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Office of Pollution Prevention and Toxics
U.S. EPA
1200 Pennsylvania Avenue NW
Washington, DC 20460-0001

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8EHQ-80-374

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:



296281

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.

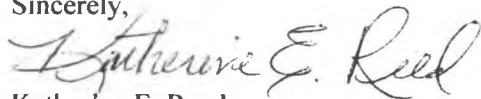
There are a two additional items pertaining to Report No. E06-0199 that merit discussion. First, the report represents a subreport for a series of samples collected adjacent to, but off of, the 3M Decatur facility. Analytical results for additional off-site samples are pending but not final at this time. 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

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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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.
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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.



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

Certificate #2052-01

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 quantitation (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	ETSSan
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 suitability 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.

Sample Description	Lab ID	PFOA			PFOS			PFHS			PFBS		
		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 $100 \pm 25\%$; however, the recovery was included in the calculation of the overall average and %RSD.
- (5) Recovery was outside the method acceptance criteria of $100 \pm 25\%$; 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:

$$101 \times (0.097) = 9.84$$

$$101 + 9.84 = 111.1; 101 - 9.84 = 91.45$$

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.

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

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 $100\pm 30\%$ 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 $100\pm 30\%$ 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 $100\pm 30\%$ 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 $100\pm 30\%$ 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 $100\pm 30\%$ 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 $100\pm 30\%$ (134%). However, the PFBS results for this sample are still considered accurate within $100\pm 30\%$ because the criteria exceedance 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 $100\pm 30\%$ 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, PFHS, 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 $100\pm 30\%$ 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 $100\pm 30\%$ 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.

3M LIMS ID	Description	PFOA		PFOS		PFHS		PFBS	
		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.

3M LIMS ID	Description	PFOA		PFOS		PFHS		PFBS	
		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.

3M LIMS ID	Description	PFOA		PFOS		PFHS		PFBS	
		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.

3M LIMS ID	Description	PFOA		PFOS		PFHS		PFBS	
		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	63.5	388	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.

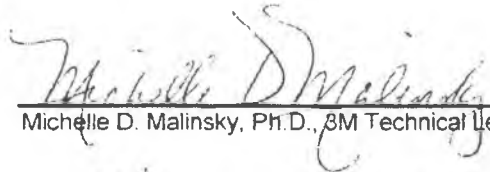
5 Conclusion

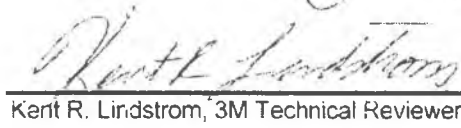
Sample matrix QC samples for the four samples reported herein did not meet method acceptance criteria of $100\pm30\%$ for PFOS. ***Therefore, the values listed in Table 1 and Tables 7-10 for PFOS should be considered estimated minimums. The sample analytical accuracy is arbitrarily estimated to be within 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 $100\pm30\%$ 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 Table 1. ***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.***

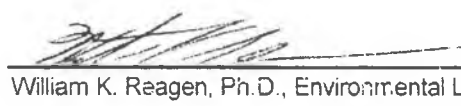
6 Data / Sample Retention

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
5/1/06
Date


Kerit R. Lindstrom, 3M Technical Reviewer
05/01/06
Date


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

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


Quality Assurance Representative
5-1-06
Date

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.

File	Vial Position	Area Counts			
		PF2A	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
1/2*LOQ Standard		81687	7300	6588	12883

(1) Area counts of the solvent blank were greater than 1/2*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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Phone: 814-272-1039

STUDY SPONSOR

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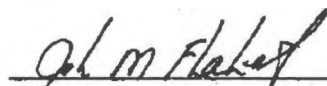
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Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 132

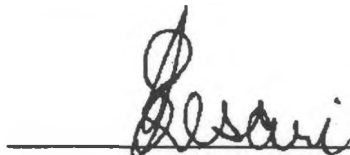
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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.



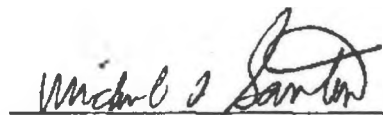
John Flaherty
Principal Investigator
Exygen Research

5/9/06
Date



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

5/10/06
Date



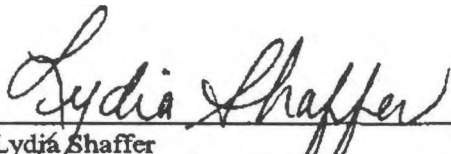
Michael A. Santoro
Sponsor Representative
3M Company

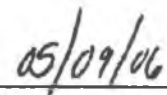
5/12/06
Date

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 phases¹ were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

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


Lydia Shaffer
Technical Lead, Quality Assurance Unit


Date

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

CERTIFICATION OF AUTHENTICITY

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

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

Principal Investigator, Exygen:



John Flaherty
Vice President
Exygen Research

5/9/06

Date

Exygen Research Facility Management:

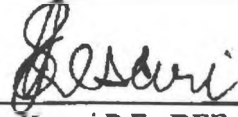


Richard A. Grazzini
President
Exygen Research

9-11-2006

Date

Study Director, Weston Solutions, Inc.

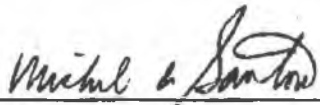


Jaisimha Kesari P.E., DEE
Weston Solutions, Inc.

5/10/06

Date

Sponsor Representative, 3M Company:



Michael A. Santoro
Director of Regulatory Affairs

5/12/06

Date

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
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PERFORMING LABORATORY: Exygen Research
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State College, PA 16801

ANALYTICAL PHASE
TIMETABLE: Study Initiation Date: 11/05/04
Interim Analytical Start Date: 12/27/04
Interim Analytical Termination Date: 04/17/06
Interim Report Completion Date: 05/09/06

PROJECT PERSONNEL

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

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

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1.0 SUMMARY

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

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

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

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

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

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

The second string defines the general category of the biota sample and the sampling round where:

F01 = Fish sample first round

I01 = Invertebrate sample first round

The third string defines the species (first two characters), the sample type (third character), and the sample number (last three characters) within that sampling area and type of sample where:

IP = channel catfish (*Ictalurus punctatus*)

MS = largemouth bass (*Micropterus salmoides*)

CF = Asiatic clam (*Corbicula fluminea*)

F = Filet tissue sample

W = Whole body sample

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

The fourth string (single character) additionally defines the sample type where:

0 = Primary field sample

1 = Field duplicate sample

2 = Equipment rinse/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.

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

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

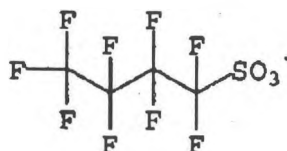
PFBS

Chemical Name: Perfluorobutanesulfonate

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

Transitions Monitored: 299 → 99

Structure:

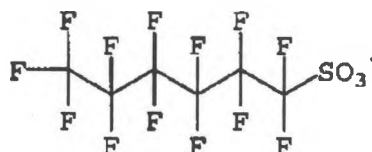
**PFHS**

Chemical Name: Perfluorohexanesulfonate

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

Transitions Monitored: 399 → 80

Structure:

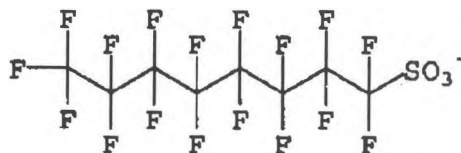
**PFOS**

Chemical Name: Perfluorooctanesulfonate

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

Transitions Monitored: 499 → 80

Structure:



6.0 DESCRIPTION OF ANALYTICAL METHOD

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

6.1 Extraction Procedure

6.1.1 *Sample Preparation*

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

6.1.2 *Glassware Preparation*

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

6.1.3 *Column Preparation*

The 20 mL columns were packed at Exygen in sequence with 2 grams florisil, 2 grams silica gel, 2 grams of carbon, and 1 gram LC-NH₂. The columns were conditioned on the day of extraction with 20 mL of methanol followed by 20 mL of acetonitrile. All washes were discarded. These clean-up columns were used to remove matrix interference and not to retain PFBS, PFHS, and PFOS. Additional details about the column packing materials can be found in the raw data package associated with this report.

6.1.4 *Sample Extraction*

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

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

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

6.2 Preparation of Standards and Fortification Solutions

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

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

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

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

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

¹ of PFBS, PFHS and PFOS

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

6.3 Chromatography

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

6.4 Instrument Sensitivity

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

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

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

HP Column Oven

HPLC Column: Thermo Fluophase RP, 50 mm x 2.1 mm

Column Temp.: ~30° C

Injection Vol.: 15 µL

Mobile Phase (A): 2 mM Ammonium Acetate in water

Mobile Phase (B): Methanol

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

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

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

6.6 Quantitation and Example Calculation

Fifteen microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x fit weighted linear regression) by Analyst software using six concentrations of standards. The concentration was determined from the following equations.

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

Equation 1:

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

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

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

Equation 2:

Analyte found (ppb, wet weight) =

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

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

Equation 3:

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

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

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

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

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

From equation 1:

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

From equation 2:

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

From equation 3:

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

7.0 EXPERIMENTAL DESIGN

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

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

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

8.0 RESULTS

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

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

9.0 CONCLUSIONS

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

10.0 RETENTION OF DATA AND SAMPLES

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

TABLES

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048126	DL3-F01-IPF001-0-041021	0.308	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*	0.362	40	ND	30	4.56	40
C0048128	DMC-F01-IPF001-0-041021	0.330	50	ND	50	7.40	50
C0048128 Rep	DMC-F01-IPF001-0-041021*	0.366	50	ND	50	6.36	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	0.968	50	ND	30	34.4	30
C0048131 Rep	DMC-F01-IPW002-0-041021*	1.34	50	ND	30	34.2	30
C0048132	DMC-F01-MSW001-0-041021	0.218	30	0.524	30	270	30
C0048132 Rep	DMC-F01-MSW001-0-041021*	0.202	30	0.484	30	280	30
C0048133 ^a	DMC-F01-MSW002-0-041021	ND ^a	30	ND ^a	30	530	30
C0048133 Rep ^a	DMC-F01-MSW002-0-041021*	ND ^a	30	ND ^a	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	2.06	30
C0048137	DL2-F01-IPF004-0-041021	0.346	40	ND	30	4.52	40
C0048137 Rep	DL2-F01-IPF004-0-041021*	0.330	40	ND	30	4.12	40
C0048138	DL2-F01-IPF005-0-041021	0.338	40	ND	30	2.46	30
C0048138 Rep	DL2-F01-IPF005-0-041021*	0.318	40	ND	30	2.60	30
C0048139	DL2-F01-IPW001-0-041021	0.326	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

^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.

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048144	DL1-F01-IPF001-0-041021	0.432	30	0.261	30	33.4	40
C0048144 Rep	DL1-F01-IPF001-0-041021*	0.428	30	0.252	30	32.8	40
C0048145	DL3-F01-IPF003-0-041021	0.448	30	ND	30	3.55	30
C0048145 Rep	DL3-F01-IPF003-0-041021*	0.440	30	0.228	30	3.35	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*	1.38	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	0.696	50	0.844	30	NR	NR
C0048150 Rep	DBC-F01-IPF003-0-041021*	0.652	50	0.824	30	NR	NR
C0048151	DBC-F01-IPW001-0-041021	0.864	40	2.21	30	NR	NR
C0048151 Rep	DBC-F01-IPW001-0-041021*	0.508	40	2.20	30	NR	NR
C0048152	DBC-F01-IPW002-0-041021	0.700	40	9.12	30	NR	NR
C0048152 Rep	DBC-F01-IPW002-0-041021*	0.736	40	8.96	30	NR	NR
C0048153	DBC-F01-IPW003-0-041021	0.736	30	6.76	30	NR	NR
C0048153 Rep	DBC-F01-IPW003-0-041021*	0.760	30	7.44	30	NR	NR
C0048154	DOU-F01-IPF001-0-041021	4.38	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	6.78	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	8.12	30	2.30	30	NR	NR
C0048157 Rep	DOU-F01-IPF004-0-041021*	8.28	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	0.882	30	NR	NR
C0048159	DOU-F01-IPW001-0-041021	2.89	30	0.948	30	NR	NR
C0048159 Rep	DOU-F01-IPW001-0-041021*	2.81	30	0.964	30	NR	NR
C0048160	DOU-F01-IPW002-0-041021	18.4	50	3.69	30	NR	NR
C0048160 Rep	DOU-F01-IPW002-0-041021*	19.3	50	3.70	30	NR	NR
C0048161	DL2-F01-MSW001-0-041021	0.203	30	ND	30	120	50
C0048161 Rep	DL2-F01-MSW001-0-041021*	ND	30	ND	30	120	50
C0048162	DL2-F01-MSW002-0-041021	ND	30	ND	30	102	30
C0048162 Rep	DL2-F01-MSW002-0-041021*	ND	30	ND	30	100	30

*Laboratory Duplicate

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

NR = Not reported due to quality control result failures.

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0048163	DL2-F01-MSW003-0-041021	ND	30	ND	30	92.4	30
C0048163 Rep	DL2-F01-MSW003-0-041021*	ND	30	ND	30	91.6	30
C0048164	DL2-F01-MSW004-0-041021	ND	30	ND	30	88.0	30
C0048164 Rep	DL2-F01-MSW004-0-041021*	ND	30	ND	30	94.8	30
C0048165	DL2-F01-MSW005-0-041021	ND	30	ND	30	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	3.36	30	NR	NR
C0049452	DBC-F01-MSW003-0-041104	0.880	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	8.72	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	2.16	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	3.48	30	7.12	30	NR	NR
C0049462 Rep	DOU-F01-IPW004-0-041104*	3.11	30	6.84	30	NR	NR
C0049463	DOU-F01-IPW005-0-041104	2.97	30	5.12	30	NR	NR
C0049463 Rep	DOU-F01-IPW005-0-041104*	2.90	30	5.32	30	NR	NR
C0049464	DL2-F01-IPW002-0-041104	1.04	30	ND	30	8.20	40
C0049464 Rep	DL2-F01-IPW002-0-041104*	0.980	30	ND	30	6.80	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

*Laboratory Duplicate

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
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	0.580	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	0.808	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	0.632	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	0.568	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	0.616	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	DL1-F01-IPF003-0-041104	ND	30	0.265	30	20.9	30
C0049480 Rep	DL1-F01-IPF003-0-041104*	ND	30	0.262	30	21.4	30
C0049481	DL1-F01-IPF004-0-041104	ND	30	ND	30	7.60	30
C0049481 Rep	DL1-F01-IPF004-0-041104*	ND	30	ND	30	7.36	30
C0049482	DL1-F01-IPF005-0-041104	ND	30	0.373	30	8.72	30
C0049482 Rep	DL1-F01-IPF005-0-041104*	ND	30	0.366	30	8.56	30
C0049483	DL3-F01-MSW001-0-041105	ND	30	ND	30	136	30
C0049483 Rep	DL3-F01-MSW001-0-041105*	ND	30	ND	30	137	30
C0049484	DL3-F01-MSW002-0-041105	ND	30	ND	30	180	30
C0049484 Rep	DL3-F01-MSW002-0-041105*	ND	30	ND	30	182	30

*Laboratory Duplicate

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049485	DL3-F01-MSW003-0-041105	ND	30	ND	30	148	30
C0049485 Rep	DL3-F01-MSW003-0-041105*	ND	30	ND	30	144	30
C0049486	DL3-F01-MSW004-0-041105	ND	30	0.285	30	198	30
C0049486 Rep	DL3-F01-MSW004-0-041105*	ND	30	0.282	30	199	30
C0049487	DL3-F01-MSW005-0-041105	ND	30	0.366	30	173	30
C0049487 Rep	DL3-F01-MSW005-0-041105*	ND	30	0.341	30	168	30
C0049488	DL3-F01-MSF001-0-041105	ND	30	ND	30	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	0.366	30	68.8	30
C0049494 Rep	DMC-F01-IPW004-0-041105*	0.345	30	0.464	30	69.6	30
C0049495	DMC-F01-IPW005-0-041105	ND	30	0.270	30	64.4	30
C0049495 Rep	DMC-F01-IPW005-0-041105*	ND	30	0.255	30	62.8	30
C0049496	DMC-F01-MSW003-0-041105	ND	30	0.582	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	0.980	30	NR	NR
C0049498 Rep	DMC-F01-MSW005-0-041105*	ND	30	0.936	30	NR	NR
C0049499	DMC-F01-MSF002-0-041105	ND	30	ND	30	NR	NR
C0049499 Rep	DMC-F01-MSF002-0-041105*	ND	30	0.367	30	NR	NR
C0049500	DMC-F01-MSF003-0-041105	ND	30	ND	30	56.8	40
C0049500 Rep	DMC-F01-MSF003-0-041105*	ND	30	ND	30	66.0	40
C0049501	DMC-F01-MSF004-0-041105	ND	30	0.254	30	116	30
C0049501 Rep	DMC-F01-MSF004-0-041105*	ND	30	0.228	30	119	30
C0049502	DMC-F01-MSF005-0-041105	ND	30	0.420	30	146	30
C0049502 Rep	DMC-F01-MSF005-0-041105*	ND	30	0.448	30	147	30
C0049503	DMC-F01-IPF003-0-041105	ND	30	ND	30	7.92	30
C0049503 Rep	DMC-F01-IPF003-0-041105*	ND	30	ND	30	7.92	30

*Laboratory Duplicate

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

NR = Not reported due to quality control result failures.

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0049504	DMC-F01-IPF004-0-041105	ND	30	ND	30	44.0	30
C0049504 Rep	DMC-F01-IPF004-0-041105*	ND	30	ND	30	45.6	30
C0049505	DMC-F01-IPF005-0-041105	ND	30	ND	30	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-IPW006-0-041215*	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	10.8	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	6.44	40
C0056465 Rep	DL3-F01-IPW003-0-041130*	ND	30	ND	30	5.26	40
C0056466	DOU-F01-MSF003-0-041216	1.72	30	6.56	30	NR	NR
C0056466 Rep	DOU-F01-MSF003-0-041216*	1.73	30	6.68	30	NR	NR
C0056467	DBC-F01-MSF005-1-041216	0.220	30	1.79	30	NR	NR
C0056467 Rep	DBC-F01-MSF005-1-041216*	ND	30	1.76	30	NR	NR
C0056468	DBC-F01-MSF006-0-041216	ND	30	1.68	30	NR	NR
C0056468 Rep	DBC-F01-MSF006-0-041216*	ND	30	1.59	30	NR	NR
C0056469	DOU-F01-MSF002-0-041216	0.888	30	4.56	30	NR	NR
C0056469 Rep	DOU-F01-MSF002-0-041216*	0.872	30	4.56	30	NR	NR
C0056470	DL3-F01-IPW001-0-041130	ND	30	0.246	30	9.04	30
C0056470 Rep	DL3-F01-IPW001-0-041130*	ND	30	0.219	30	8.80	30

*Laboratory Duplicate

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

NR = Not reported due to quality control result failures.

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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS <i>Perfluorobutanesulfonate</i>		C6 Sulfonate PFHS <i>Perfluorohexanesulfonate</i>		C8 Sulfonate PFOS <i>Perfluorooctanesulfonate</i>	
		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-MSF002-1-041218	0.840	30	4.88	30	NR	NR
C0056471 Rep	DOU-F01-MSF002-1-041218*	0.880	30	4.72	30	NR	NR
C0056472	DOU-F01-MSF004-0-041218	0.792	30	2.35	40	NR	NR
C0056472 Rep	DOU-F01-MSF004-0-041218*	0.816	30	2.22	40	NR	NR
C0056473	DOU-F01-MSF001-0-041218	2.18	30	10.9	30	NR	NR
C0056473 Rep	DOU-F01-MSF001-0-041218*	2.28	30	10.7	30	NR	NR
C0056474	DBC-F01-MSF003-0-041218	ND	30	2.08	30	NR	NR
C0056474 Rep	DBC-F01-MSF003-0-041218*	ND	30	2.08	30	NR	NR
C0056475	DBC-F01-MSF004-0-041218	ND	30	3.87	30	NR	NR
C0056475 Rep	DBC-F01-MSF004-0-041218*	ND	30	3.77	30	NR	NR
C0056476	DMC-F01-MSF001-0-041218	ND	30	0.420	30	NR	NR
C0056476 Rep	DMC-F01-MSF001-0-041218*	ND	30	0.456	30	NR	NR
C0056477	DL1-F01-IPW005-0-041218	0.253	30	0.644	30	88.8	30
C0056477 Rep	DL1-F01-IPW005-0-041218*	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	1680	50
C0048154	DOU-F01-IPF001-0-041021	596	30
C0048155	DOU-F01-IPF002-0-041021	838	30
C0048158	DOU-F01-IPF005-0-041021	544	30
C0048180	DOU-F01-IPW002-0-041021	1210	40
C0049450	DBC-F01-MSW001-0-041104	9480	40
C0049451	DBC-F01-MSW002-0-041104	7520	30
C0049452	DBC-F01-MSW003-0-041104	12800	30
C0049453	DBC-F01-MSW004-0-041104	3440	30
C0049454	DBC-F01-MSW005-0-041104	5240	30
C0049455	DBC-F01-MSF001-0-041104	5240	30
C0049456	DBC-F01-MSF002-0-041104	4760	30
C0049458	DBC-F01-IPF005-0-041104	808	30
C0049459	DBC-F01-IPW004-0-041104	1230	40
C0049460	DBC-F01-IPW005-0-041104	2620	30
C0049461	DOU-F01-IPW003-0-041104	NR	NR
C0049462	DOU-F01-IPW004-0-041104	1740	30
C0049463	DOU-F01-IPW005-0-041104	2130	30
C0049465	DL1-F01-MSW001-0-041104	988	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	580	30
C0049499	DMC-F01-MSF002-0-041105	620	30
C0056456	DOU-F01-MSW002-0-041216	28200	60
C0056458	DOU-F01-MSW003-0-041216	4040	30
C0056460	DOU-F01-MSW001-0-041216	NR	NR
C0056461	DOU-F01-MSW004-0-041216	5120	30
C0056462	DOU-F01-MSW005-0-041216	496	30
C0056463	DOU-F01-MSF005-0-041216	720	30
C0056466	DOU-F01-MSF003-0-041216	848	30
C0056467	DBC-F01-MSF005-1-041216	NR	NR
C0056468	DBC-F01-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

