MS/MS electrospray.

Using this method acceptable data was obtained for PFOS, but the recoveries for PFOA and ^{13}C PFOA were still poor. Another alternative method was then used for PFOA and ^{13}C PFOA, as follows:

Alternative SPE Method: The samples that were prepared in 1% acetic acid for the direct injection method were used for this extraction. Five milliliters of each sample was aliquoted into a 50-mL polypropylene centrifuge tube and the volume was taken to 40 mL with water. The samples were then centrifuged for ~10 minutes at ~3000 rpm. The supernatant was then loaded onto a C_{18} SPE cartridge conditioned with 10 mL of methanol and 5 mL of water. The eluate was discarded. Approximately five milliliters of methanol was added to the cartridge. Five milliliters of eluate was collected into a graduated 15 mL polypropylene centrifuge tube. Each sample was analyzed by LC/MS/MS electrospray.

Cause: ___ Preparation ___ Analysis ___ Instrument ___ Client Request X Other

Impact:
More usable data was obtained.

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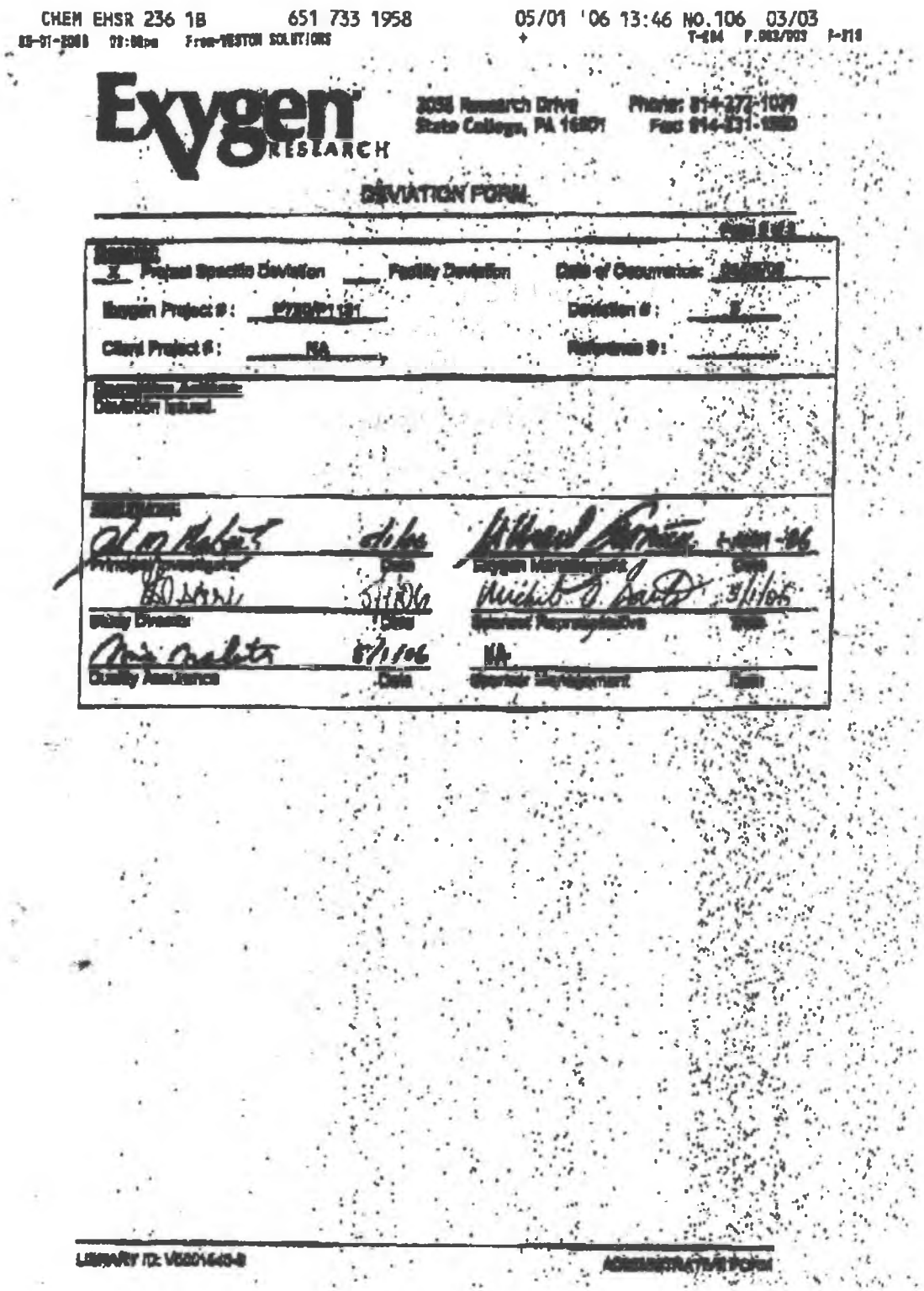