Exygen ID	Client Sample ID	C4 Sulfonate PFBS Perfluorobutanesulfonate		C6 Sulfonate PFHS Perfluorohexanesulfonate		C8 Sulfonate PFOS Perfluorooctanesulfonate	
		Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)	Analyte Found (ng/g) wet wt.	Assessed Accuracy (+/- %)
C0056480	DL3-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	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 (%)
DL3-F01-IPF001-0-041021 (C0048126 Spk C, 16 ppb)	10	0.309	6.56	63	ND	8.28	83	5.16	12.8	74
DL3-F01-IPF001-0-041021 (C0048126 Spk F, 160 ppb)	100	0.309	62.4	62	ND	73.2	73	5.16	78.4	73
DL3-F01-IPF002-0-041021 (C0048127 Spk D, 16 ppb)	10	0.265	6.52	63	ND	7.48	75	4.16	10.3	61
DL3-F01-IPF002-0-041021 (C0048127 Spk G, 160 ppb)	100	0.265	66.8	66	ND	79.8	80	4.16	60.8	77
DMC-F01-IPF001-0-041021 (C0048126 Spk E, 16 ppb)	10	0.330	4.48	42	ND	5.24	52	7.40	9.96	26
DMC-F01-IPF001-0-041021 (C0048126 Spk H, 160 ppb)	100	0.330	66.4	66	ND	74.8	75	7.40	81.8	74
DMC-F01-IPF002-0-041021 (C0048126 Spk C, 16 ppb)	10	0.712	7.68	70	ND	8.60	88	14.1	29.8	156
DMC-F01-IPF002-0-041021 (C0048126 Spk H, 160 ppb)	100	0.712	78.0	77	ND	98.0	98	14.1	104	90
DMC-F01-IPW001-0-041021 (C0048130 Spk D, 16 ppb)	10	1.08	7.60	35	ND	7.84	78	70.0	71.8	*
DMC-F01-IPW001-0-041021 (C0048130 Spk I, 160 ppb)	100	1.08	64.8	64	ND	80.0	80	70.0	131	81
DMC-F01-IPW002-0-041021 (C0048131 Spk E, 16 ppb)	10	0.888	6.76	58	ND	7.86	80	34.4	44.8	*
DMC-F01-IPW002-0-041021 (C0048131 Spk J, 160 ppb)	100	0.888	80.8	60	ND	74.8	76	34.4	101	67
DMC-F01-MSW001-0-041021 (C0048132 Spk E, 16 ppb Spikes)	10	0.216	9.76	96	0.524	9.44	89	270	270	*
DMC-F01-MSW001-0-041021 (C0048132 Spk F, 160 ppb Spikes)	100	0.216	81.2	81	0.524	93.2	93	270	375	106
DMC-F01-MSW002-0-041021 ^A (C0048133 Spk G, 50 ppb Spikes)	50	ND ^A	45.2	90	ND ^A	52.0	104	530	470	*
DMC-F01-MSW002-0-041021 ^A (C0048133 Spk H, 600 ppb Spikes)	500	ND ^A	432	86	ND ^A	482	96	530	924	79
DL2-F01-IPF001-0-041021 (C0048134 Spk F, 16 ppb Spikes)	10	0.680	5.80	51	ND	6.56	66	3.45	8.36	49
DL2-F01-IPF001-0-041021 (C0048134 Spk K, 160 ppb Spikes)	100	0.680	46.4	46	ND	58.8	59	3.45	56.4	53
DL2-F01-IPF002-0-041021 (C0048136 Spk G, 16 ppb Spikes)	10	0.816	5.84	46	ND	6.40	64	4.72	6.56	38
DL2-F01-IPF002-0-041021 (C0048136 Spk L, 160 ppb Spikes)	100	0.816	64.8	54	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				C8 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 (C0048138 Spk C, 10 ppb Spikes)	10	0.247	7.00	68	ND	7.68	80	2.01	9.20	72
DL2-F01-IPF003-0-041021 (C0048138 Spk H, 100 ppb Spikes)	100	0.247	82.8	63	ND	73.2	73	2.01	74.0	72
DL2-F01-IPF004-0-041021 (C0048137 Spk D, 10 ppb Spikes)	10	0.346	6.84	65	ND	7.60	76	4.52	11.3	68
DL2-F01-IPF004-0-041021 (C0048137 Spk L, 100 ppb Spikes)	100	0.346	67.6	67	ND	76.8	77	4.52	60.4	76
DL2-F01-IPF005-0-041021 (C0048138 Spk E, 10 ppb Spikes)	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 Spikes)	100	0.338	68.4	68	ND	80.8	81	2.46	82.0	80
DL2-F01-IPW001-0-041021 (C0048139 Spk F, 10 ppb Spikes)	10	0.326	7.04	67	ND	7.60	76	5.86	13.9	80
DL2-F01-IPW001-0-041021 (C0048139 Spk K, 100 ppb Spikes)	100	0.326	66.0	66	ND	77.2	77	5.86	65.2	79
DL2-F01-MSF001-0-041021 (C0048140 Spk G, 10 ppb Spikes)	10	0.333	7.36	70	ND	8.20	82	77.2	78.0	*
DL2-F01-MSF001-0-041021 (C0048140 Spk L, 100 ppb Spikes)	100	0.333	64.8	64	ND	77.8	78	77.2	158	81
DL2-F01-MSF002-0-041021 (C0048141 Spk C, 10 ppb Spikes)	10	0.408	6.68	65	ND	7.48	75	94.8	101	*
DL2-F01-MSF002-0-041021 (C0048141 Spk H, 100 ppb Spikes)	100	0.408	61.6	61	ND	68.0	66	94.8	158	81
DL2-F01-MSF003-0-041021 (C0048142 Spk D, 10 ppb Spikes)	10	0.416	6.52	61	ND	6.78	68	41.8	41.2	*
DL2-F01-MSF003-0-041021 (C0048142 Spk L, 100 ppb Spikes)	100	0.416	64.8	64	ND	70.8	71	41.8	104	62
DL2-F01-MSF004-0-041021 (C0048143 Spk E, 10 ppb Spikes)	10	0.345	7.08	67	ND	7.56	76	83.6	91.2	*
DL2-F01-MSF004-0-041021 (C0048143 Spk J, 100 ppb Spikes)	100	0.345	71.6	71	ND	81.2	81	83.6	167	83
DL1-F01-IPF001-0-041021 (C0048144 Spk F, 10 ppb Spikes)	10	0.432	7.44	70	0.281	8.16	78	33.4	40.0	*
DL1-F01-IPF001-0-041021 (C0048144 Spk K, 100 ppb Spikes)	100	0.432	87.2	87	0.281	76.4	78	33.4	108.0	73
DL3-F01-IPF003-0-041021 (C0048145 Spk G, 10 ppb Spikes)	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 Spikes)	100	0.440	69.2	69	ND	81.8	82	3.55	80.0	78

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

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

NR = Not reported due to quality control result 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 PFH8			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found In Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPF004-0-041021 (C0048146 Spk C, 10 ppb Spike)	10	0.784	6.84	81	ND	10.8	108	0.382	8.98	88
DL3-F01-IPF004-0-041021 (C0048146 Spk H, 100 ppb Spike)	100	0.784	69.6	88	ND	88.4	88	0.382	81.2	81
DL3-F01-IPF005-0-041021 (C0048147 Spk D, 10 ppb Spike)	10	1.53	5.44	36	ND	9.16	92	5.48	11.7	82
DL3-F01-IPF005-0-041021 (C0048147 Spk I, 100 ppb Spike)	100	1.53	60.4	59	ND	93.2	93	5.48	85.8	81
DBC-F01-IPF001-0-041021 (C0048148 Spk E, 10 ppb Spike)	10	1.34	6.24	49	0.602	10.8	101	NR	NR	NR
DBC-F01-IPF001-0-041021 (C0048148 Spk J, 100 ppb Spike)	100	1.34	56.4	56	0.602	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	0.998	6.72	57	0.644	10.4	98	NR	NR	NR
DBC-F01-IPF003-0-041021 (C0048150 Spk L, 100 ppb Spike)	100	0.998	60.8	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	82	2.21	9.88	75	NR	NR	NR
DBC-F01-IPW001-0-041021 (C0048151 Spk H, 100 ppb Spike)	100	0.864	70.0	88	2.21	88.0	86	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048152 Spk D, 10 ppb Spike)	10	0.700	7.56	88	9.12	18.2	91	NR	NR	NR
DBC-F01-IPW002-0-041021 (C0048152 Spk I, 100 ppb Spike)	100	0.700	69.6	86	9.12	96.0	87	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048153 Spk E, 10 ppb Spike)	10	0.736	8.32	78	6.78	16.5	97	NR	NR	NR
DBC-F01-IPW003-0-041021 (C0048153 Spk J, 100 ppb Spike)	100	0.736	70.8	70	6.78	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	8.48	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	6.76	13.1	63	0.792	9.72	89	NR	NR	NR
DOU-F01-IPF002-0-041021 (C0048155 Spk L, 100 ppb Spike)	100	6.76	78.0	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	88	1.02	10.8	96	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-analyze results are shown in Table II.

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHS				C8 Sulfonate PFOS			
	Amount Spiked (ng/g)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Recovery (%)
DOU-F01-IPF004-0-041021 (C0040157 Spk D, 10 ppb Spike)	10	6.12	13.2	71	2.30	12.0	97	NR	NR	NR		
DOU-F01-IPF004-0-041021 (C0040157 Spk I, 100 ppb Spike)	100	3.12	78.4	70	2.30	86.0	84	NR	NR	NR		
DOU-F01-IPF005-0-041021 (C0040158 Spk E, 10 ppb Spike)	10	4.56	12.2	78	0.956	11.2	102	NR	NR	NR		
DOU-F01-IPF005-0-041021 (C0040158 Spk J, 100 ppb Spike)	100	4.56	78.0	71	0.956	85.8	85	NR	NR	NR		
DOU-F01-IPW001-0-041021 (C0040159 Spk F, 10 ppb Spike)	10	2.89	10.2	73	0.948	10.7	98	NR	NR	NR		
DOU-F01-IPW001-0-041021 (C0040159 Spk K, 100 ppb Spike)	100	2.89	72.4	70	0.948	83.8	83	NR	NR	NR		
DOU-F01-IPW002-0-041021 (C0040160 Spk G, 10 ppb Spike)	10	16.4	34.4	100	3.88	13.2	95	NR	NR	NR		
DOU-F01-IPW002-0-041021 (C0040160 Spk L, 100 ppb Spike)	100	16.4	94.8	76	3.88	88.2	88	NR	NR	NR		
DL2-F01-MSW001-0-041021 (C0040161 Spk I, 10 ppb Spike)	10	0.203	9.32	91	ND	8.88	87	120	126	*		
DL2-F01-MSW001-0-041021 (C0040161 Spk J, 100 ppb Spike)	100	0.203	76.8	77	ND	85.2	85	120	177	57		
DL2-F01-MSW002-0-041021 (C0040162 Spk K, 10 ppb Spike)	10	ND	9.66	87	ND	9.12	91	102	110	*		
DL2-F01-MSW002-0-041021 (C0040162 Spk L, 100 ppb Spike)	100	ND	86.4	88	ND	93.6	94	102	185	83		
DL2-F01-MSW003-0-041021 (C0040163 Spk C, 10 ppb Spike)	10	ND	9.24	82	ND	9.08	91	92.4	103	*		
DL2-F01-MSW003-0-041021 (C0040163 Spk D, 100 ppb Spike)	100	ND	81.2	81	ND	95.2	95	92.4	182	90		
DL2-F01-MSW004-0-041021 (C0040164 Spk E, 10 ppb Spike)	10	ND	7.56	78	ND	7.28	73	88.0	75.6	*		
DL2-F01-MSW004-0-041021 (C0040164 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	93.6	94	88.0	188	78		
DL2-F01-MSW005-0-041021 (C0040165 Spk G, 10 ppb Spike)	10	ND	7.88	80	ND	8.08	81	168	135	*		
DL2-F01-MSW005-0-041021 (C0040165 Spk H, 100 ppb Spike)	100	ND	80.8	81	ND	100	103	168	234	86		
DBC-F01-MSW001-0-041104 (C0040650 Spk C, 10 ppb Spike)	10	0.888	10.3	94	8.32	17.6	93	NR	NR	NR		
DBC-F01-MSW001-0-041104 (C0040650 Spk D, 100 ppb Spike)	100	0.888	90.4	90	8.32	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				C8 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 (C0040451 Spk E, 10 ppb Spike)	10	0.868	9.96	91	3.43	12.8	94	NR	NR	NR
DBC-F01-MSW002-0-041104 (C0040451 Spk F, 100 ppb Spike)	100	0.868	94.8	94	3.43	113	110	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0040452 Spk G, 10 ppb Spike)	10	0.860	9.92	91	3.62	13.6	100	NR	NR	NR
DBC-F01-MSW003-0-041104 (C0040452 Spk H, 100 ppb Spike)	100	0.860	86.0	86	3.62	104	100	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0040453 Spk I, 10 ppb Spike)	10	0.884	9.96	91	9.72	19.5	98	NR	NR	NR
DBC-F01-MSW004-0-041104 (C0040453 Spk J, 100 ppb Spike)	100	0.884	97.2	96	9.72	124	114	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0040454 Spk K, 10 ppb Spike)	10	0.832	8.20	74	2.77	10.2	74	NR	NR	NR
DBC-F01-MSW005-0-041104 (C0040454 Spk L, 100 ppb Spike)	100	0.832	91.6	91	2.77	108	106	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0040455 Spk M, 10 ppb Spike)	10	0.820	8.80	88	3.58	13.7	101	NR	NR	NR
DBC-F01-MSF001-0-041104 (C0040455 Spk N, 100 ppb Spike)	100	0.820	88.4	88	3.58	108	102	NR	NR	NR
DBC-F01-MBF002-0-041104 (C0040456 Spk O, 10 ppb Spike)	10	0.768	9.92	92	1.94	12.5	108	NR	NR	NR
DBC-F01-MBF002-0-041104 (C0040456 Spk P, 100 ppb Spike)	100	0.768	90.8	90	1.94	102	100	NR	NR	NR
DBC-F01-IPF004-0-041104 (C0040457 Spk Q, 10 ppb Spike)	10	0.504	10.5	100	0.584	11.6	110	142	152	*
DBC-F01-IPF004-0-041104 (C0040457 Spk R, 100 ppb Spike)	100	0.504	94.8	94	0.584	108	106	142	254	112
DBC-F01-IPF005-0-041104 (C0040458 Spk S, 10 ppb Spike)	10	1.60	10.4	88	2.15	12.2	101	NR	NR	NR
DBC-F01-IPF005-0-041104 (C0040458 Spk T, 100 ppb Spike)	100	1.60	90.8	88	2.15	105	103	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0040459 Spk U, 10 ppb Spike)	10	0.484	9.72	92	3.28	13.0	97	NR	NR	NR
DBC-F01-IPW004-0-041104 (C0040459 Spk V, 100 ppb Spike)	100	0.484	87.6	87	3.28	104	101	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0040460 Spk W, 10 ppb Spike)	10	0.572	10.4	98	2.10	13.2	111	NR	NR	NR
DBC-F01-IPW005-0-041104 (C0040460 Spk X, 100 ppb Spike)	100	0.572	92.8	92	2.10	102	100	NR	NR	NR

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

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

NR = Not reported due to quality control result failures. Re-analysis results are shown in Table 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 Spikes)	10	3.12	13.1	100	8.00	16.3	103	NR	NR	NR
DOU-F01-IPW003-0-041104 (C0049461 Spk L, 100 ppb Spikes)	100	3.12	88.0	85	8.00	107	101	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk C, 10 ppb Spikes)	10	3.49	12.8	93	7.12	17.4	103	NR	NR	NR
DOU-F01-IPW004-0-041104 (C0049462 Spk D, 100 ppb Spikes)	100	3.49	88.4	85	7.12	108	101	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk E, 10 ppb Spikes)	10	2.97	10.5	75	5.12	13.5	84	NR	NR	NR
DOU-F01-IPW005-0-041104 (C0049463 Spk F, 100 ppb Spikes)	100	2.97	83.6	81	5.12	109	104	NR	NR	NR
DL2-F01-IPW002-0-041104 (C0049464 Spk G, 10 ppb Spikes)	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 Spikes)	100	1.04	80.0	79	ND	97.8	98	8.20	124	116
DL1-F01-MSW001-0-041104 (C0049465 Spk I, 10 ppb Spikes)	10	1.20	10.6	94	0.908	11.3	104	NR	NR	NR
DL1-F01-MSW001-0-041104 (C0049465 Spk J, 100 ppb Spikes)	100	1.20	89.6	88	0.908	112	111	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk K, 10 ppb Spikes)	10	1.30	9.76	85	1.72	12.4	107	NR	NR	NR
DL1-F01-MSW002-0-041104 (C0049466 Spk L, 100 ppb Spikes)	100	1.30	80.4	79	1.72	100	98	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk C, 10 ppb Spikes)	10	0.560	8.92	84	1.13	10.2	91	NR	NR	NR
DL1-F01-MSW003-0-041104 (C0049467 Spk D, 100 ppb Spikes)	100	0.560	85.2	85	1.13	98.8	98	NR	NR	NR
DL1-F01-MSW004-0-041104 (C0049468 Spk E, 10 ppb Spikes)	10	0.908	8.52	76	0.908	9.76	89	181	201	*
DL1-F01-MSW004-0-041104 (C0049468 Spk F, 100 ppb Spikes)	100	0.908	84.8	84	0.908	99.6	99	181	286	108
DL1-F01-MSW006-0-041104 (C0049469 Spk G, 10 ppb Spikes)	10	1.18	9.80	78	1.18	10.4	92	NR	NR	NR
DL1-F01-MSW006-0-041104 (C0049469 Spk H, 100 ppb Spikes)	100	1.18	82.4	81	1.18	98.8	98	NR	NR	NR
DL1-F01-MSF001-0-041104 (C0049470 Spk I, 10 ppb Spikes)	10	ND	7.76	78	0.315	8.52	92	283	303	*
DL1-F01-MSF001-0-041104 (C0049470 Spk J, 100 ppb Spikes)	100	ND	78.4	78	0.315	90.8	90	283	394	101

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

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

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

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHs			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0049471 Spk K, 10 ppb Spikes)	10	ND	8.60	86	0.824	10.5	89	NR	NR	NR
DL1-F01-MSF002-0-041104 (C0049471 Spk L, 100 ppb Spikes)	100	ND	84.8	85	0.824	98.4	98	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0049472 Spk C, 10 ppb Spikes)	10	0.203	8.78	86	0.852	10.2	95	NR	NR	NR
DL1-F01-MSF003-0-041104 (C0049472 Spk D, 100 ppb Spikes)	100	0.203	84.4	84	0.852	95.2	95	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0049473 Spk E, 10 ppb Spikes)	10	ND	8.86	89	0.532	10.4	98	NR	NR	NR
DL1-F01-MSF004-0-041104 (C0049473 Spk F, 100 ppb Spikes)	100	ND	86.8	87	0.532	101	100	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0049474 Spk G, 10 ppb Spikes)	10	ND	8.64	86	0.684	10.1	94	NR	NR	NR
DL1-F01-MSF005-0-041104 (C0049474 Spk H, 100 ppb Spikes)	100	ND	82.4	82	0.684	94.0	83	NR	NR	NR
DL1-F01-IPW001-0-041104 (C0049475 Spk I, 10 ppb Spikes)	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 Spikes)	100	0.772	84.0	83	0.556	97.8	97	17.4	113	98
DL1-F01-IPW002-0-041104 (C0049476 Spk K, 10 ppb Spikes)	10	0.468	8.88	82	0.536	9.96	84	15.7	27.4	117
DL1-F01-IPW002-0-041104 (C0049476 Spk L, 100 ppb Spikes)	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 Spikes)	10	ND	9.08	91	0.209	9.44	92	9.36	16.7	73
DL1-F01-IPW003-0-041104 (C0049477 Spk D, 100 ppb Spikes)	100	ND	90.4	90	0.209	106	106	9.36	109	100
DL1-F01-IPW004-0-041104 (C0049478 Spk E, 10 ppb Spikes)	10	ND	8.48	85	0.238	9.80	96	58.6	72.0	*
DL1-F01-IPW004-0-041104 (C0049478 Spk F, 100 ppb Spikes)	100	ND	71.6	72	0.238	84.8	85	58.6	140	80
DL1-F01-IPF002-0-041104 (C0049479 Spk G, 10 ppb Spikes)	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 Spikes)	100	ND	86.4	86	0.222	98.4	98	11.1	108	97
DL1-F01-IPF003-0-041104 (C0049480 Spk I, 10 ppb Spikes)	10	ND	8.80	88	0.265	10.8	103	20.9	30.5	98
DL1-F01-IPF003-0-041104 (C0049480 Spk J, 100 ppb Spikes)	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 PFH6			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL-1-F01-IPF004-0-041104 (C0040481 Spk K, 10 ppb Spikes)	10	ND	8.00	80	ND	9.48	95	7.80	15.8	82
DL-1-F01-IPF004-0-041104 (C0040481 Spk L, 100 ppb Spikes)	100	ND	84.8	85	ND	98.8	98	7.80	102	94
DL-1-F01-IPF005-0-041104 (C0040482 Spk C, 10 ppb Spikes)	10	ND	7.44	74	0.373	9.00	88	8.72	15.9	72
DL-1-F01-IPF005-0-041104 (C0040482 Spk D, 100 ppb Spikes)	100	ND	84.8	85	0.373	98.4	98	8.72	102	93
DL3-F01-MSW001-0-041105 (C0040483 Spk E, 10 ppb Spikes)	10	ND	8.36	84	ND	9.20	92	138	165	*
DL3-F01-MSW001-0-041105 (C0040483 Spk F, 100 ppb Spikes)	100	ND	85.6	86	ND	99.6	100	138	256	119
DL3-F01-MSW002-0-041105 (C0040484 Spk G, 10 ppb Spikes)	10	ND	8.44	84	ND	9.92	98	180	150	*
DL3-F01-MSW002-0-041105 (C0040484 Spk H, 100 ppb Spikes)	100	ND	80.4	80	ND	106	106	180	263	109
DL3-F01-MSW003-0-041105 (C0040485 Spk I, 10 ppb Spikes)	10	ND	8.80	88	ND	9.58	96	146	154	*
DL3-F01-MSW003-0-041105 (C0040485 Spk J, 100 ppb Spikes)	100	ND	88.4	88	ND	102	102	146	264	108
DL3-F01-MSW004-0-041105 (C0040486 Spk K, 10 ppb Spikes)	10	ND	8.48	85	0.286	9.88	94	198	225	*
DL3-F01-MSW004-0-041105 (C0040486 Spk L, 100 ppb Spikes)	100	ND	88.4	88	0.286	102	102	198	301	103
DL3-F01-MSW005-0-041105 (C0040487 Spk C, 10 ppb Spikes)	10	ND	9.56	96	0.386	11.1	107	173	183	*
DL3-F01-MSW005-0-041105 (C0040487 Spk D, 100 ppb Spikes)	100	ND	100	100	0.386	105	105	173	246	73
DL3-F01-MSF001-0-041105 (C0040488 Spk E, 10 ppb Spikes)	10	ND	9.48	95	ND	11.4	114	116	138	*
DL3-F01-MSF001-0-041105 (C0040488 Spk F, 100 ppb Spikes)	100	ND	84.8	85	ND	109	109	116	234	118
DL3-F01-MSF002-0-041105 (C0040489 Spk G, 10 ppb Spikes)	10	ND	9.86	98	ND	10.4	104	116	96.8	*
DL3-F01-MSF002-0-041105 (C0040489 Spk H, 100 ppb Spikes)	100	ND	89.6	90	ND	103	103	116	218	100
DL3-F01-MSF003-0-041105 (C0040490 Spk I, 10 ppb Spikes)	10	ND	9.32	93	ND	11.0	110	75.6	92.0	*
DL3-F01-MSF003-0-041105 (C0040490 Spk J, 100 ppb Spikes)	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 PFH8				C8 Sulfonate PFO3			
	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 (C0040401 Spk K, 10 ppb Spike)	10	ND	8.80	96	ND	11.0	110	130	144	*		
DL3-F01-MSF004-0-041105 (C0040401 Spk L, 100 ppb Spike)	100	ND	90.4	90	ND	104	104	130	236	108		
DL3-F01-MSF005-0-041105 (C0040402 Spk C, 10 ppb Spike)	10	ND	8.92	88	ND	10.3	103	81.8	97.2	*		
DL3-F01-MSF005-0-041105 (C0040402 Spk D, 100 ppb Spike)	100	ND	85.6	86	ND	98.4	98	81.8	181	99		
DMC-F01-IPW003-0-041105 (C0040403 Spk E, 10 ppb Spike)	10	ND	8.26	83	0.366	9.52	91	81.2	79.0	*		
DMC-F01-IPW003-0-041105 (C0040403 Spk F, 100 ppb Spike)	100	ND	84.4	84	0.366	99.6	99	81.2	156	95		
DMC-F01-IPW004-0-041105 (C0040404 Spk G, 10 ppb Spike)	10	0.300	8.04	77	0.366	9.28	96	68.8	107	*		
DMC-F01-IPW004-0-041105 (C0040404 Spk H, 100 ppb Spike)	100	0.300	90.4	90	0.366	104	104	68.8	168	99		
DMC-F01-IPW005-0-041105 (C0040405 Spk I, 10 ppb Spike)	10	ND	8.98	90	0.270	10.1	98	84.4	75.8	*		
DMC-F01-IPW005-0-041105 (C0040405 Spk J, 100 ppb Spike)	100	ND	88.4	88	0.270	105	105	84.4	153	89		
DMC-F01-MSW003-0-041105 (C0040406 Spk K, 10 ppb Spike)	10	ND	9.84	98	0.602	11.0	104	354	389	*		
DMC-F01-MSW003-0-041105 (C0040406 Spk L, 100 ppb Spike)	100	ND	92.4	92	0.602	108	106	364	484	110		
DMC-F01-MSW004-0-041105 (C0040407 Spk C, 10 ppb Spike)	10	0.318	9.52	92	1.90	12.1	102	259	259	*		
DMC-F01-MSW004-0-041105 (C0040407 Spk D, 100 ppb Spike)	100	0.318	82.4	82	1.90	101	99	259	313	54		
DMC-F01-MSW005-0-041105 (C0040408 Spk E, 10 ppb Spike)	10	ND	2.16	92	0.960	11.8	106	NR	NR	NR		
DMC-F01-MSW005-0-041105 (C0040408 Spk F, 100 ppb Spike)	100	ND	93.6	94	0.960	118	117	NR	NR	NR		
DMC-F01-MSF002-0-041105 (C0040409 Spk G, 10 ppb Spike)	10	ND	8.36	84	ND	10.4	104	NR	NR	NR		
DMC-F01-MSF002-0-041105 (C0040409 Spk H, 100 ppb Spike)	100	ND	97.6	98	ND	104	104	NR	NR	NR		
DMC-F01-MSF003-0-041105 (C0040410 Spk I, 10 ppb Spike)	10	ND	8.20	82	ND	10.4	104	68.8	108	*		
DMC-F01-MSF003-0-041105 (C0040410 Spk J, 100 ppb Spike)	100	ND	87.2	87	ND	110	110	68.8	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 failure. Re-analysis results are shown in Table II.

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

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

Sample Description	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C8 Sulfonate PFHS			C8 Sulfonate PFOS		
		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 (C0048801 Spk K, 10 ppb Spike)	10	ND	8.76	88	0.254	11.1	108	118	123	*
DMC-F01-MSF004-0-041106 (C0048801 Spk L, 100 ppb Spike)	100	ND	88.0	88	0.254	107	107	118	228	110
DMC-F01-MSF005-0-041105 (C0048802 Spk C, 10 ppb Spike)	10	ND	8.52	85	0.420	12.2	118	146	193	*
DMC-F01-MSF005-0-041105 (C0048802 Spk D, 100 ppb Spike)	100	ND	83.8	84	0.420	110	110	146	280	114
DMC-F01-IPF003-0-041106 (C0048803 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	10.8	108	7.92	16.0	81
DMC-F01-IPF003-0-041106 (C0048803 Spk F, 100 ppb Spike)	100	ND	82.0	82	ND	108	108	7.92	101	83
DMC-F01-IPF004-0-041105 (C0048804 Spk G, 10 ppb Spike)	10	ND	7.92	79	ND	10.7	107	44.0	56.2	*
DMC-F01-IPF004-0-041105 (C0048804 Spk H, 100 ppb Spike)	100	ND	83.2	83	ND	107	107	44.0	134	90
DMC-F01-IPF005-0-041105 (C0048805 Spk I, 10 ppb Spike)	10	ND	7.88	79	ND	10.3	103	46.4	54.4	*
DMC-F01-IPF005-0-041105 (C0048805 Spk J, 100 ppb Spike)	100	ND	75.8	76	ND	96.4	96	46.4	121	75
DL2-F01-MSF005-0-041216 (C0054454 Spk E, 10 ppb Spike)	10	ND	8.44	84	ND	10.0	100	49.2	59.2	100
DL2-F01-IPW005-0-041215 (C0054454 Spk F, 100 ppb Spike)	100	ND	83.2	83	ND	102	102	49.2	142	93
DL2-F01-IPW005-0-041215 (C0054454 Spk G, 10 ppb Spike)	10	ND	8.00	80	ND	11.5	115	13.2	22.7	95
DL2-F01-IPW005-0-041215 (C0054454 Spk D, 100 ppb Spike)	100	ND	84.8	85	ND	107	107	13.2	121	108
DOU-F01-MSW002-0-041216 (C0054458 Spk I, 10 ppb Spike)	10	0.208	8.82	87	1.72	10.8	92	NR	NR	NR
DOU-F01-MSW002-0-041216 (C0054458 Spk J, 100 ppb Spike)	100	0.208	87.2	87	1.72	92.8	91	NR	NR	NR
DL2-F01-IPW004-0-041215 (C0054457 Spk C, 10 ppb Spike)	10	ND	8.08	81	ND	10.4	104	6.80	14.6	80
DL2-F01-IPW004-0-041215 (C0054457 Spk D, 100 ppb Spike)	100	ND	76.8	77	ND	95.2	95	6.80	97.2	90
DOU-F01-MSW003-0-041216 (C0054458 Spk K, 10 ppb Spike)	10	ND	8.12	81	10.4	19.4	90	NR	NR	NR
DOU-F01-MSW003-0-041216 (C0054458 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	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPW004-0-041130 (C0000438 Spk E, 10 ppb Spike)	10	ND	7.52	75	ND	11.0	110	7.92	17.3	94
DL3-F01-IPW004-0-041130 (C0000438 Spk F, 100 ppb Spike)	100	ND	66.4	66	ND	118	118	7.92	114	106
DOU-F01-MSW001-0-041218 (C0000440 Spk G, 10 ppb Spike)	10	7.08	14.6	75	15.4	27.5	121	NR	NR	NR
DOU-F01-MSW001-0-041218 (C0000440 Spk H, 100 ppb Spike)	100	7.08	83.2	76	15.4	122.0	107	NR	NR	NR
DOU-F01-MSW004-0-041218 (C0000441 Spk E, 10 ppb Spike)	10	4.72	10.8	61	21.7	29.3	76	NR	NR	NR
DOU-F01-MSW004-0-041218 (C0000441 Spk F, 100 ppb Spike)	100	4.72	72.8	68	21.7	104	82	NR	NR	NR
DOU-F01-MSW005-0-041218 (C0000442 Spk G, 10 ppb Spike)	10	0.364	7.08	67	1.34	8.52	72	NR	NR	NR
DOU-F01-MSW005-0-041218 (C0000442 Spk H, 100 ppb Spike)	100	0.364	72.0	72	1.34	67.6	68	NR	NR	NR
DOU-F01-MSF005-0-041218 (C0000443 Spk C, 10 ppb Spike)	10	1.41	8.80	72	8.40	17.8	82	NR	NR	NR
DOU-F01-MSF005-0-041218 (C0000443 Spk D, 100 ppb Spike)	100	1.41	72.0	71	8.40	94.0	88	NR	NR	NR
DL3-F01-IPW002-0-041130 (C0000444 Spk E, 10 ppb Spike)	10	ND	7.92	79	ND	9.04	90	12.0	16.6	46
DL3-F01-IPW002-0-041130 (C0000444 Spk F, 100 ppb Spike)	100	ND	81.2	81	ND	110	110	12.0	106	93
DL3-F01-IPW003-0-041130 (C0000445 Spk G, 10 ppb Spike)	10	ND	7.98	80	ND	8.88	89	5.44	12.3	69
DL3-F01-IPW003-0-041130 (C0000445 Spk H, 100 ppb Spike)	100	ND	84.4	84	ND	102	102	5.44	92.0	87
DOU-F01-MSF003-0-041218 (C0000446 Spk K, 10 ppb Spike)	10	1.72	9.00	73	8.56	14.3	77	NR	NR	NR
DOU-F01-MSF003-0-041218 (C0000446 Spk L, 100 ppb Spike)	100	1.72	86.6	86	8.56	107	100	NR	NR	NR
DBC-F01-MSF005-1-041218 (C0000447 Spk G, 10 ppb Spike)	10	0.220	8.36	81	1.78	11.6	97	NR	NR	NR
DBC-F01-MSF005-1-041218 (C0000447 Spk H, 100 ppb Spike)	100	0.220	83.6	83	1.78	86.6	95	NR	NR	NR
DBC-F01-MSF005-0-041218 (C0000448 Spk K, 10 ppb Spike)	10	ND	8.36	84	1.88	11.4	97	NR	NR	NR
DBC-F01-MSF005-0-041218 (C0000448 Spk L, 100 ppb Spike)	100	ND	80.8	81	1.88	99.6	98	NR	NR	NR

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

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

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

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

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

Sample Description	C4 Sulfonate PFBS				C6 Sulfonate PFHxS			C8 Sulfonate PFOS		
	Amount Spiked (ng/g)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Am't Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DOU-F01-MSF002-0-041218 (C0086466 Spk G, 10 ppb Spikes)	10	0.688	8.24	84	4.56	14.5	98	NR	NR	NR
DOU-F01-MSF002-0-041218 (C0086466 Spk H, 100 ppb Spikes)	100	0.688	83.6	83	4.56	104	99	NR	NR	NR
DL3-F01-IPW001-0-041130 (C0086470 Spk C, 10 ppb Spikes)	10	ND	7.80	78	0.246	9.80	96	9.04	17.2	82
DL3-F01-IPW001-0-041130 (C0086470 Spk D, 100 ppb Spikes)	100	ND	82.4	82	0.246	101	101	9.04	102	93
DOU-F01-MSF002-1-041218 (C0086471 Spk I, 10 ppb Spikes)	10	0.840	9.20	84	4.68	14.5	98	NR	NR	NR
DOU-F01-MSF002-1-041218 (C0086471 Spk J, 100 ppb Spikes)	100	0.840	82.0	81	4.68	103	98	NR	NR	NR
DOU-F01-MSF004-0-041218 (C0086472 Spk K, 10 ppb Spikes)	10	0.792	7.76	70	2.35	8.80	65	NR	NR	NR
DOU-F01-MSF004-0-041218 (C0086472 Spk L, 100 ppb Spikes)	100	0.792	81.2	80	2.35	86.8	83	NR	NR	NR
DOU-F01-MSF001-0-041218 (C0086473 Spk E, 10 ppb Spikes)	10	2.18	11.1	89	10.9	20.8	98	NR	NR	NR
DOU-F01-MSF001-0-041218 (C0086473 Spk F, 100 ppb Spikes)	100	2.18	82.8	81	10.9	104	93	NR	NR	NR
DBC-F01-MSF003-0-041218 (C0086474 Spk G, 10 ppb Spikes)	10	ND	8.48	86	2.09	11.9	98	NR	NR	NR
DBC-F01-MSF003-0-041218 (C0086474 Spk H, 100 ppb Spikes)	100	ND	84.0	84	2.09	108	104	NR	NR	NR
DBC-F01-MSF004-0-041218 (C0086475 Spk I, 10 ppb Spikes)	10	ND	7.72	77	3.87	12.8	90	NR	NR	NR
DBC-F01-MSF004-0-041218 (C0086475 Spk J, 100 ppb Spikes)	100	ND	75.8	78	3.87	98.8	95	NR	NR	NR
DMC-F01-MSF001-0-041218 (C0086476 Spk C, 10 ppb Spikes)	10	ND	9.06	91	0.420	9.08	87	NR	NR	NR
DMC-F01-MSF001-0-041218 (C0086476 Spk D, 100 ppb Spikes)	100	ND	88.0	88	0.420	102	102	NR	NR	NR
DL1-F01-IPW005-0-041218 (C0086477 Spk I, 10 ppb Spikes)	10	0.253	7.40	71	0.644	9.96	83	68.8	83.2	*
DL1-F01-IPW005-0-041218 (C0086477 Spk J, 100 ppb Spikes)	100	0.253	72.4	72	0.644	88.8	86	68.8	145	76
DL2-F01-IPW003-0-041201 (C0086478 Spk K, 10 ppb Spikes)	10	ND	7.04	70	ND	9.04	90	7.48	13.7	62
DL2-F01-IPW003-0-041201 (C0086478 Spk L, 100 ppb Spikes)	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	Amount Spiked (ng/g)	C4 Sulfonate PFBS			C6 Sulfonate PFHS			C8 Sulfonate PFOS		
		Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)	Amount Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL3-F01-IPW005-0-041201 (C8056476 Spk 1, 10 ppb Spike)	10	ND	8.16	82	ND	8.96	90	4.88	14.0	91
DL3-F01-IPW005-0-041201 (C8056476 Spk 1, 100 ppb Spike)	100	ND	84.8	85	ND	97.2	97	4.88	94.4	90
		Average: 79			Average: 94			Average: 86		
		Standard Deviation: 13			Standard Deviation: 12			Standard Deviation: 20		

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

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

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

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

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

Sample Description	Amount Spiked (ng/g)	C8 Sulfonate PFOS		
		Amt Found in Sample (ng/g) wet wt	Amount Recovered (ng/g) wet wt	Recovery (%)
DBC-F01-IPW001-0-041021 (C0048151 Spk C, 1000 ppb)	1000	2080	2880	78
DBC-F01-IPW002-0-041021 (C0048152 Spk D, 5000 ppb)	5000	3330	8680	107
DBC-F01-IPW003-0-041021 (C0048153 Spk E, 1000 ppb)	1000	1660	3180	150
DOU-F01-IPF001-0-041021 (C0048154 Spk F, 1000 ppb)	1000	598	1780	116
DOU-F01-IPF002-0-041021 (C0048155 Spk G, 1000 ppb)	1000	636	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 (C0049490 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	16200	100
DBC-F01-MSF001-0-041104 (C0049455 Spk O, 10,000 ppb)	10000	5240	14400	92
DBC-F01-MSF002-0-041104 (C0049456 Spk P, 10,000 ppb)	10000	4760	15200	104
DBC-F01-IPF005-0-041104 (C0049458 Spk E, 1000 ppb)	1000	608	1800	98
DBC-F01-IPW004-0-041104 (C0049459 Spk F, 1000 ppb)	1000	1230	2590	138
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	88
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	604	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)**

Sample Description	Amount Spiked (ng/g)	C8 Sulfonate PFOS		
		Amt Found in Sample (ng/g) wet wt.	Amount Recovered (ng/g) wet wt.	Recovery (%)
DL1-F01-MSF002-0-041104 (C0048471 Spk F, 1000 ppb)	1000	568	1090	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 (C0048488 Spk I, 1000 ppb)	1000	580	1780	122
DMC-F01-MSF002-0-041105 (C0048498 Spk J, 1000 ppb)	1000	620	1500	88
DOU-F01-MSW002-0-041218 (C0058486 Spk K, 10,000 ppb)	10000	28200	32700	45
DOU-F01-MSW003-0-041218 (C0058488 Spk L, 5000 ppb)	5000	4040	10200	123
DOU-F01-MSW001-0-041218 (C0058480 Spk C, 10000 ppb)	10000	7800	25000	174*
DOU-F01-MSW004-0-041218 (C0058481 Spk D, 5000 ppb)	5000	5120	9880	95
DOU-F01-MSW005-0-041218 (C0058482 Spk E, 1000 ppb)	1000	486	1780	128
DOU-F01-MSF005-0-041218 (C0058483 Spk F, 1000 ppb)	1000	720	1820	110
DOU-F01-MSF003-0-041218 (C0058488 Spk G, 1000 ppb)	1000	848	1720	87
DBC-F01-MSF005-1-041218 (C0058487 Spk H, 10000 ppb)	10000	9320	32400	230*
DBC-F01-MSF005-0-041218 (C0058488 Spk I, 10000 ppb)	10000	8980	24100	151
DOU-F01-MSF002-0-041218 (C0058489 Spk J, 1000 ppb)	1000	640	1720	108
DOU-F01-MSF002-1-041218 (C0058471 Spk K, 1000 ppb)	1000	692	1950	126
DOU-F01-MSF004-0-041218 (C0058472 Spk L, 1000 ppb)	1000	1820	2700	88
DOU-F01-MSF001-0-041218 (C0058473 Spk C, 5000 ppb)	5000	2070	7980	118
DBC-F01-MSF003-0-041218 (C0058474 Spk D, 10000 ppb)	10000	4640	15500	108
DBC-F01-MSF004-0-041218 (C0058475 Spk E, 10000 ppb)	10000	9280	24500	152
DMC-F01-MSF001-0-041218 (C0058478 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 (C0048158 Spk I, 1000 ppb)	1000	359	1400	104

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

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

Sample Description	C4 Sulfonate PFBS				C8 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-CFW001-0-041218 (C0086480 Spk C, 10 ppb)	10	ND	6.68	60	ND	6.18	62	1.82	6.32	46		
DL3-I01-CFW001-0-041218 (C0086480 Spk D, 100 ppb)	100	ND	82.4	82	ND	95.8	96	1.82	85.2	83		
DMC-I01-CFW001-0-041218 (C0086481 Spk E, 10 ppb)	10	ND	9.04	90	ND	9.44	94	6.16	11.3	51		
DMC-I01-CFW001-0-041218 (C0086481 Spk F, 100 ppb)	100	ND	82.8	83	ND	98.8	98	6.16	92.8	87		
DL2-I01-CFW001-0-041218 (C0086482 Spk G, 10 ppb)	10	ND	7.28	73	ND	7.84	78	1.81	7.52	57		
DL2-I01-CFW001-0-041218 (C0086482 Spk H, 100 ppb)	100	ND	83.6	84	ND	101	101	1.81	88.4	87		
DBC-I01-CFW001-0-041218 (C0086483 Spk I, 10 ppb)	10	ND	10.3	103	ND	10.8	108	4.48	13.0	86		
DBC-I01-CFW001-0-041218 (C0086483 Spk J, 100 ppb)	100	ND	84.4	84	ND	97.2	97	4.48	88.4	82		
DL1-I01-CFW001-0-041218 (C0086484 Spk K, 10 ppb)	10	ND	6.96	90	ND	9.32	93	8.80	16.0	62		
DL1-I01-CFW001-0-041218 (C0086484 Spk L, 100 ppb)	100	ND	86.2	86	ND	103	103	8.80	98.8	88		
DOU-I01-CFW001-0-041218 (C0086485 Spk M, 10 ppb)	10	ND	6.48	86	0.202	8.32	81	11.4	17.2	58		
DOU-I01-CFW001-0-041218 (C0086485 Spk N, 100 ppb)	100	ND	84.4	84	0.202	98.0	98	11.4	98.0	87		
Average:				84	Average:				Average:			
Standard Deviation:				10	Standard Deviation:				Standard Deviation:			

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

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

FIGURES

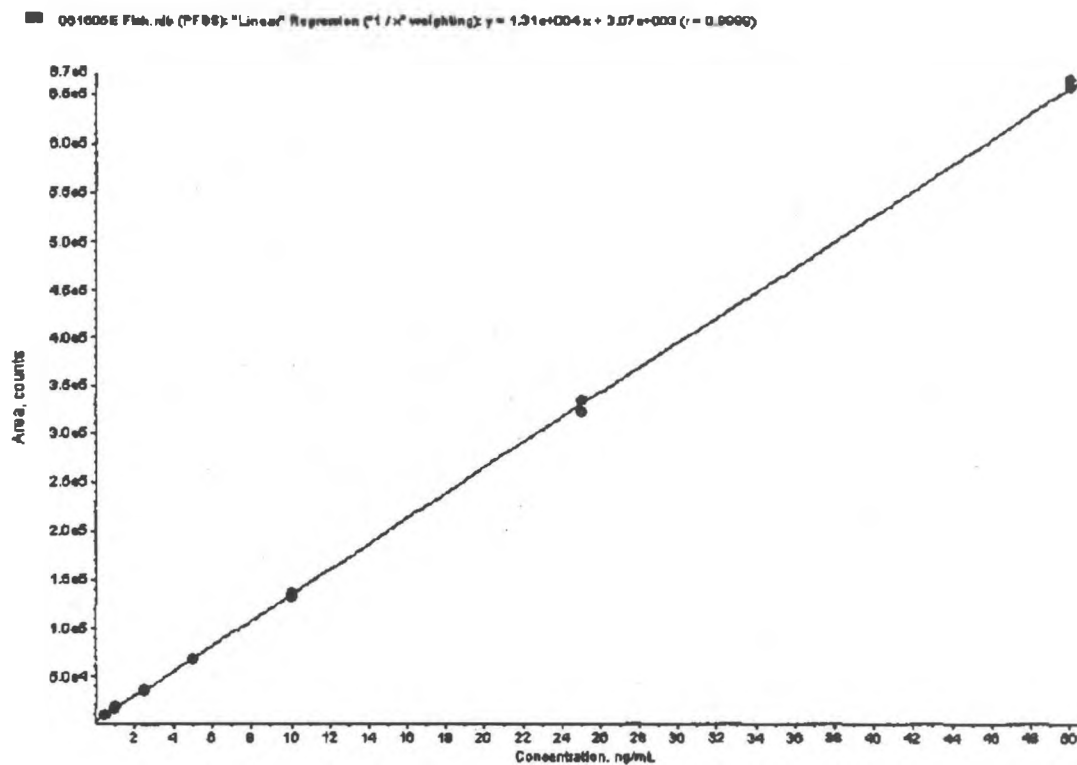
Figure 1. Typical Calibration Curve for PFBS in Methanol