CHEM EHSR 236 1B 651 733 1958 05/01 '06 13:46 NO.106 03/03

Exygen RESEARCH

DEVIATION FORM

Project Specific Deviation ___ Facility Deviation

Exygen Project #: P0001131

Deviation #: 1

Client Project #: NA

Reference #:

Deviation Issued

Date

Date

Date

Date

Date

Date

Date

Date

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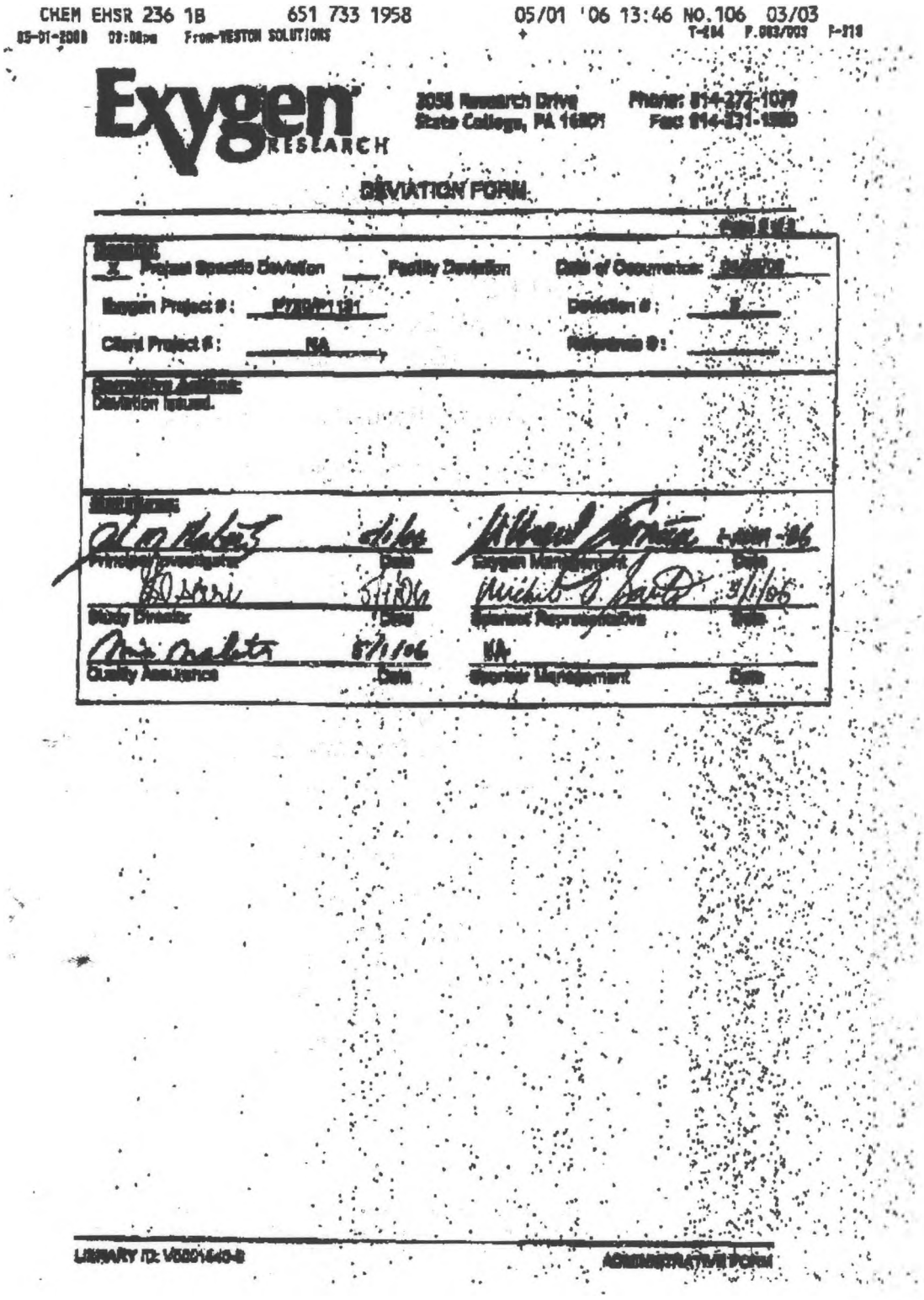

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Exygen RESEARCH

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DEVIATION FORM

Page 1 of 1

Type:
X Project Specific Deviation ___ Facility Deviation Date of Occurrence: 5/1/06

Exygen Project #: P0001131 Deviation #: 1

Client Project #: NA Reference #:

Description/Justification:
Deviation Issued.

Signatures:

Principal Investigator — Date: 5/1/06
Exygen Management — Date: 5/1/06
Study Director — Date: 5/1/06
Sponsor Representative — Date:
Quality Assurance — Date: 5/1/06
Sponsor Management — Date: NA

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