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

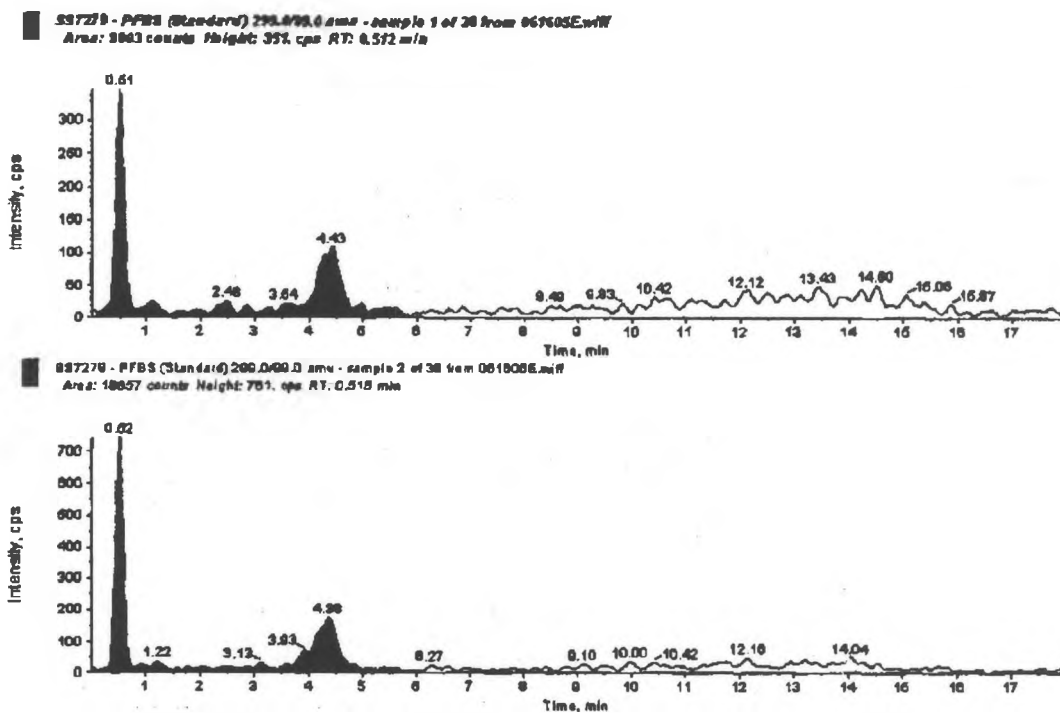


Figure 3. PFBS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

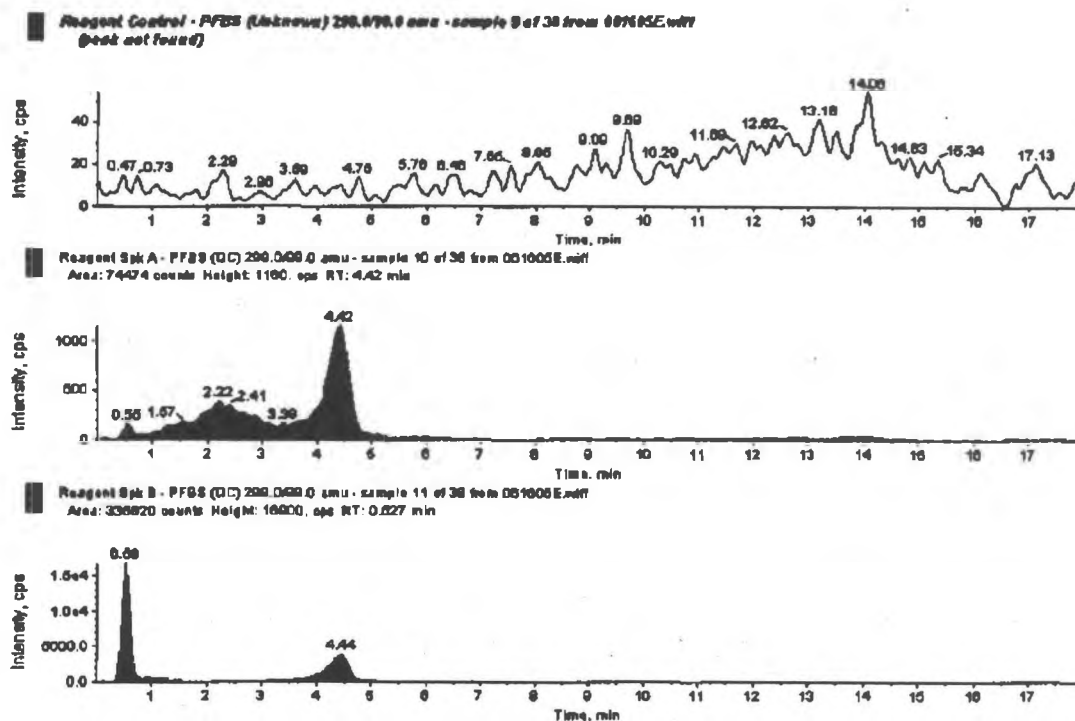


Figure 4. Chromatogram Representing a Fish Sample Analyzed for PFBS (Exygen ID: C0049478, Data Set:061605E)

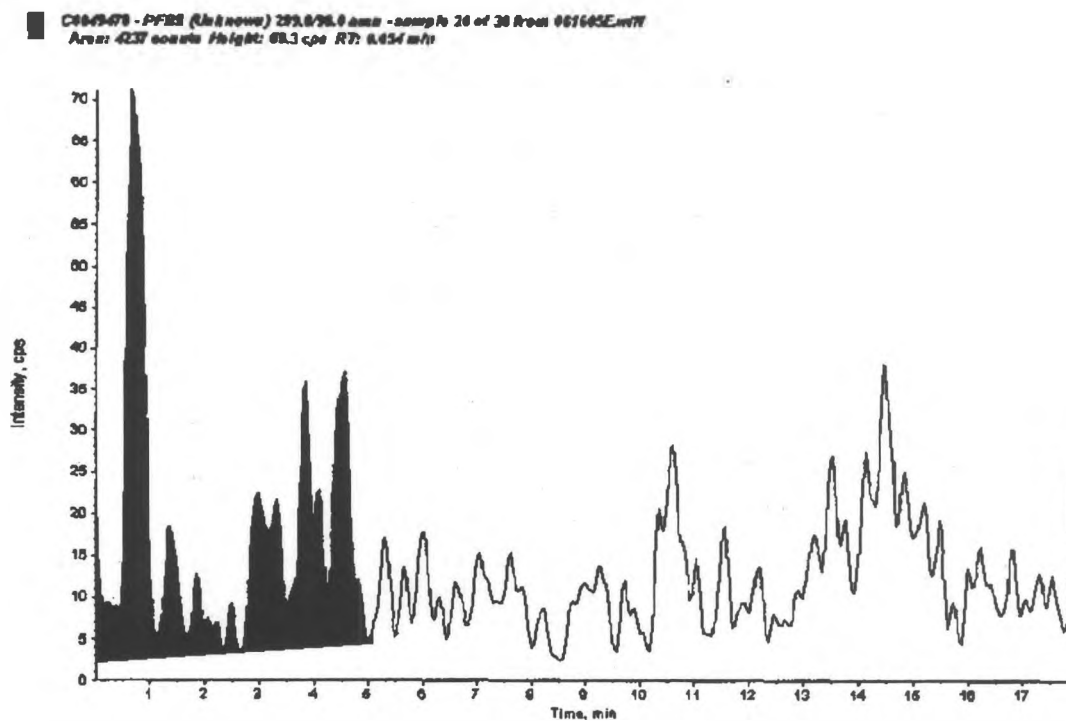


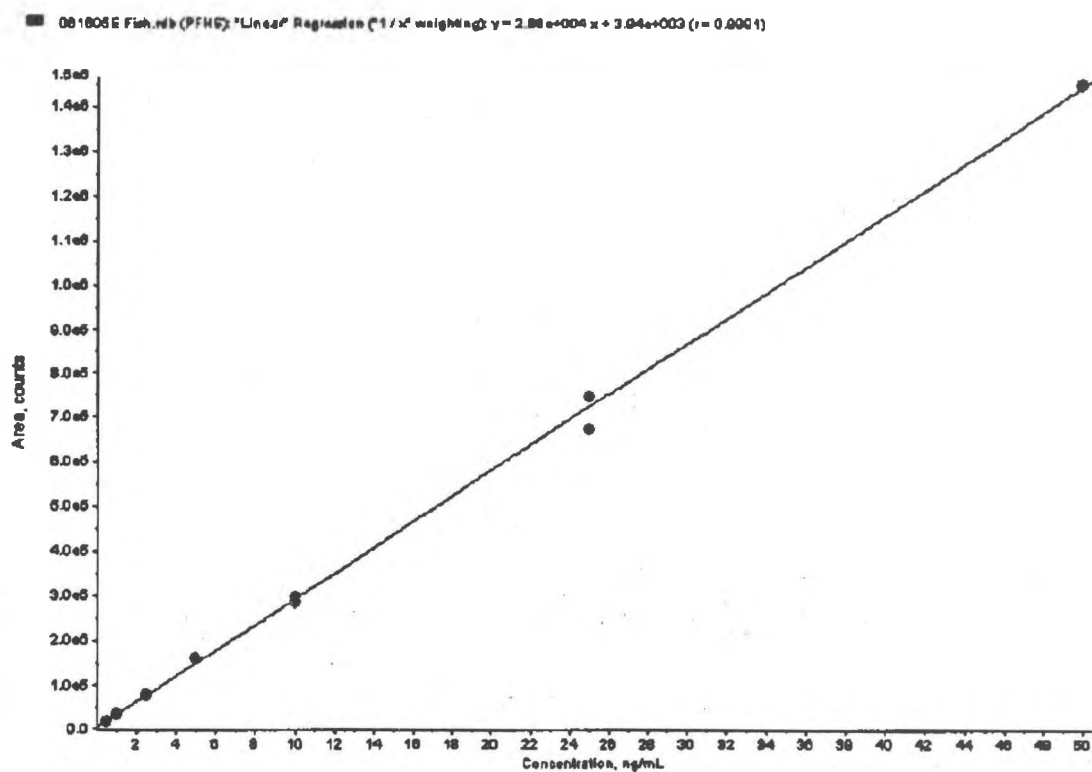
Figure 5. Typical Calibration Curve for PFHS in Methanol

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

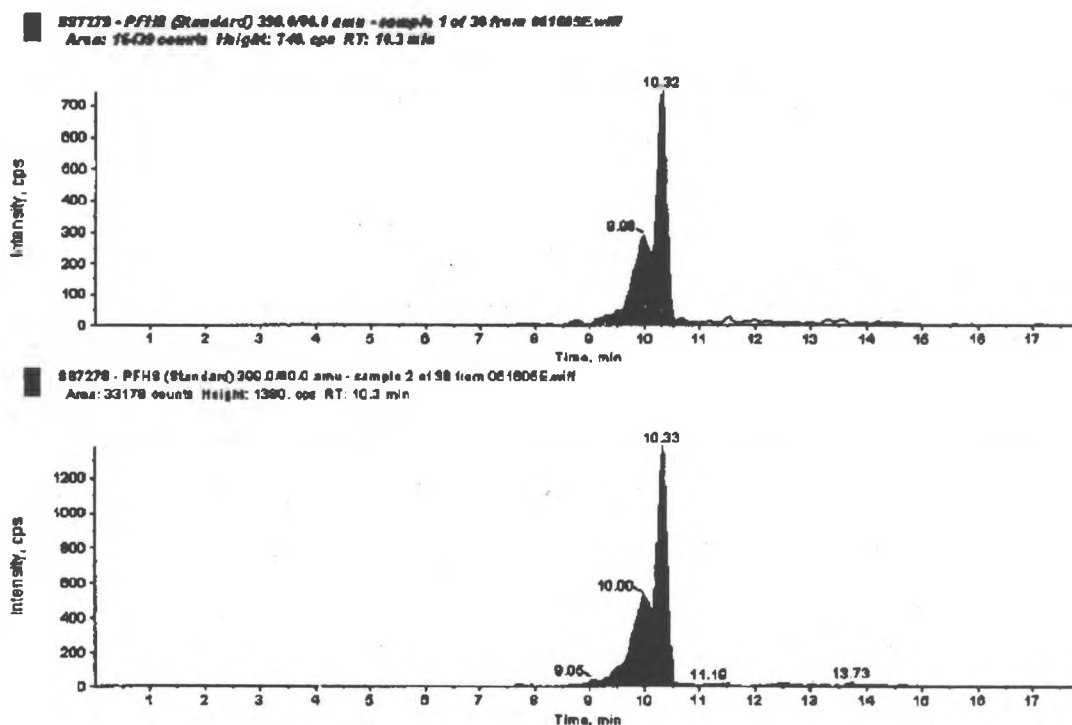


Figure 7. PFHS in Control Fish Blank, 0.00625 $\mu\text{g/mL}$ Fortified Control Fish Spk A, and 0.025 $\mu\text{g/mL}$ Fortified Control Fish Spk B, Respectively

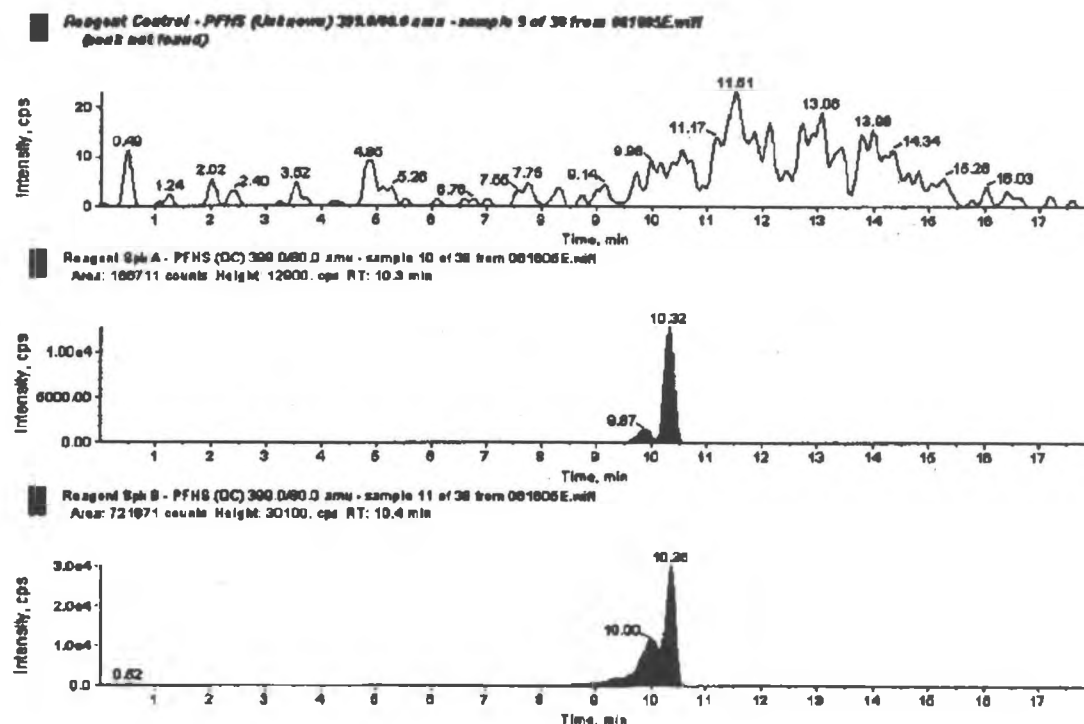


Figure 3. Chromatogram Representing a Fish Sample Analyzed for PFHS (Exygen ID: C0049478, Data Set: 061605E)

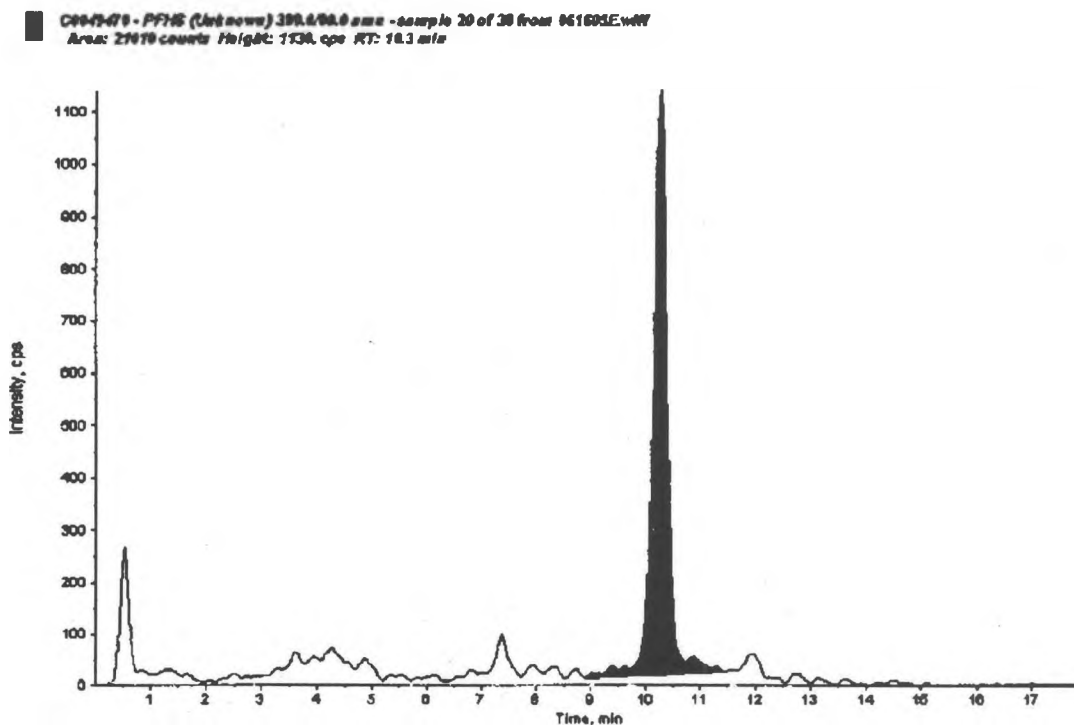


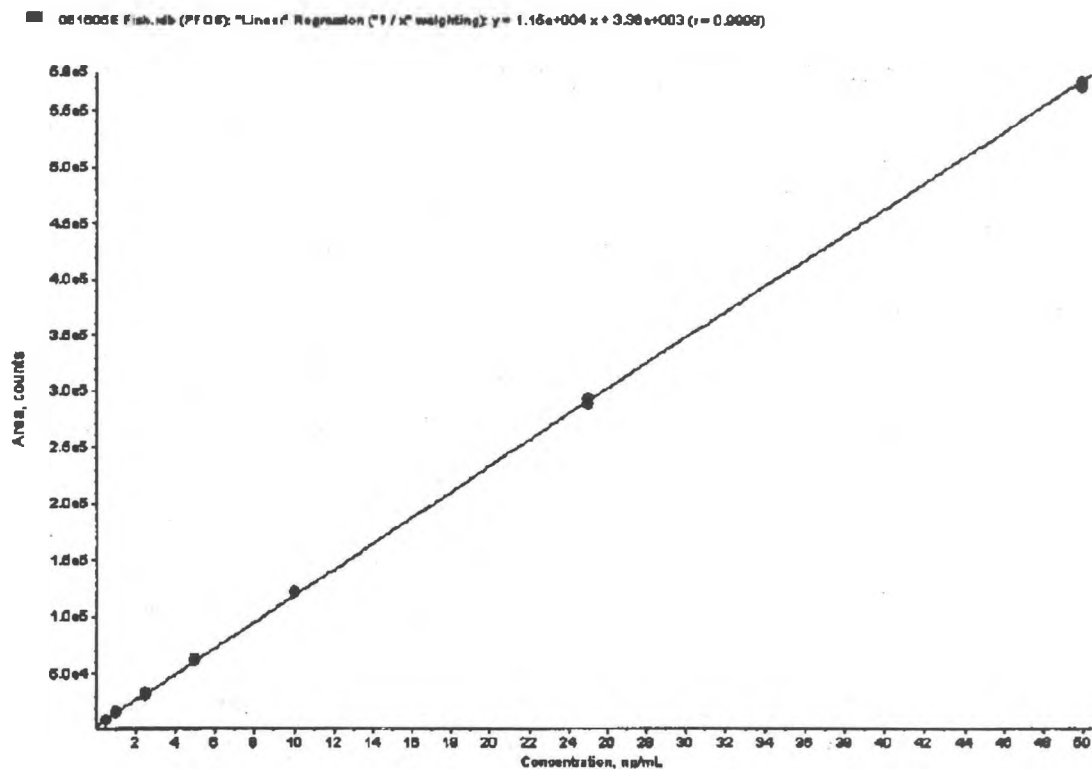
Figure 9. Typical Calibration Curve for PFOS in Methanol

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

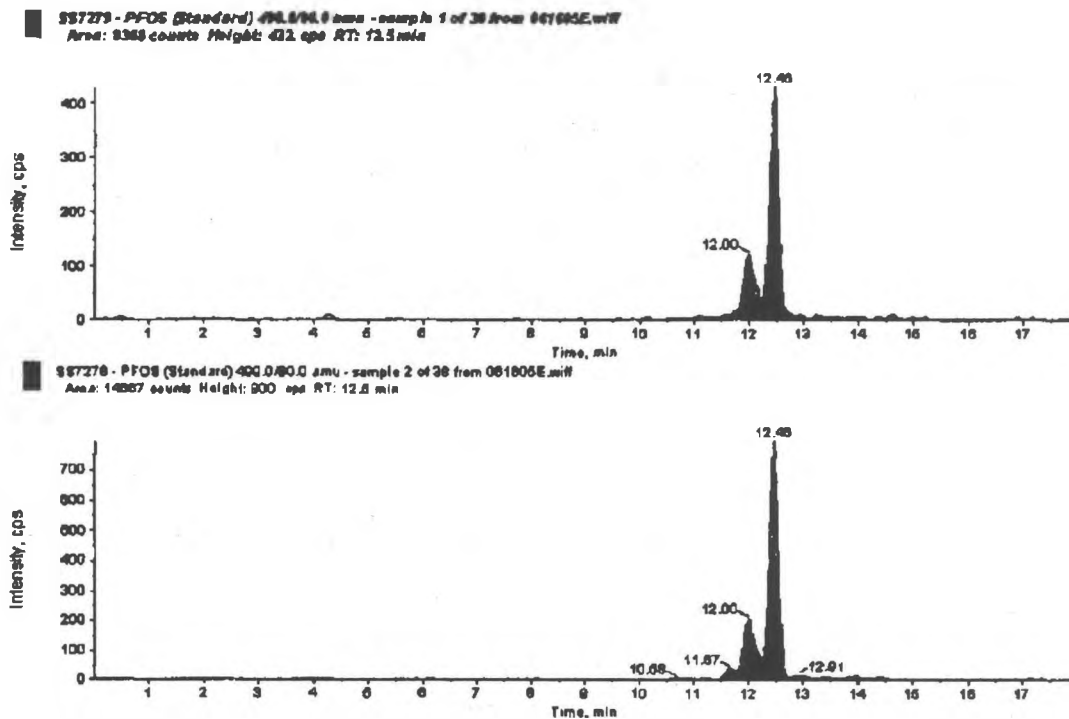


Figure 11. PFOS in Control Fish Blank, 0.00625 µg/mL Fortified Control Fish Spk A, and 0.025 µg/mL Fortified Control Fish Spk B, Respectively

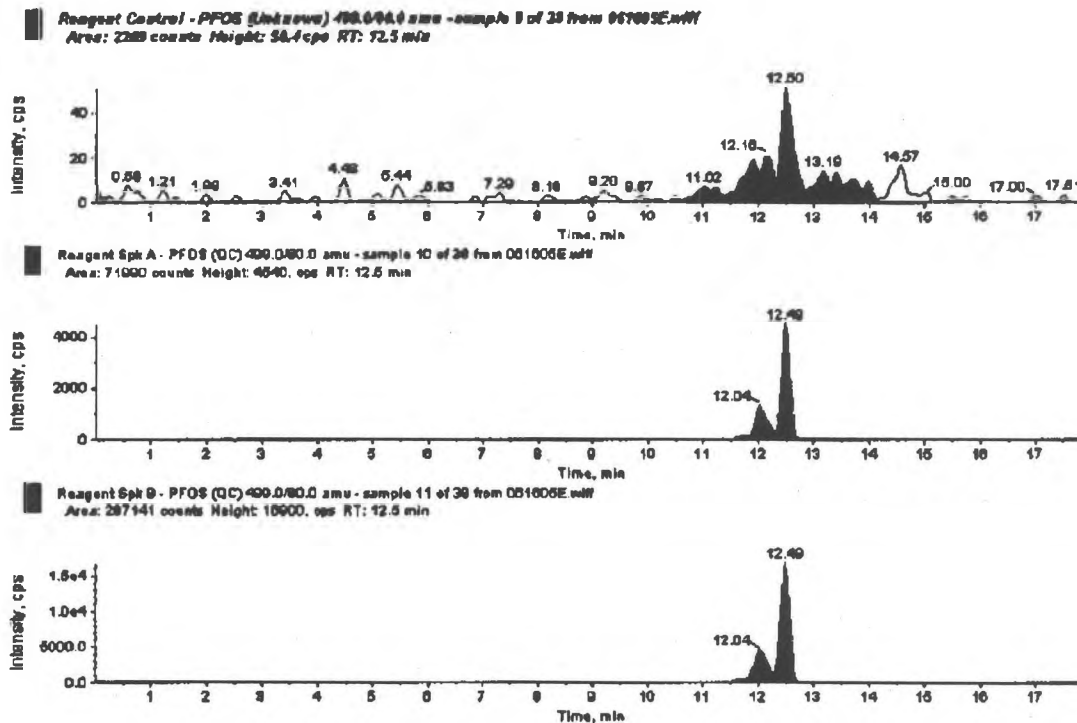
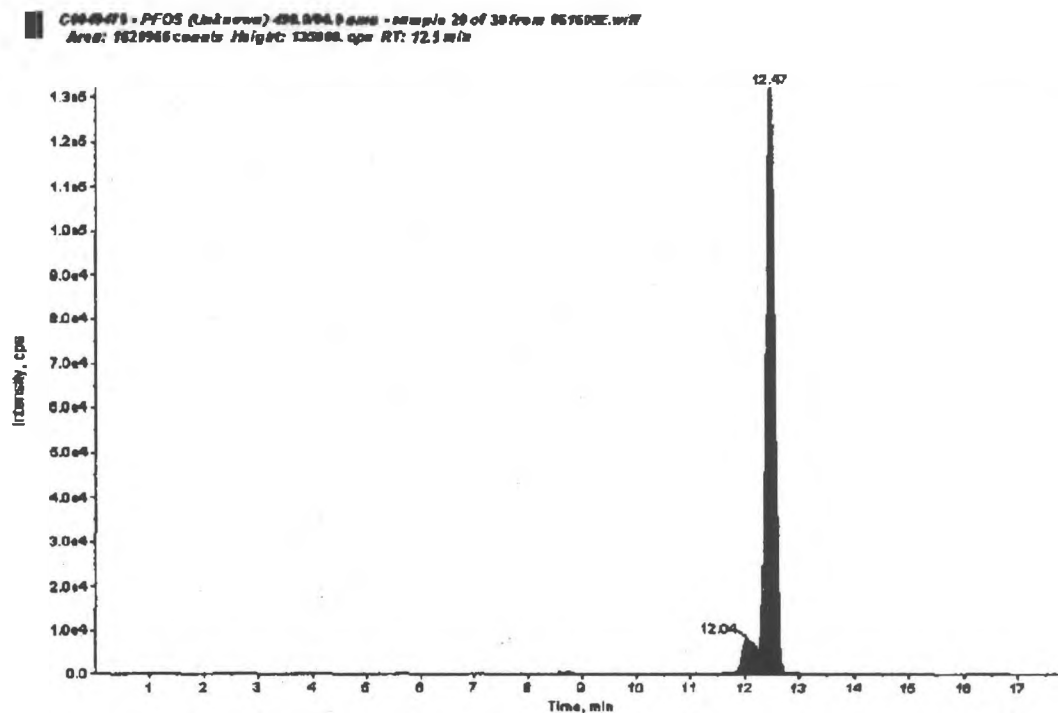


Figure 12. Chromatogram Representing a Fish Sample Analyzed for PFOS (Exygen ID: C0049478, Data Set:061605E)



APPENDIX A

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

Exygen Protocol Number: P0001131

STUDY PROTOCOL

Study Title:

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

Exygen Protocol Number: P0001131

Performing Laboratory:

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

Sponsor Representative:

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

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Exygen Protocol Number: P0001131

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

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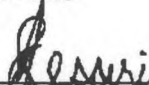
Exygen Protocol Number: P0001131

PROTOCOL APPROVAL

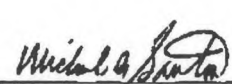
Study Title: Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Livers and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program

Exygen Protocol Number: P0001131

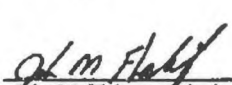
APPROVALS


Jaishma Kesari, Study Director
Weston Solutions

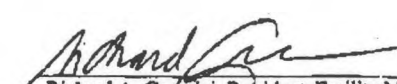
11/5/04
Date


Michael A. Santoro, Sponsor Representative
3M Company

11/11/04
Date


John M. Flaherty, Principal Investigator
Exygen Research

10/29/04
Date


Richard A. Grizzini, President, Facility Management
Exygen Research

29-Oct-2004
Date


Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research

10/29/04
Date

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Exygen Protocol Number: P0001131

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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

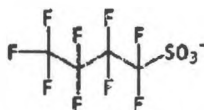
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3^- K^+$)

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:



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PFOS

Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 338 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

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State College, PA 16801
Phone: (814) 272-1039

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Exygen Protocol Number: P0001131

STUDY DIRECTOR

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PRINCIPAL INVESTIGATOR

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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.

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IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

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

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

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

SAMPLE PROCUREMENT, RECEIPT AND RETENTION

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

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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

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

For soil, sediment, clams, and vegetation, Exygen will supply one 500 mL pre-cleaned 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

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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).

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- 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).

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AMENDMENTS TO PROTOCOL

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

DATA RECORD KEEPING

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

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

Chromatograms- All chromatograms will contain the following:

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

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- 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.

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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"

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ANALYTICAL METHOD
Method Number: V9001780

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

Analytical Testing Facility: Exygen Research
3038 Research Drive
State College, PA 16801

Approved By:

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

08/26/04
Date

John Fishery
John Fishery
Vice President, Operations, Exygen Research

08/26/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: Y0001788

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 Acetonitrile - A.C.S. Reagent Grade
- 4.4 Perfluorooctanoic Acid - Sigma-Aldrich

5.0 Instruments 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 utilize raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polystyrene centrifuge tubes.
- 5.5 15 mL disposable polystyrene centrifuge tubes.
- 5.6 Disposable microcentrifuge (10-100 µL, 100-200 µL).
- 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 or (1g) C18 SPE cartridges.

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Exygen Research

Method Number V9001788

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: Fluoropak RP (Krytox Scientific), 3.1 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 0.134 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

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Exygen Research

Method Number V0001756

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water by LC/MS/MS****9.0 Standard Preparation****9.1 Standard Stock/Portification 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 (µg/L)	Fortification Volume (µL)	Volume of Purified Control Sample (mL)	Final Concentration of Calibration Standard (µg/L)*	Calibration Standard ID (example)
0	0	40	0	XCumidivvy-0
10	100	40	25	XCumidivvy-1
10	200	40	50	XCumidivvy-2
10	400	40	100	XCumidivvy-3
100	100	40	250	XCumidivvy-4
100	200	40	500	XCumidivvy-5
100	400	40	1000	XCumidivvy-6

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0801180

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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.
- 11.0 Sample Extraction
- 11.1 Measure 40 mL of sample or a portion of sample diluted to 40 mL with water into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and stir well).
- 11.3 Condition the C_{18} SPE cartridge (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.
- 11.5 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 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. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001786

ANALYTICAL METHOD

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

various calibration standard concentrations using MassLynx 3.3 (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 50 ng/L, then a new blank sample must be obtained and the entire set must be re-extracted.
- 13.3 Replication 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 spikes outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Grubbs 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the run must be reanalyzed.

14.0 Calculations

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

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

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0807720

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method Number: V0001781

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

Analytical Testing Facility:

Exygen Research
1038 Research Drive
State College, PA 16801

Approved By:

Paul C. Kelly
Paul C. Kelly
Technical Leader, LC-MS, Exygen Research

10/11/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/11/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number Y0001781

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirements

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

- 5.1 A high performance liquid chromatograph capable of pumping up to 2 solvents equipped with a variable volume injector capable of injecting 5-200 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable pol. propylene centrifuge tubes.
- 5.5 15 mL. dry coat 15 polypropylene centrifuge tubes.
- 5.6 Disposable micro pipets (50-100 μ L, 100-200 μ L).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pk Vac 6 on (1g) μ C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Ultrasonic bath.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001761

ANALYTICAL METHOD

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

- 5.14 Write-solen driver.
 5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 5000 rpm.
- 6.0 Chromatographic System
- 5.1 Analytical Column: Fluorophen RP (KeyStone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 825d3-052130)
 6.2 Temperature: 30°C
 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
 6.4 Mobile Phase (B): Methanol
 5.9 Gradient Program:

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

- 6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL)
 6.7 Quantitation: Peak Area - external standard calibration curve.
 6.8 Run Time: ~ 25 minutes.

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions

8.1 Mobile Phase

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

Alternate volumes may be prepared.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V6001761

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 (µg/L)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (ng/L)	Calibration Standard ID (Reference)
0	0	40	0	XCalib00000-0
10	100	40	25	XCalib00000-1
10	200	40	50	XCalib00000-2
10	400	40	100	XCalib00000-3
100	100	40	250	XCalib00000-4
100	200	40	500	XCalib00000-5
100	400	40	1000	XCalib00000-6

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

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Exygen Research	Method Number V0001781
ANALYTICAL METHOD	
Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS	
9.2.3	A zero standard solution (reagent blank) must be prepared with each set of standards analyzed.
9.2.4	Store all extracted calibration standards in 15-mL polypropylene tubes at 2°C to 6°C, up to two weeks.
9.2.5	Alternate volumes and concentrations of standards may be prepared as needed.
10.0	Batch Set Up
10.1	Each batch of samples extracted (typically 20 or less) must include at least one reagent control (method blank using 5 mL of methanol) and two reagent controls fortified at known concentrations (lab control spikes) to verify procedural recovery for the batch.
10.2	Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.
11.0	Sample Extraction
11.1	Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, vialson 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 cartridge (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 via the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
12.2	Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
12.3	An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of

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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

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 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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.

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

13.3 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 Hughes River Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.

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

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0051731

ANALYTICAL METHODMethod 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.

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).

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

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

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001782

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

Analytical Testing Facility: Exygen Research
3038 Research Drive
State College, PA 16801

Approved By:

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

10/28/04
Date

John Fishery
John Fishery
Vice President, Operations, Exygen Research

10/28/04
Date

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Exygen Protocol Number: P0001131

Exygen Research	Method Number V8001713
ANALYTICAL METHOD	
Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Sediment by LC/MS/MS	
1.0	Scope
	This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometer (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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
5.2	A device to collect raw data for peak integration and quantitation.
5.3	A 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 microcylinders (50-100 μ L, 100-200 μ L).
5.7	125-mL LDPE narrow-mouth bottles.
5.8	2 mL glass HPLC vial kit.
5.9	Disposable pipettes.
5.10	Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
5.11	Waters Sep Pak Vac 6 or (1g) AC18 SPE cartridges.
5.12	SPE vacuum manifold.

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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: Fluoropak RP (Krytox Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-052120)
 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)
0.5	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 → 360 m/z for PFOA.

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number Y00017E2

ANALYTICAL METHOD

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

8.2 Extraction Solutions

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

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/fortification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125 mL LDPE bottle.
- 9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

- 9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.
- 9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

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

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Exygen Research

Method Number V0001782

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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.
- 11.0 Sample Extraction
- 11.1 Weigh 5 g of sample into 50 mL polystyrene 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~2 drops/sec). Do not let column run dry.
- 11.5 Load (decant) the sample on the conditioned C₁₈ 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~2 drops/sec). Do not let column run dry.
- 11.11 Add the sediment to ~200 mL of water and load on the second conditioned SPE cartridge.
- 11.12 Rinse with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 5 mL polystyrene centrifuge tubes (final volume ~5 mL).
- 11.13 Analyze samples using electrospray LC/MS/MS.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0801182

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 sample injections.
- 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 estimated 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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.
- 13.3 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hugh 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 30% of the total number of extracted standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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Exygen Research

Method Number V0001782

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

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

14.0 Calculations

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

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

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

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

Recovery (%) =

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

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

$$\text{PFOA found (ppb)} = \frac{\text{PFOA found (ng/mL)} \times \text{final volume (1 mL)}}{\text{sample weight (1 g)}}$$

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

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

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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
3038 Research Drive
State College, PA 16801

Approved By:

P. J. Cull
Paul Cull
Technical Leader, LC-MS, Exygen Research

11/14/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

11/14/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001783

ANALYTICAL METHOD

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

1.0 Scope

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

2.0 Safety

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

3.0 Sample Requirement

- 3.1 At least 20 g of 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 frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis.
- 3.3 Sample collection procedures will be specified in the sampling plan for this project.

4.0 Reagents and Standards

- 4.1 Water - HPLC grade
- 4.2 Acetonitrile - HPLC grade
- 4.3 Carbon (120-400 mesh) - Reagent grade
- 4.4 Methanol - HPLC grade
- 4.5 Nitrogen gas (60-200 mesh) - Reagent grade
- 4.6 Picric acid (60-100 mesh) - Reagent grade
- 4.7 Supraclean LC-NH₂ - Reagent grade
- 4.8 1-Octanol - HPLC grade
- 4.9 L-Ascorbic acid - Reagent grade
- 4.10 Dimethylsiloxane - Reagent grade
- 4.11 Toluene - Reagent grade
- 4.12 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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.

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ANALYTICAL METHOD

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

- 5.4 Rotary evaporator.
- 5.5 Tissue/ass.
- 5.6 125 mL pear-shaped flask.
- 5.7 50 mL disposable polypropylene centrifuge tubes.
- 5.8 15 mL disposable polypropylene centrifuge tubes.
- 5.9 Disposable micropipets (50-100µL, 100-200µL).
- 5.10 125-mL LDPE narrow-mouth bottles.
- 5.11 3 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. NS57177).
- 5.15 Vial action dilutor.
- 5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Flucphase RP (Larystone Scientific), 2.1 mm x 50 mm, 5µ (PN: E2505-052136)
- 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
20.8	25	75	0.3
22.5	65	35	0.3

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

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

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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% Dimethylchloroethane in toluene is prepared by bringing 3 mL of dimethylchloroethane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Spiking 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 µL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
9.1.5 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

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Exygen Research

Method Number Y0001743

ANALYTICAL METHOD

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

9.2 Standard Calibration Solutions

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

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

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

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 8°C, up to six months.

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

10.0 Sample Set Up

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

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

11.0 Sample Extraction

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

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

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

11.4 Thaw and condition the SPE tubes and equilibrate the pear-shaped flasks.

11.5 Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g anisole, 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 Eluate the 125 mL pear-shaped flasks by rinsing with the 50% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

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Exygen Research

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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-shaped flask.
 - 11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat phase using a douncer for ~30 seconds and rinse the douncer with ~10 mL of acetonitrile into the tube.
 - 11.10 Shake the sample again for ~10 minutes on a wrist-action shaker.
 - 11.11 Place the tubes in a freezer for ~1 hour more.
 - 11.12 Centrifuge the 50 mL polypropylene tubes containing sample at ~2000 rpm for ~10 minutes.
 - 11.13 Decant the extract onto the same SPE column. Collect the eluate into the same pear-shaped flask and combine with the eluate from the initial extraction.
 - 11.14 Pass 20 mL of acetonitrile through the SPE column and combine the eluate in the same pear-shaped flask.
 - 11.15 Add 3-4 drops of 1-octanol to the extract in the pear-shaped flask and evaporate at reduced pressure using a rotary evaporator (at < 40°C).
 - 11.16 Make the final volume, by adding 1 mL of 2% ascorbic acid in methanol in 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 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 analysis by linear regression using 1/x weighting of peak area versus calibration standard concentration using Minitab 13.3 (or equivalent) software system.

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Exygen Research

Method Number V0001131

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-analyzed.
- 13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-analyzed.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hugh Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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.

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Exygen Research

Method Number V0001131

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method Number: V0001784

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

Analytical Testing Facility:

Exygen Research
3851 Ransack Drive
State College, PA 16801

Approved By:


Paul Cosandry
Technical Leader, LC-MS, Exygen Research


Date


John Flaherty
Vice President, Operations, Exygen Research


Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001754

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS****1.0 Scope**

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

- 4.1 Water - HPLC grade
- 4.2 Acetonitrile - HPLC grade
- 4.3 Carbon (120-400 mesh) - Reagent grade
- 4.4 Methanol - HPLC grade
- 4.5 Silica gel (60-200 mesh) - Reagent grade
- 4.6 Florisil (60-100 mesh) - Reagent grade
- 4.7 Superclean LC-NH₂ - 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 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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001784

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

- 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 (50-100µL, 100-200µL).
- 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. N857177).
- 5.14 What column shaker.
- 5.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Fluorophase RP (Kayton Scientific), 2.1 mm x 50 mm, 5µ (P/N: 02505-052130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 1 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

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

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

7.0 MS/MS System

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001784

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

8.2 Extraction Solutions

- 8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.
- 8.2.2 30% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Fortification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (assumed 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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001784

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

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

Concentration of Perfluorooctanoic Acid (ppm)	Volume (ml)	Diluted to (ml)	Final Concentration (ppm)
1.0	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	0.01
0.005	10	100	0.0005

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

9.3.4 Adequate 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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 5 g of 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 Rack and condition the SPE tubes and silanize the pear-shaped flasks.
- 11.5 Peak the 20 mL SPE tubes in sequence with 2 g florist, 2 g silica gel, 2 g carbon, and 1 g LC-NH₂. Condition the column with 20 mL of methanol, then 20 mL of acetonitrile. Discard all wastes. Do not allow the column to dry.
- 11.6 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyl-dichlorosilane 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-shaped flask.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001794

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

- 11.8 Add 20 mL of methanol 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 extractions.
 - 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 standard spray 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 analysis 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 m/z from a parent of 413 m/z. The 413 m/z parent ion corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001764

ANALYTICAL METHOD

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

- 13.3 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 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hoge River Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run, then the 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.

Recovery (%) =

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001783

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

Analytical Testing Facility: Exygen Research
3658 Research Drive
State College, PA 16801

Approved By:

P.J. Connelly
Paul Connelly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date

A.M. Field
A.M. Field
Vice President, Operations, Exygen Research

10/26/04
Date

Total Pages: 7

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001765

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Marine 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 marine liver.

2.0 Safety

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

3.0 Sample Requirements

- 3.1 At least 5 g of test sample for extraction.
- 3.2 Samples should be processed before extraction. Place the frozen sample in a food processor and homogenize with dry ice. Place the samples in containers and leave open in frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternatively, 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 mL/min equipped with a variable volume injector capable of injecting 5-20 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 (50-100 µL, 100-200 µL).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001181

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Marine 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 or (1g) C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Titration.
- 5.14 Vortex-action shaker.
- 5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Phosphor RP (Kymene Scientific), 2.1 mm x 50 mm, 3µ (P/N: 02305-022130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

- 6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
- 6.7 Quantitation: Peak Area - external standard calibration curve.
- 6.8 Run Time: ~ 25 minutes.

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

7.0 MS/MS System

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

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001785

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS****8.0 Preparation of Solutions****8.1 Mobile Phase**

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

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Fortification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute in 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 µL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a minimum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

- 9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.
- 9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (µL)	Diluted to (mL)	Final Concentration (µg/mL)
100	1.0	100	1.0
100	2.0	100	2.0
100	1.0	100	1.0
1.0	10	100	0.1
1.0	10	100	0.1

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

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number Y0001793

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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 1 g of sample into a 50 mL polypropylene centrifuge tubes (fortify as desired, replace lid and mix well). Note that alternate weights of liver may be necessary depending on the sample size available for use.
- 11.2 Add water to the sample for a final volume of 10 mL.
- 11.3 Homogenize sample using a homogenizer for ~1 minute.
- 11.4 Transfer 1 mL of the sample using a disposable pipette into a 15 mL disposable centrifuge tube.
- 11.5 Add 5 mL of acetonitrile and shake for ~30 minutes on a wrist-action shaker.
- 11.6 Centrifuge the tubes at ~3000 rpm for ~5 minutes.
- 11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
- 11.8 Condition the C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~3 drops/sec). Do not let column run dry.
- 11.9 Load the sample on conditioned C₁₈ 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 Quantities 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 analysis by linear regression using 1/x weighting of peak area.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001785

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Mammal Liver by LC/MS/MSrun on calibration standard concentration using MammLynx 3.3 (or equivalent)
software system.13.5 Sample responses should not exceed standard responses. Any samples that
exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

13.1 Chromatogram must show a peak of a daughter ion at 369 m/z from a parent
of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the
daughter ion (369 m/z) represents the loss of carbon dioxide.13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a
blank contains PFOA at levels greater than 10 ng/g, then a new blank sample
must be obtained and the entire set must be re-extracted.13.3 Recovery of control spikes and matrix spikes must be between 70-130% of
their known values. If a control spike falls outside the acceptable limits, the
entire set of samples should be re-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 Nage
Beyer Test, may be excluded from the calculation of the calibration curve.
However, the total number of calibration standards that could be excluded
must not exceed 20% of the total number of standards injected.13.5 The correlation coefficient (R) for calibration curves generated must be
≥0.992 (R² ≥0.983). 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 Mamm 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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001784

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

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

Recovery (%) =

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

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

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD

Method Number: V0001786

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

Analytical Testing Facility:

Exygen Research
3038 Research Drive
State College, PA 16801

Approved By:

Paul Connolly
Paul Connolly
Technical Lead/LC-MS, Exygen Research

10/16/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/16/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001754

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS****1.0 Scope**

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

2.0 Safety

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

3.0 Sample Requirements

- 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 Acetonitrile Acetate - A.C.S. Reagent Grade
- 4.5 Perfluorooctanoic Acid - Sigma-Aldrich

5.0 Instrument and Equipment

- 5.1 A high performance liquid chromatograph capable of pumping up to 2 mL/min equipped with a variable volume injector capable of injecting 5-200 μ L, connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polypropylene centrifuge tubes.
- 5.5 15 mL disposable polypropylene centrifuge tubes.
- 5.6 Disposable micropipets (50-100 μ L, 100-200 μ L).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Vortexer.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001766

ANALYTICAL METHOD

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

5.14 Vial-action shaker.

5.15 Centrifuge capable of spinning 15 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System6.1 Analytical Column: Platisphere RP (Kymene Scientific), 2.1 mm x 50 mm, 5 μ (P/N: 82505-032130)

6.2 Temperature: 30°C

6.3 Mobile Phase (A): 1 mM Ammonium Acetate in Water

6.4 Mobile Phase (B): Methanol

6.5 Gradient Program:

Time (min)	%A	%B	Flow Rate (mL/min)
0.5	45	35	0.3
1.0	45	35	0.3
8.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

6.6 Injection Volume: 15 μ L (can be increased to as much as 50 μ L).

6.7 Quantitation: Peak Area - external standard calibration curve.

6.8 Run Time: ~ 23 minutes.

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

7.0 MS/MS System7.1 Mode: Electrospray Negative MRM mode, monitoring 413 $\rightarrow$ 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 1 mM ammonium acetate in water is prepared by adding 0.154 g of ammonium acetate to 1000 mL of water.

Alternate volumes may be prepared.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0801786

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/Portification Solutions**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 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 (µL)	Diluted to (mL)	Final Concentration (ng/mL)
100	5.0	100	5.0
100	2.5	100	2.5
100	1.0	100	1.0
1.0	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 (in control spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicate and spikes will be specified in the quality assurance plan for this project.

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Exygen Protocol Number: P0001131

Exygen Extract

Method Number: V0001766

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 tube (verify 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 1 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
- 11.6 Centrifuge the tube at ~3000 rpm for ~5 minutes.
- 11.7 Remove the supernatant into a 50 mL disposable centrifuge tube and add 15 mL of water.
- 11.8 Condition the C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~2 drops/sec). Do not let column run dry.
- 11.9 Load the sample on conditioned C₁₈ 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 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 A 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/n weighting of peak area versus calibration standard concentration using Minitab 3.3 (or equivalent) software system.
- 12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001796

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS****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-analyzed.
- 13.3 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spikes outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hugs Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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 = 20

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

Recovery (%) =

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

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Page 64 of 65

Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001706

ANALYTICAL METHOD

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

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

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

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Page 65 of 65



3058 Research Drive
State College, PA 16801

Phone: 814-272-1039
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PROTOCOL AMENDMENT

Amendment Number: 1

Effective Date: 01/19/05

Exygen Study Number: P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

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

AMENDED TO

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

RATIONALE

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

IMPACT ON STUDY

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

Study Director Signature

Principal Investigator Signature

Study Director/Amendment Signature

Sponsor Signature (if required)

3/9/05

Date 2/2/05

Date 2/2/05

Date 2/18/05

Date 1

Exygen QAU Review LJS 02/18/05

LIBRARY ID: VV0001228-6

ADMINISTRATIVE FORM

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

Amendment Number:

2

Page 1 of 1

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, *max 10/15/05*
- 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

Principal Investigator Signature

Date

Study Director Management Signature

Date

Exygen Management Signature

Date

Sponsor Signature (if required)

Date

Exygen QAU Review

LJS 03/08/05

LIBRARY ID: V0001226-8

ADMINISTRATIVE FORM

09-20-2005 02:35pm From: NESTON SOLUTIONS

T-304 P.003/004 F-427

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

DEVIATION FORM

Page 1 of 1

General:	
☒ Project Specific Deviation	☐ Facility Deviation
Date of Occurrence: 08/29/05	
Exygen Project # : P780/P1131	Deviation # : 2/2
Client Project # : NA	Reference # : 05-133 <i>from 9/20/05</i>
Regulatory Driver:	Deviation Type: (include V# for methods and SOPs)
☒ GMP ☒ GLP ☐ Other ☐ None	☒ Protocol ☐ Method ☐ SOP V#: NA Notebook reference: NA
Sample Description:	
Lot # : L4264/L4265	Container # : C0056480-85 Lot # : NA
Summary of Deviation:	
The protocol states that control and fortified control samples of each matrix will be analyzed; however, control clam was not obtained. Fish was used as the control for the analysis of these six clam samples.	
Cause: ☐ Preparation ☐ Analysis ☐ Instrument ☐ Client Request ☒ Other	
Impact:	
No negative impact on this study.	
Corrective Actions:	
Deviation issued.	
Signatures:	
<i>[Signature]</i> Principal Investigator	<i>[Signature]</i> Exygen Management
<i>[Signature]</i> Study Director	<i>[Signature]</i> Sponsor Representative
<i>[Signature]</i> Quality Assurance	<i>[Signature]</i> Sponsor Management
Date: 9/20/05	Date: 9/20/05

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

T-384 P.084/004 F-437

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

DEVIATION FORM

Page 1 of 1

General:			
☒ Project Specific Deviation		☐ Facility Deviation	
Date of Occurrence: 12/27/04			
Exygen Project #: P760/P1131		Deviation #: 3/3	
Client Project #: NA		Reference #: 05-134 ^{Rev. 1} 9/20/05	
Regulatory Driver:		Deviation Type: (Include V# for methods and SOPs)	
☒ GMP ☐ GLP ☐ Other ☐ None		☐ Protocol ☐ Method ☒ SOP V#: 202-20 Section 5.2.3 Notebook reference: NA	
Sample Description:			
Login #: 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 ☒ 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		 Exygen Management	
Date: 9/20/05		Date: 9/20/05	
 Study Director		 Sponsor Representative	
Date: 9/20/05		Date: NA	
 Quality Assurance		 Sponsor Management	
Date: 9/20/05		Date: NA	

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

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

STUDY SPONSOR

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

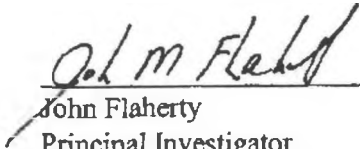
PROJECT

Protocol Number: P0000760
Exygen Study Number: P0000760

Total Pages: 110

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

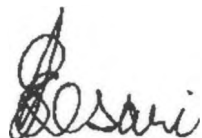
Exygen Study Number P0000760, entitled "Analysis of Perfluorooctanoic Acid (PFOA) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," conducted for 3M Company, is being performed in compliance with EPA TSCA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



John Flaherty
Principal Investigator
Exygen Research

5/2/06

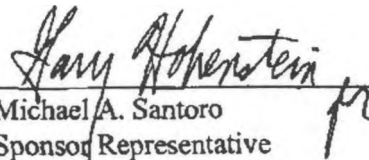
Date



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

5/3/06

Date



Michael A. Santoro
Sponsor Representative
3M Company

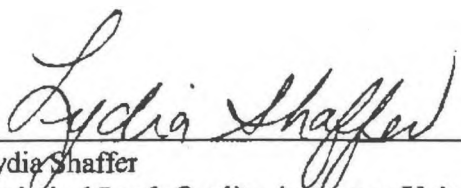
5/4/06

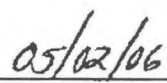
Date

QUALITY ASSURANCE STATEMENT

Exygen Research's Quality Assurance Unit reviewed Exygen Study Number 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 phases¹ were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

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


Date

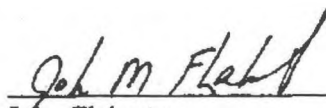
¹Note: All in-lab inspections will be documented in the QA statement for the final analytical report at the conclusion of the study. This QA statement involves only the review of the interim report and associated raw data.

CERTIFICATION OF AUTHENTICITY

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

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

Principal Investigator, Exygen:

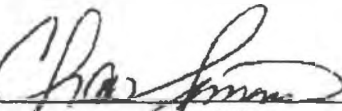


John Flaherty
Vice President
Exygen Research

5/2/06

Date

Exygen Research Facility Management:

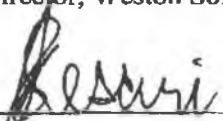
 FOR

Richard A. Grazzini
President
Exygen Research

5/2/06

Date

Study Director, Weston Solutions, Inc.



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

5/3/06

Date

Sponsor Representative, 3M Company:



Michael A. Santoro
Director of Regulatory Affairs

5/4/06

Date

STUDY IDENTIFICATION

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

PROTOCOL NUMBER: P0000760

EXYGEN STUDY NUMBER: P0000760

TYPE OF STUDY: Residue

SAMPLE MATRIX: 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:

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

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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 $\pm$ standard deviation for PFOA in sediment samples based on wet weight was $73 \pm 16\%$. 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 $\pm$ standard deviation for ^{13}C PFOA in sediment samples based on wet weight was $97 \pm 27\%$. 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, ¹³C labeled perfluorooctanoic acid (¹³C 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 ¹³C PFOA was stored frozen.

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

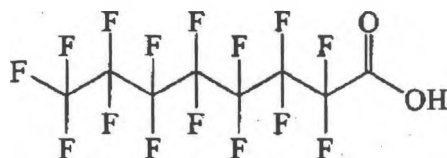
PFOA

Chemical Name: Perfluorooctanoic acid

Molecular Weight: 414

Transitions Monitored: 413 → 369 (for quantification) and
413 → 219 (for confirmation)

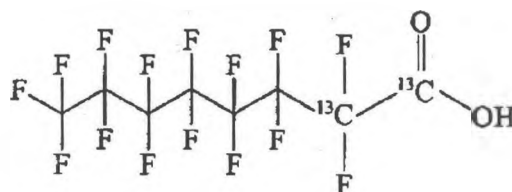
Structure:

**¹³C 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 C₁₈ 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 C₁₈ 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 C₁₈ 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 C₁₈ 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 ¹³C 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 ($\mu\text{g/mL}$) ¹	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Fortification Std. ($\mu\text{g/mL}$)
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 ¹³C PFOA

A stock solution of only ¹³C PFOA was also prepared at a concentration of 100 $\mu\text{g/mL}$ 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 ($\mu\text{g/mL}$) ¹	Fort Volume (mL)	Final Volume (mL)	Final Conc. of Fortification Std. ($\mu\text{g/mL}$)
100	10	100	10
10	10	100	1.0
1.0	10	100	0.1

¹ of ¹³C PFOA

A set of non-extracted calibration standards containing PFOA and ¹³C 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 ¹³C 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 ($4^{\circ} \pm 2^{\circ}\text{C}$) 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

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

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

<u>Analyte</u>	<u>Mode</u>	<u>Transition Monitored</u>	<u>Approximate Retention Time (min)</u>
PFOA	negative	413 → 369	~11.1 min.
PFOA Confirm Ion	negative	413 → 219	~11.1 min.
¹³ C 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 ¹³C 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:

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:

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

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:

$$\begin{aligned} \text{Analyte found (ng/L)} &= \frac{[236588 - 4630] \times 100}{420} \\ &= 55200 \text{ ng/L} \end{aligned}$$

From equation 3:

$$\begin{aligned} \text{PFOA found (ppb)} &= \frac{(55200 \text{ ng/L} \times 0.04 \text{ L})}{[5 \text{ mL} \times (1 \text{ g} / 10 \text{ mL})]} \\ &= 4420 \text{ ppb} \end{aligned}$$

From equation 3:

$$\begin{aligned} \% \text{ Recovery} &= \frac{(4420 \text{ ng/g} - 586 \text{ ng/g})}{4000 \text{ ng/g}} \times 100\% \\ &= 96\% \end{aligned}$$

From equation 4:

$$\begin{aligned}\text{PFOA found (ppb) dry weight} &= 4416 \text{ ppb} \times (100\% / 66.54\%) \\ &= 6640 \text{ ppb}\end{aligned}$$

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 $\pm$ standard deviation for PFOA in sediment samples based on wet weight was $73 \pm 16\%$. 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 $\pm$ standard deviation for ^{13}C PFOA in sediment samples based on wet weight was $97 \pm 27\%$. 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		Assessed Accuracy (+/- %)
		C8 Acid PFOA	Perfluorooctanoic Acid	
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		Assessed Accuracy (+/- %)
		C8 Acid PFOA	Perfluorooctanoic Acid	
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

Sample Description	Amount Spiked (ng/g)	C8 Acid PFOA		
		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

Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	C8 Acid PFOA	
			Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172882 Spk D, 4000 ppb Spike)	4000	NR	NR	NR
DAL-SD-BPP01-0-0000 (C0172882 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

Sample Description	Amount Spiked (ng/g)	Amt Found in Sample (ng/g) wet weight	CB Acid PFOA	
			Amount Recovered (ng/g) wet weight	Recovery (%)
DAL-SD-BPP01-0-0000 (C0172882 Spk D, 4000 ng/g Spike)	4000	588	4420	96
DAL-SD-BPP01-0-0000 (C0172882 Spk E, 40000 ng/g Spike)	40000	588	25800	83
DAL-SD-BPP02-0-0000 (C0172883 Spk G, 4000 ng/g Spike)	4000	504	3880	84
DAL-SD-BPP02-0-0000 (C0172883 Spk H, 40000 ng/g Spike)	40000	504	25100	61
DAL-SD-BPP03-0-0000 (C0172884 Spk J, 4000 ng/g Spike)	4000	1080	4140	77
DAL-SD-BPP03-0-0000 (C0172884 Spk K, 40000 ng/g Spike)	40000	1080	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

Exygen ID	Sample Description	Amount Spiked (ng/g)	^{13}C -PFOA	
			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

Exygen ID	Sample Description	Amount Spiked (ng/g)	^{13}C -PFOA	
			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

Exygen ID	Sample Description	Amount Spiked (ng/g)	^{13}C -PFOA	
			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	25800	65
C0172893	DAL-SD-BPP02-0-0000	40	47.8	120
C0172893 Rep	DAL-SD-BPP02-0-0000	40	51.8	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

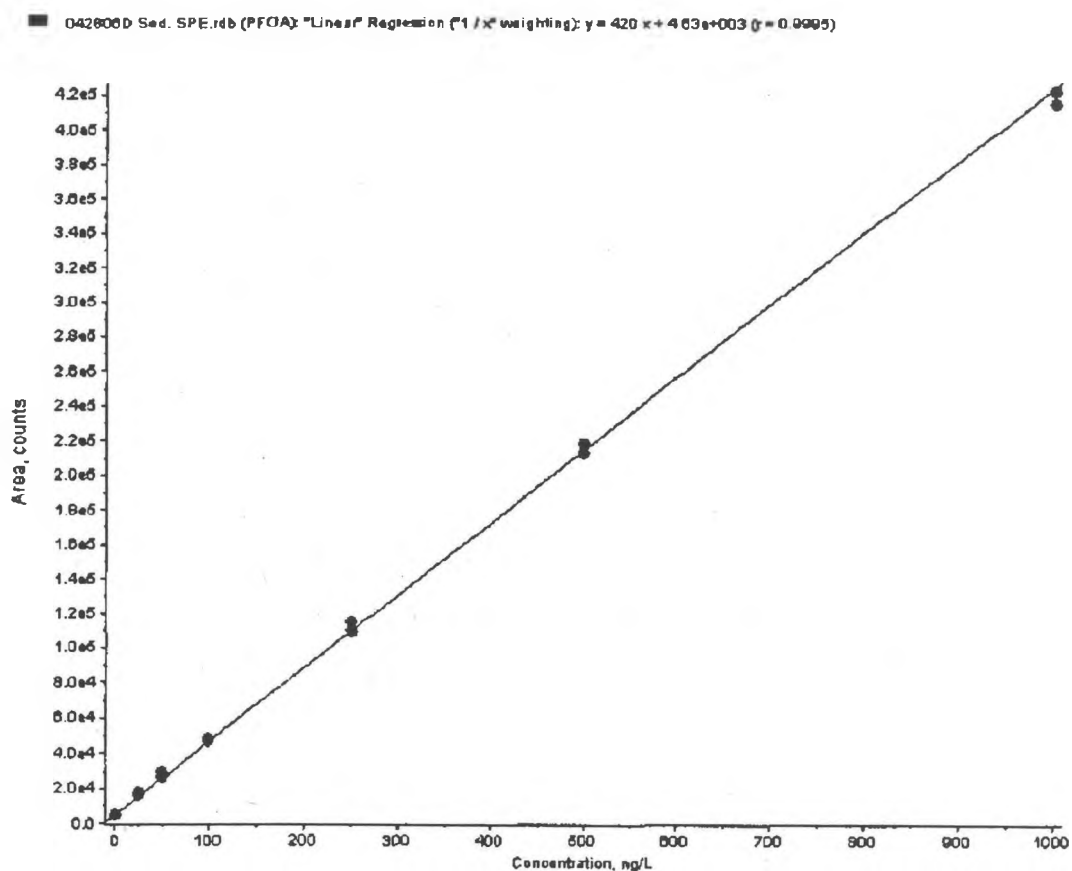
Figure 1. Typical Calibration Curve for PFOA in Methanol

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

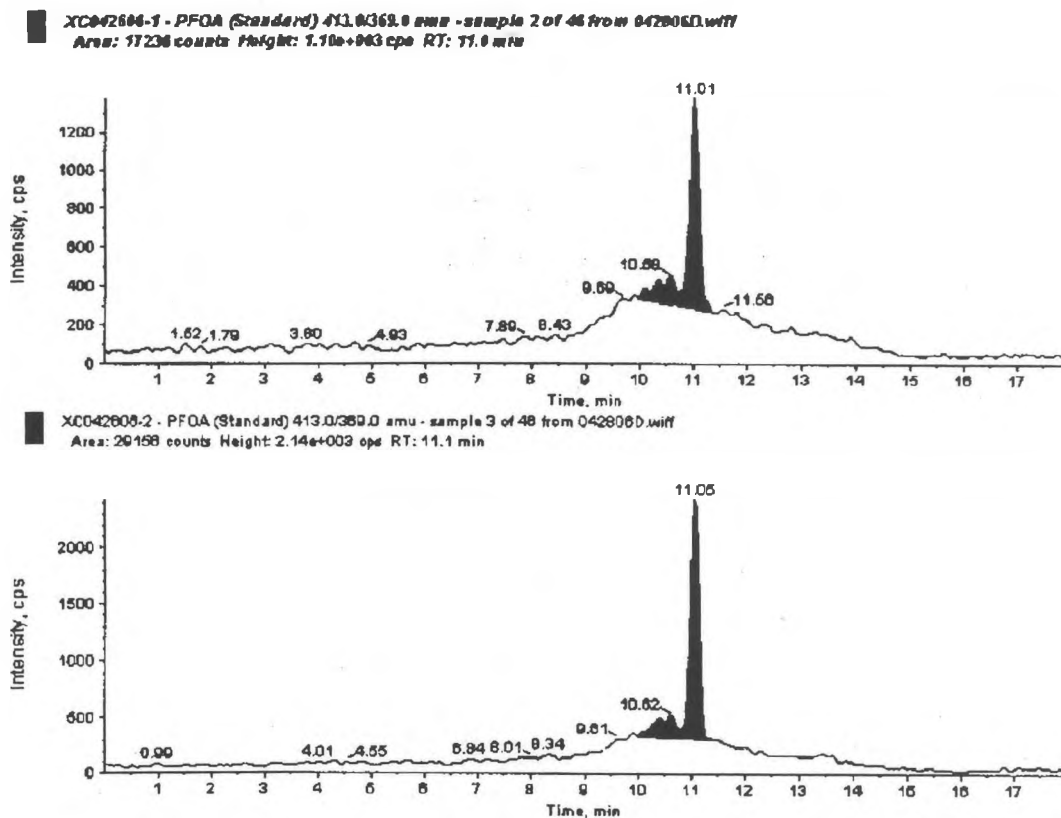


Figure 3. PFOA in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

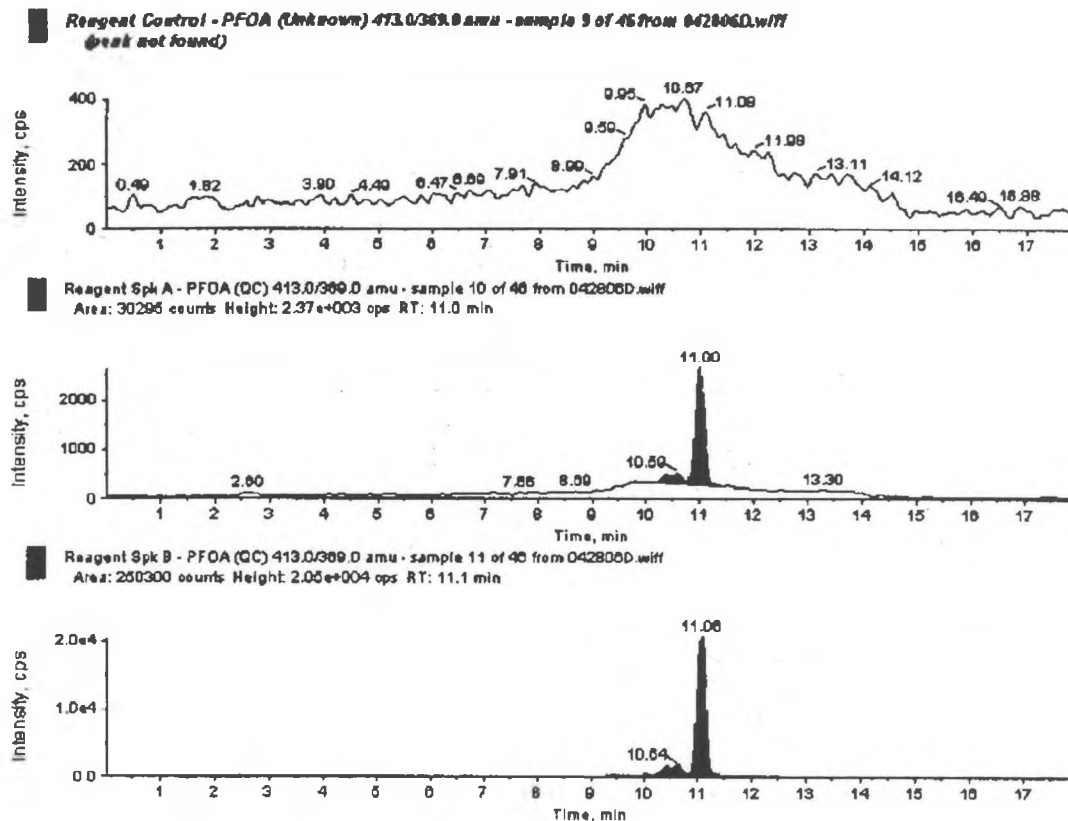


Figure 4. Chromatogram Representing a Sediment Sample Analyzed for PFOA (Exygen ID: C0172892, Data Set: 042806D)

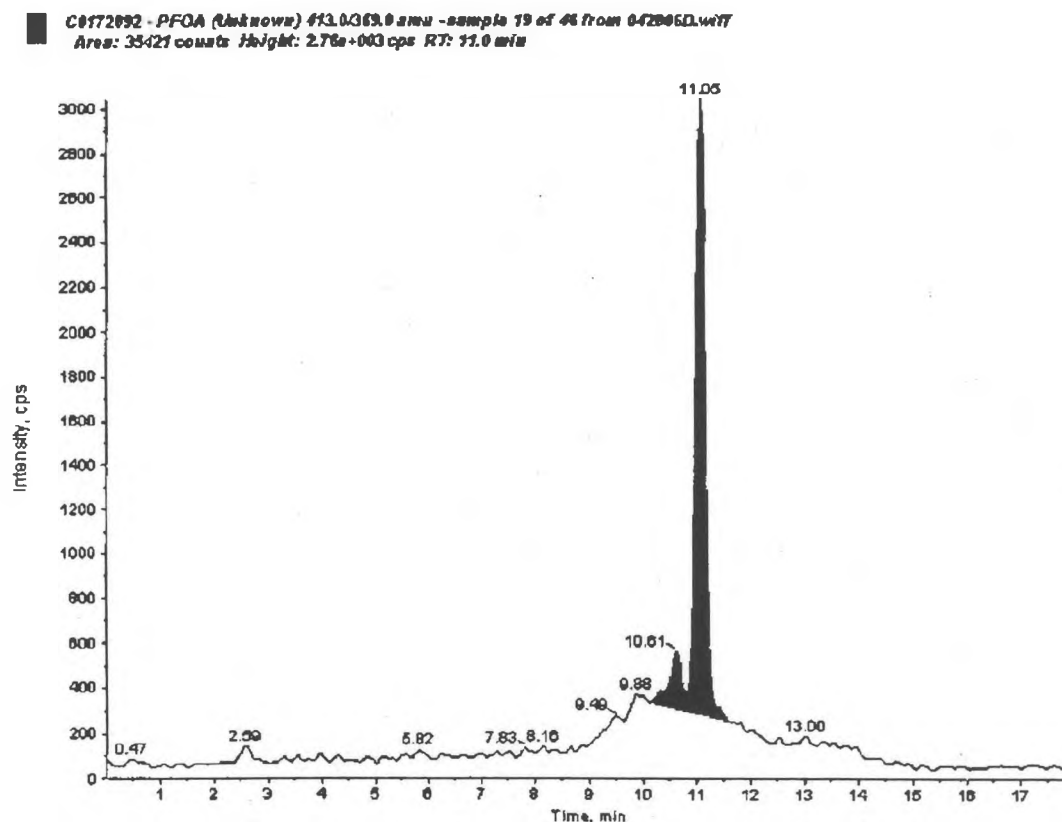


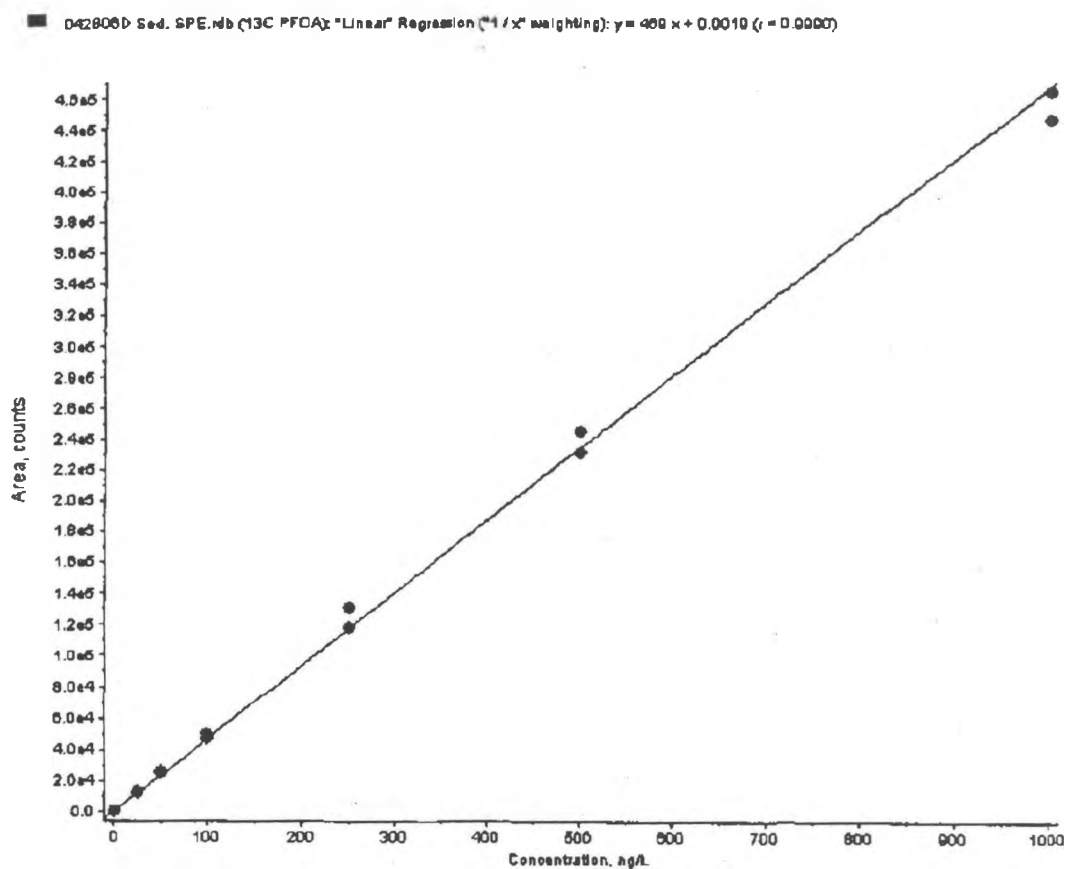
Figure 5. Typical Calibration Curve for ^{13}C PFOA in Methanol

Figure 6. Extracted Standards of ^{13}C PFOA in Methanol, 25 ng/L and 50 ng/L, Respectively

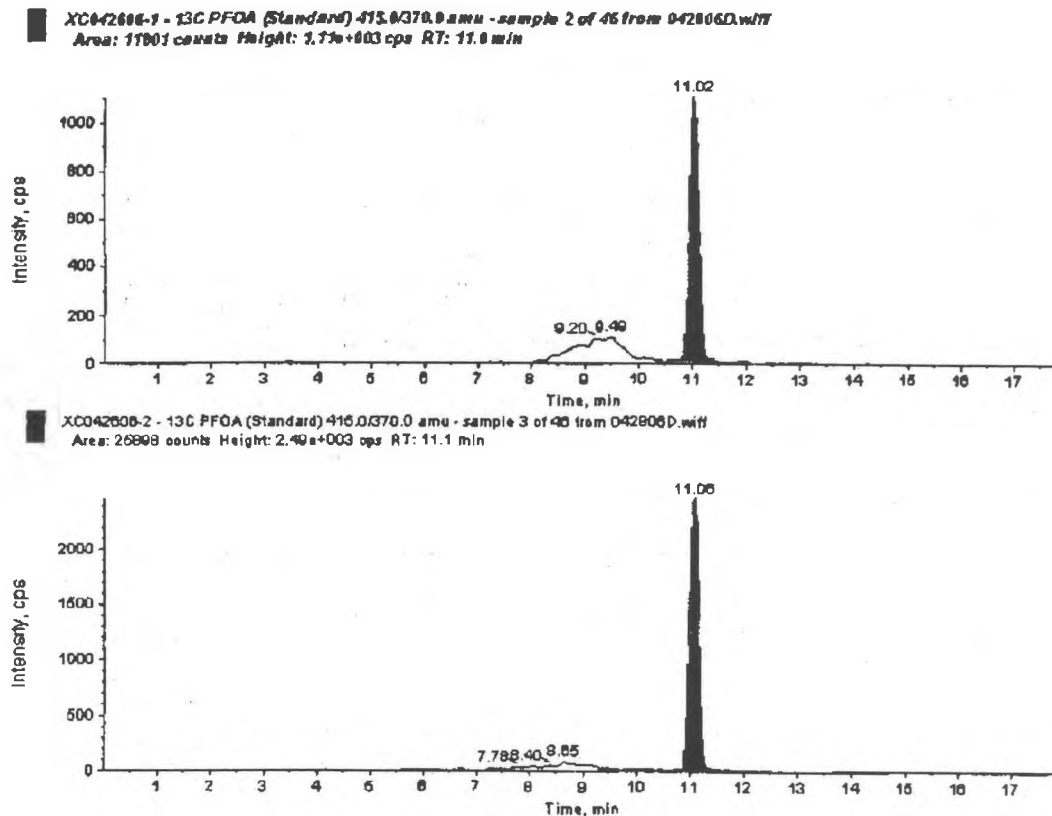


Figure 7. ^{13}C PFOA in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

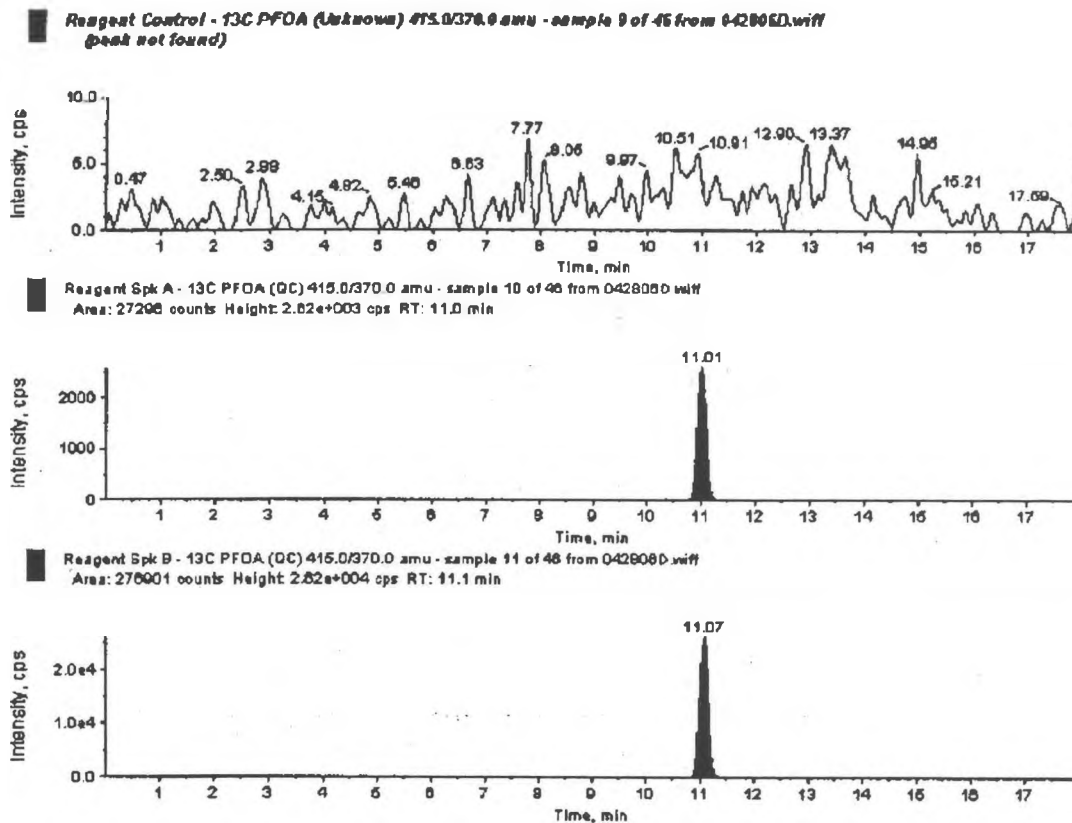
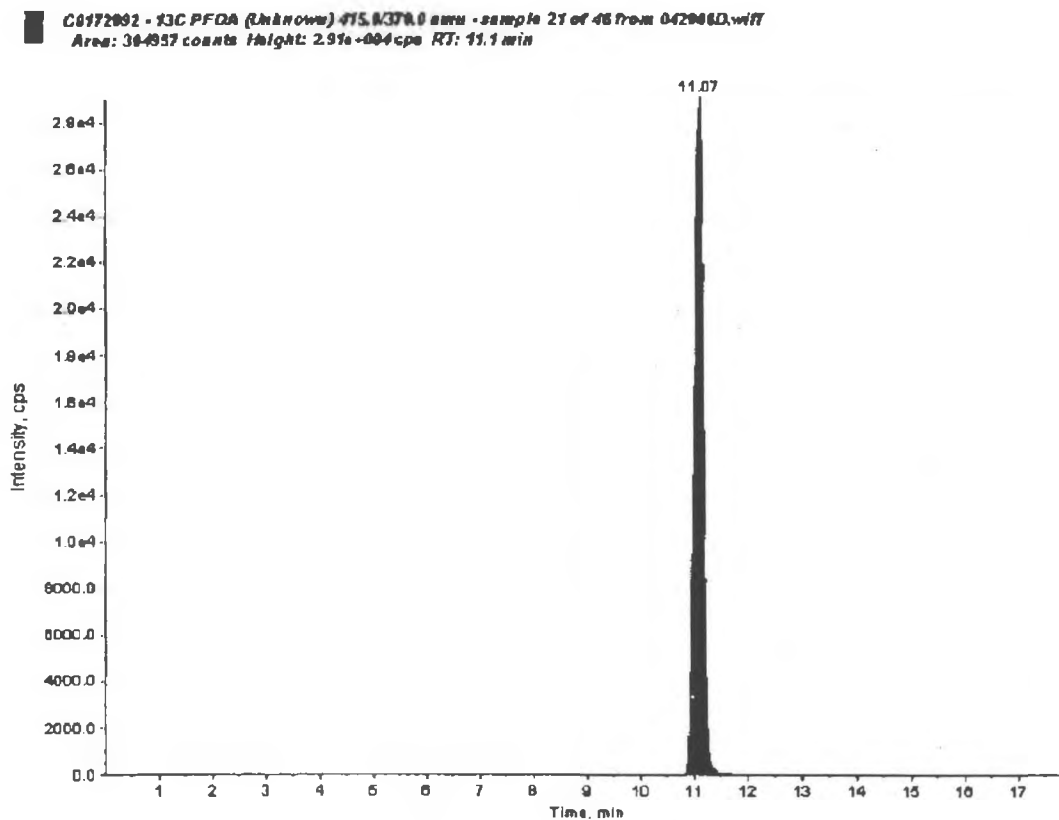


Figure 8. Chromatogram Representing a Sediment Sample Analyzed for ^{13}C PFOA (Exygen ID: C0172892, Data Set: 042806D)



APPENDIX A

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

Exygen Protocol Number: P0000760

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

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Exygen Protocol Number: P0000760

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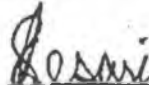
Exygen Protocol Number: P0000760

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

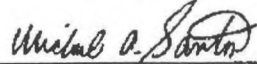
APPROVALS



Jaisimha Kesari, Study Director
Weston Solutions



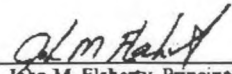
Date



Michael A. Sanjoro, Sponsor Representative
3M Company



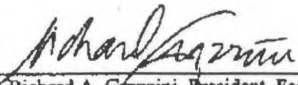
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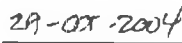
John M. Flaherty, Principal Investigator
Exygen Research



Date



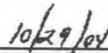
Richard A. Grazzini, President, Facility Management
Exygen Research



Date



Lydia Shaffer, Technical Lead, Quality Assurance Unit
Exygen Research



Date

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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

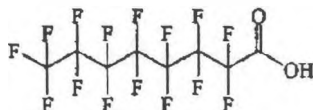
Molecular Weight: 414

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 ¹³C labeled perfluorooctanoic acid (¹³C PFOA) and was purchased by 3M from Perkin-Elmer Life and Analytical Sciences.

¹³C PFOA

Chemical Name: 1,2-¹³C perfluorooctanoic acid

Molecular Weight: 416

Lot Number: 3507-195

Purity: 97%

Transition Monitored: 415 → 370

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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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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

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

The test systems were chosen to assess the environmental impact of 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 on 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.

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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 ¹³C 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

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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:

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- 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:

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- 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).

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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).

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- 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 Oxygen 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, Oxygen 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 Oxygen Research.

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

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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"

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ANALYTICAL METHOD

Method Number: V0001780

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

Analytical Testing Facility:

Exygen Research
1088 Research Drive
State College, PA 16801

Approved By:

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

10/24/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/24/04
Date

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Exygen Research

Method Number V0001780

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFDA) 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 Requirements

- 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 Acetic Acid – 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 5-100 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polypropylene centrifuge tubes.
- 5.5 15 mL disposable polypropylene centrifuge tubes.
- 5.6 Disposable micropipettes (50-100 μ L, 100-200 μ L).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges.

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Exygen Research

Method Number V0001780

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 (Kaysons Scientific), 2.1 mm x 50 mm, 5µ (PN: 82505-052130)

6.2 Temperature: 30°C

6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water

6.4 Mobile Phase (B): Methanol

6.5 Gradient Program:

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

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).

6.7 Quantitation: Peak Area – external standard calibration curve.

6.8 Run Time: ~ 23 minutes.

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

Alternate volumes may be prepared.

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Exygen Research

Method Number V000770

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 (µg/L)	Fortification Volume (µL)	Volume of Fortified Control Sample (mL)	Pinal Concentration of Calibration Standard (ppt)*	Calibration Standard ID (example)
0	0	40	0	XChromdly-0
10	100	40	25	XChromdly-1
10	200	40	50	XChromdly-2
10	400	40	100	XChromdly-3
100	100	40	250	XChromdly-4
100	200	40	500	XChromdly-5
100	400	40	1000	XChromdly-6

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

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Exygen Research

Method Number V0001750

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

- 9.2.3 A new 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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Measure 40 mL of sample or a portion of sample diluted to 40 mL with water into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
- 11.2 Condition the C₁₈ SPE cartridge (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 C₁₈ SPE cartridge. Discard eluate.
- 11.4 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
- 11.5 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

- 12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
- 12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
- 12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area

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Method Number: V0001760

ANALYTICAL METHOD

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

versus calibration standard concentration using Mass Lynx 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 Hugs 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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:

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

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

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Exygen Research

Method Number: V0001180

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001781

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

Analytical Testing Facility:

Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

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

10/24/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/24/04
Date

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001781

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS****1.0 Scope**

This method is to be employed for the isolation and quantization 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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantization.
- 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 micropress (50-100 μ L, 100-200 μ L).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipette.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pak Vac 6 cc (12) μ C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Ultrasonic bath.

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ANALYTICAL METHODMethod 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: Fluorophase RP (Kinesis Scientifics), 2.1 mm x 50 mm, 5 μ (P/N: 82505-032130)
6.2 Temperature: 30°C
6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4 Mobile Phase (B): Methanol
6.5 Gradient Program:

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

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

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

7.0 MS/MS System

- 7.1 Mode: Electrospray Negative MRM mode, monitoring 413 $\rightarrow$ 369 m/z for PFOA.

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

Alternate volumes may be prepared.

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Exygen 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 (ppb)*	Calibration Standard ID (example)
0	0	40	0	XCm00dyy-0
10	100	40	25	XCm00dyy-1
10	200	40	50	XCm00dyy-2
10	400	40	100	XCm00dyy-3
100	100	40	250	XCm00dyy-4
100	200	40	500	XCm00dyy-5
100	400	40	1000	XCm00dyy-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.

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Exygen Research

Method Number V0001721

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 Absolute 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 tubes.
- 11.5 Centrifuge for ~10 minutes at ~3000 rpm.
- 11.6 Condition the C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~2 drops/sec). Do not let column run dry.
- 11.7 Load (decant) the sample on the conditioned C₁₈ SPE cartridge. Discard eluate.
- 11.8 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume ~5 mL).
- 11.9 Analyze samples using electrospray LC/MS/MS.
- 12.0 **Chromatography**
- 12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
- 12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
- 12.3 An entire set of extracted calibration standards must be included at the beginning and at the end of a sample set. Extracted standards must be interspersed between every 5-10 samples. As an alternative, an entire set of

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Exygen Research

Method Number V0001781

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

- extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (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 spikes outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

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Exygen Research

Method Number: V0001781

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.

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).

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

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

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

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Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001782

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

Analytical Testing Facility:

Exygen Research
3038 Research Drive
State College, PA 16801

Approved By:

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

10/16/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/16/04
Date

Total Pages: 9

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001182

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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polypropylene centrifuge tubes.
- 5.5 15 mL disposable polypropylene centrifuge tubes.
- 5.6 Disposable micropipets (50-100 μ L, 100-200 μ L).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges.
- 5.12 SPE vacuum manifold.

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Exygen Research

Method Number V0001782

ANALYTICAL METHODMethod 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: Fluorophase RP (Keyscience Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82563-052130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0881762

ANALYTICAL METHODMethod 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 reagent standard (assessed for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 10 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 10 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.5 A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.6 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

- 9.2.1 LC/MS/MS calibration standards are prepared in 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

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Exygen Research

Method Number V0001782

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 LIXPE 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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 5 g of sample into 50 mL polypropylene centrifuge tubes (fortify as needed, replace lid and mix well).
- 11.2 Add 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 ~30 minutes.
- 11.4 Condition the C₁₈ 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 (desorb) the sample on the conditioned C₁₈ 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 ~30 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~2 drops/sec). Do not let column run dry.
- 11.11 Add the methanol to ~200 mL of water and load on the second conditioned SPE cartridge.
- 11.12 Elute with ~5 mL 100% methanol. Collect 5 mL of eluate into graduated 15 mL polypropylene centrifuge tubes (final volume = 5 mL).
- 11.13 Analyze samples using electrospray LC/MS/MS.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001712

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 Minitab 3.3 (or equivalent) software system.
- 12.5 Sample responses should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

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 Repetition 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 Hughes-Box 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0061763

ANALYTICAL METHOD

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

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

14.0 Calculations

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

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

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

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

Recovery (%) =

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

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

$$\text{PFOA found (ppb)} = \frac{(\text{PFOA found (ng/mL)} \times \text{final volume (1 mL)})}{\text{sample weight (1 g)}}$$

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

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

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Exygen Protocol Number: P0000760

ANALYTICAL METHOD

Method Number: V000178J

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

Analytical Testing Facility:

Exygen Research
1058 Research Drive
State College, PA 16801

Approved By:

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

11/26/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

11/26/04
Date

Total Pages: 5

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Exygen Protocol Number: P0000760

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 Requirements

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

4.0 Reagents and Standards

- 4.1 Water - HPLC grade
- 4.2 Acetonitrile - HPLC grade
- 4.3 Carbon (120-400 mesh) - Reagent grade
- 4.4 Methanol - HPLC grade
- 4.5 Silica gel (60-200 mesh) - Reagent grade
- 4.6 Florisil (60-100 mesh) - Reagent grade
- 4.7 Supellean LC-NH₂ - Reagent grade
- 4.8 1-Octanol - HPLC grade
- 4.9 L-Ascorbic acid - Reagent grade
- 4.10 Dimethyldichlorosilane - Reagent grade
- 4.11 Toluene - Reagent grade
- 4.12 Ammonium Acetate - A.C.S. Reagent Grade
- 4.13 Perfluorooctanoic Acid - Sigma-Aldrich

5.0 Instrument and Equipment

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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001731

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 Thermometer.
- 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 (50-100 μ L, 100-200 μ L).
- 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 μ L and 10-100 μ L), 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: Fluorophase RP (Keystone Scientific), 2.1 mm i.d. 50 mm, S_{μ} (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 μ L (can be increased to as much as 50 μ L).
- 6.7 Quantitation: Peak Area – external standard calibration curve
- 6.8 Run Time: ~ 23 minutes

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

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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****7.0 MS/MS System**

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

8.2 Extraction Solutions

- 8.2.1** 2% acetic acid in methanol is prepared by dissolving 2 g of acetic acid in 100 mL of methanol.
- 8.2.2** 10% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Portification 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.

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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
0.25	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 spikes) to verify procedural recovery for the batch.

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

11.0 Sample Extraction

11.1 Weigh 5 g of 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-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 Silanize the 125 mL pear-shaped flasks by rinsing with the 30% dimethyldichlorosilane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V8001783

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 standardized pear-shaped flask.
- 11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the frozen fat phase using a tissueizer for ~30 seconds and rinse the tissueizer 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 ($\leq 40^{\circ}\text{C}$).
- 11.16 Make the final volume, by adding 2 mL of 2% ascorbic acid in methanol to the pear-shaped flask and swirl to mix/dissolve.
- 11.17 Transfer the extracts to HPLC vials using disposable pipets.
- 11.18 Analyze samples using electrospray LC/MS/MS.

12.0 Chromatography

- 12.1 Inject the same amount of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all analyzed samples.
- 12.2 Standards of PFOA corresponding to at least five or more concentration levels must be included in an analytical set.
- 12.3 An entire set of calibration standards must be included at the beginning and at the end of a sample set. Standards must be interspersed between every 5-10 samples. As an alternative, an entire set of calibration standards may be injected at the beginning of a set followed by calibration standards interspersed every 5-10 samples (to account for a second set of standards). In either case, calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.

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Exygen Protocol Number: P0000760

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

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

13.0 Acceptance Criteria

- 13.1 Chromatograms must show a peak of a daughter ion at 369 amu from a parent of 413 amu. The 413 amu parent corresponds to the PFOA anion, while the daughter ion (369 amu) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 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 Hughes-Box Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards on the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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.

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Exygen Protocol Number: P0000760

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**

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

Recovery (%) =

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

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Oxygen 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:

Oxygen Research
3058 Research Drive
State College, PA 16801

Approved By:

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

10/26/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Oxygen Research

10/26/04
Date

Total Pages: 7

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001784

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS****1.0 Scope**

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

- 4.1 Water – HPLC grade
4.2 Acetonitrile – HPLC grade
4.3 Carbon (120-400 mesh) – Reagent grade
4.4 Methanol – HPLC grade
4.5 Silica gel (60-200 mesh) – Reagent grade
4.6 Florisil (60-100 mesh) – Reagent grade
4.7 Superclean LC-NH₂ – 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 μ L connected to a tandem Mass Spectrometer (LC/MS/MS)
5.2 A device to collect raw data for peak integration and quantitation.
5.3 Analytical balance capable of reading to 0.00001 g.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001784

ANALYTICAL METHOD

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

- 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 (50-100 μ L, 100-200 μ L).
- 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 μ L and 10-100 μ L), 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: Fluorobond RP (Keystone Scientific), 2.1 mm x 50 mm, 5 μ m (P/N: K2505-052130)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

- 6.6 Injection Volume: 15 μ L (can be increased to as much as 50 μ L).
- 6.7 Quantitation: Peak Area – external standard calibration curve.
- 6.8 Run Time: ~ 25 minutes.

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

7.0 MS/MS System

- 7.1 Mode: Electrospray Negative MRM mode, monitoring 413 $\rightarrow$ 369 m/z for PFOA.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V000178

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 optimise 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% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Fortification Solutions**

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

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

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

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

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

9.2 Standard Calibration Solutions

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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0081794

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 (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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (sterilize 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 minimize the pear-shaped flasks.
- 11.5 Pack the 30 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g carbon, and 1 g LC-NH₂. Condition the column 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% dimethylchlorosilane 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 fixed inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shaped flask.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001784

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

- 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 Deposit 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 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.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001734

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 Hugs Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.984$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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).

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

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0000760

ANALYTICAL METHOD
Method Number: V0001785

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

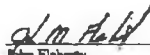
Analytical Testing Facility:

Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date


John Flaherty
Vice President, Operations, Exygen Research


Date

Total Pages: 7

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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 Requirements

- 3.1 At least 5 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 frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternatively, 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 (50-1000µL, 100-2000µL).
- 5.7 125 mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001765

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 or (1g) C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Tissue grinder.
- 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: Phosphane RP (Kaysone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82505-032130)
- 6.2 Temperature: 50°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
2.0	25	75	0.3
20.0	25	75	0.3
22.5	65	35	0.3

- 6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL)
- 6.7 Quantitation: Peak Area – external standard calibration curve.
- 6.8 Run Time: ~25 minutes.

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

7.0 MS/MS System

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

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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V9001723

ANALYTICAL METHODMethod 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 (µg/mL)	Volume (µL)	Diluted to (mL)	Final Concentration (ng/mL)
100	1.0	100	1.0
100	2.0	100	2.0
100	1.0	100	1.0
5.0	10	100	0.5
2.0	10	100	0.2
1.0	10	100	0.1

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

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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001783

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS****10.0 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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 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 tissue mixer 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 9 mL of acetonitrile and shake for ~20 minutes on a wrist-action shaker.
- 11.6 Centrifuge the tubes at ~1000 rpm for ~3 minutes.
- 11.7 Decant the supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.
- 11.8 Condition the C₁₈ 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 C₁₈ 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 = 3 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 quantification. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001783

ANALYTICAL METHOD

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

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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/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 Hoge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.983$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001781

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Marine Livers 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).

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

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Exygen Protocol Number: P0000760

ANALYTICAL METHOD

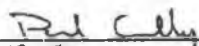
Method Number: V0001786

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

Analytical Testing Facility:

Exygen Research
3058 Research Drive
Scotts College, PA 16001

Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/01
Date


John Flaherty
Vice President, Operations, Exygen Research

11/1/01
Date

Total Pages: 7

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001726

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 Requirements

- 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 3-200 μ L connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polypropylene centrifuge tubes.
- 5.5 15 mL disposable polypropylene centrifuge tubes.
- 5.6 Disposable micropipets (50-100 μ L, 100-200 μ L)
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipettes (100-1000 μ L and 10-100 μ L), with disposable tips.
- 5.11 Waters Sep Pak Vae 6 cc (1g) C_{18} SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Vortexer.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: P000178a

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

- 5.14 Vial-action shaker.
5.15 Centrifuge capable of spinning 13 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Fluorphase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (PN: K2505-051138)
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 PFQA.

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

Alternate volumes may be prepared.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number V0001788

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS****9.0 Standard Preparation****9.1 Standard Stock/Fortification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

- 9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.
- 9.2.2 The following is a typical example: additional concentrations may be prepared as needed.

Concentration of Fortification Solution (µg/mL)	Volume (mL)	Diluted to (mL)	Final Concentration (ng/mL)
100	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 spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

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Exygen Protocol Number: P0000760

Exygen Research

Method Number: V0001726

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 C₁₈ SPE cartridge (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 C₁₈ 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/n weighting of peak area versus calibration standard concentration using MassLynx 3.3 (or equivalent) software system.
- 12.5 Sample response should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

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Exygen Protocol Number: P0000760

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

13.0 Acceptance Criteria

- 13.1 Chromatogram must show a peak of a daughter ion at 369 *m/z* from a parent of 413 *m/z*. The 413 *m/z* parent corresponds to the PFOA anion, while the daughter ion (369 *m/z*) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.
- 13.3 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-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 Hoge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (*R*) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards of the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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:

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

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

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

Recovery (%) =

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

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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

- 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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Phone: 814-272-1039
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 9.1.8, 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

Principal Investigator Signature

Safety Director/Management Signature

Sponsor Signature (If required)

Date

Date

Date

Date

Date

Date

Exygen QAU Review 1/25 02/01/05

LIBRARY ID: VV0001226-6

ADMINISTRATIVE FORM



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PROTOCOL AMENDMENT

Amendment Number: 2 Page 1 of 1
Effective Date: 03/07/05
Exogen Study Number: P0001131 Client Study Number: None

DESCRIPTION OF AMENDED SECTION

- 1) Report, page 11 of 85
- 2) Test Materials, page 6 of 85: PFO8 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 → 800.

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 ~~means~~ *offer as the*
- 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: _____

Principal Investigator Signature

Date _____

Study Director Management Signature

Date _____

Oxygen Management Signature

Date _____

Sponsor Signature (if required)

Date _____

Oxygen CAU Review: LTJ asbestos

LIBRARY ID: V0001228-8

ADMINISTRATIVE FORM

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

DEVIATION FORM

Page 1 of 2

General:		
☒ Project Specific Deviation	☐ Facility Deviation	Date of Occurrence: 04/28/08
Exygen Project #: P760/P1131		Deviation #: 5
Client Project #: NA		Reference #: 06-076
Regulatory Driver:		Deviation Type: (Include V# for methods and SOPs)
☐ GMP	☒ Protocol	☐ Method ☐ SOP
☒ GLP		
☐ Other	V#: NA	
☐ None	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 ¹³C 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 ¹³C PFOA were still poor. Another alternative method was then used for PFOA and ¹³C 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 C₁₈ 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 ☒ Other		
Impact:		
More usable data was obtained.		

LIBRARY ID: V0001640-6

ADMINISTRATIVE FORM

CHEM EHSR 236 1B 651 733 1958
06-01-2008 09:01pm Free-TESTON SOLUTIONS

05/01 '06 13:46 NO.106 03/03
+ T-214 P.003/003 F-219

Exygen

RESEARCH

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DEVIATION FORM

General		Form 214-1	
☒ Project Specific Deviation	☐ Facility Deviation	Date of Occurrence: 04/26/06	
Exygen Project #:	PTES/P1121	Deviation #:	7
Client Project #:	NA	Reference #:	
Administrative Actions: Deviation Issued.			
Signatures:			
<i>[Signature]</i>	<i>[Signature]</i>	<i>[Signature]</i>	4-26-06
Principal Investigator	Date	Exygen Management	Date
<i>[Signature]</i>	5/1/06	<i>[Signature]</i>	5/1/06
Study Director	Date	Sponsor Representative	Date
<i>[Signature]</i>	5/1/06	NA	
Quality Assurance	Date	Sponsor Management	Date

LIBRARY ID: V0001840-8

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

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INTERIM REPORT COMPLETION DATE

May 2, 2006

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PROJECT

Protocol Number: P0001131
Exygen Study Number: P0001131

Total Pages: 110

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

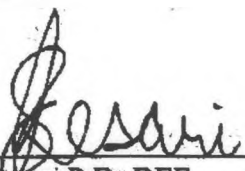
Exygen Study Number P0001131, entitled "Analysis of Perfluorobutanesulfonate (PFBS), Perfluorohexanesulfonate (PFHS), and Perfluorooctanesulfonate (PFOS) in Water, Soil, Sediment, Fish, Clams, Vegetation, Small Mammal Liver and Small Mammal Serum Using LC/MS/MS for the 3M Decatur Monitoring Program," 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

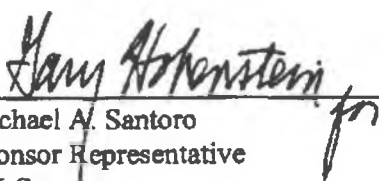
Date



Jaisimha Kesari P.E., DEE
Study Director
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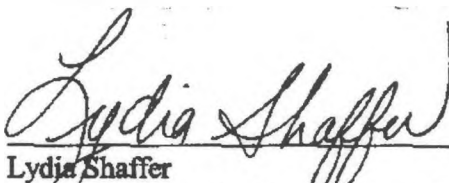
5/4/06

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 phases¹ were inspected for conduct according to Exygen Research's Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Exygen Principal Investigator and Management and to the Study Director.

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

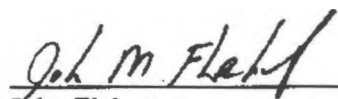
¹Note: All in-lab inspections will be documented in the QA statement for the final analytical report at the conclusion of the study. This QA statement involves only the review of the interim report and associated raw data.

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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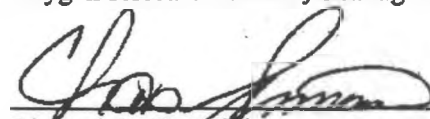
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Date

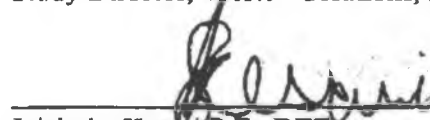
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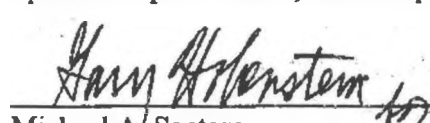
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STUDY IDENTIFICATION

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

PROTOCOL NUMBER: P0001131

EXYGEN STUDY NUMBER: P0001131

TYPE OF STUDY: Residue

SAMPLE MATRIX: Sediment

TEST SUBSTANCE: Perfluorobutanesulfonate (PFBS),
Perfluorohexanesulfonate (PFHS), and
Perfluorooctanesulfonate (PFOS)

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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:

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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

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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 $\pm$ standard deviations for PFBS, PFHS, PFOS in sediment samples were $85 \pm 19\%$, $99 \pm 29\%$, and $93 \pm 6\%$, 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.

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

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

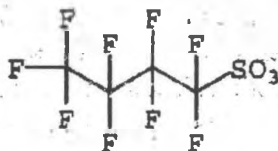
PFBS

Chemical Name: Perfluorobutanesulfonate

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

Transitions Monitored: 299 → 99

Structure:



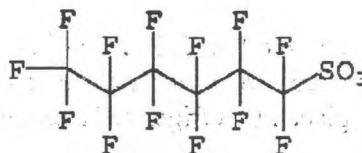
PFHS

Chemical Name: Perfluorohexanesulfonate

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

Transitions Monitored: 399 → 80

Structure:



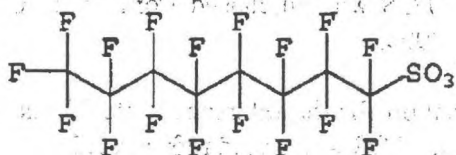
PFOS

Chemical Name: Perfluorooctanesulfonate

Molecular Weight: 538 supplied as the potassium salt (C₈F₁₇SO₃⁻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 C₁₈ 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 C₁₈ 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 C₁₈ 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 ($4^{\circ} \pm 2^{\circ}\text{C}$) 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

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

Total run time: ~18 min

Flow Rate: 0.3 mL/min

Ions monitored:

<u>Analyte</u>	<u>Mode</u>	<u>Transition Monitored</u>	<u>Approximate Retention Time (min)</u>
PFBS	negative	299 → 99	~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:

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

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

For samples fortified with known amounts of 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:

Recovery (%) =

$$\frac{(\text{analyte found (ppb)} - \text{analyte in control (ppb)})}{\text{amount added (ppb)}} \times 100\%$$

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

Equation 4:

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

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:

$$\begin{aligned}\text{Analyte found (ng/mL)} &= \frac{[253819 - (-592)] \times 1}{42300} \\ &= 6.01 \text{ ng/mL}\end{aligned}$$

From equation 2:

$$\begin{aligned}\text{Analyte found (ppb)} &= \frac{(6.01 \text{ ng/mL} \times 5 \text{ mL})}{5 \text{ g}} \\ &= 6.01 \text{ ppb}\end{aligned}$$

From equation 3:

$$\begin{aligned}\% \text{ Recovery} &= \frac{(6.01 \text{ ppb} - 2.41 \text{ ppb}) \times 100\%}{4 \text{ ppb}} \\ &= 90\%\end{aligned}$$

From equation 4:

$$\begin{aligned}\text{Analyte found (ppb) dry weight} &= 6.01 \text{ ppb} \times (100\% / 54.37\%) \\ &= 11.1 \text{ ppb}\end{aligned}$$

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:

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

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

For samples fortified with known amounts of 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 (%) =

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

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:

$$\begin{aligned} \text{Analyte found (ng/L)} &= \frac{[152371 - 3720] \times 100}{22700} \\ &= 654 \text{ ng/mL} \end{aligned}$$

From equation 2:

$$\begin{aligned} \text{Analyte found (ppb)} &= \frac{(654 \text{ ng/mL} \times 10 \text{ mL})}{1 \text{ g}} \\ &= 6540 \text{ ppb} \end{aligned}$$

From equation 3:

$$\begin{aligned} \% \text{ Recovery} &= \frac{(6540 \text{ ng/g} - 2360 \text{ ng/g})}{4000 \text{ ng/g}} \times 100\% \\ &= 105\% \end{aligned}$$

From equation 4:

$$\begin{aligned} \text{Analyte found (ppb) dry weight} &= 6540 \text{ ppb} \times (100\% / 66.54\%) \\ &= 9830 \text{ ppb} \end{aligned}$$

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 $\pm$ standard deviations for PFBS, PFHS, PFOS in sediment samples were $85 \pm 19\%$, $99 \pm 29\%$, and $93 \pm 6\%$, 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		C8 Sulfonate PFHS		C8 Sulfonate PFOS	
		Perfluorobutanesulfonate		Perfluorooctanesulfonate		Perfluorooctanesulfonate	
		Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)	Analyte Found (ppb, ng/g) Dry Weight	Assessed Accuracy (+/- %)	Analyte Found (ppb, ng/g) Dry Weight	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	80.1	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				C5 Sulfonate PFBS Wet Weight			C6 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-BPP01-0-0000 (C0172002 Spk C, 4 ppb Spikes)	4	2.09	4.47	80	-	-	-	-	-	-	
DAL-SD-BPP01-0-0000 (C0172002 Spk D, 400 ppb Spikes)	400	2.09	282	66	56.1	454	80	-	-	-	
DAL-SD-BPP01-0-0000 (C0172002 Spk E, 4000 ppb Spikes)	4000	2.09	2670	67	56.1	5190	120	NR	NR	NR	
DAL-SD-BPP02-0-0000 (C0172003 Spk F, 4 ppb Spikes)	4	2.41	8.02	90	-	-	-	-	-	-	
DAL-SD-BPP02-0-0000 (C0172003 Spk G, 400 ppb Spikes)	400	2.41	374	93	40.5	345	78	-	-	-	
DAL-SD-BPP02-0-0000 (C0172003 Spk H, 4000 ppb Spikes)	4000	2.41	3820	96	40.5	5090	120	NR	NR	NR	
DAL-SD-BPP03-0-0000 (C0172004 Spk I, 4 ppb Spikes)	4	4.39	7.71	83	-	-	-	-	-	-	
DAL-SD-BPP03-0-0000 (C0172004 Spk J, 400 ppb Spikes)	400	4.39	359	80	61.8	283	85	-	-	-	
DAL-SD-BPP03-0-0000 (C0172004 Spk K, 4000 ppb Spikes)	4000	4.39	4840	121	61.8	4500	111	NR	NR	NR	
Average:				85	Average:			88	Average:		NR
Standard Deviation:				19	Standard Deviation:			29	Standard Deviation:		NR

*Sample residue exceeds the spiking level significantly (to 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**

Sample Description	Amount Spiked (ppb)	C8 Sulfonate PFOS Wet Weight		
		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 (C0172893 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

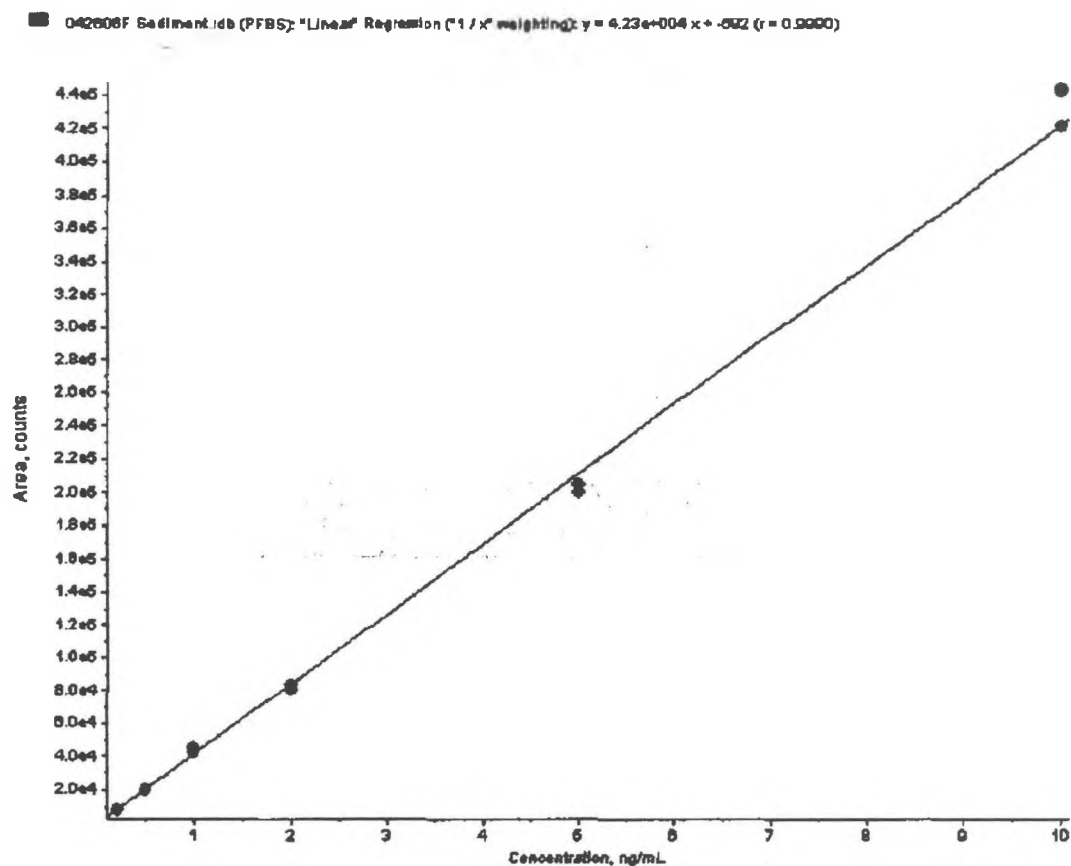
Figure 1. Typical Calibration Curve for PFBS in Methanol

Figure 2. Non-Extracted Standards of PFBS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

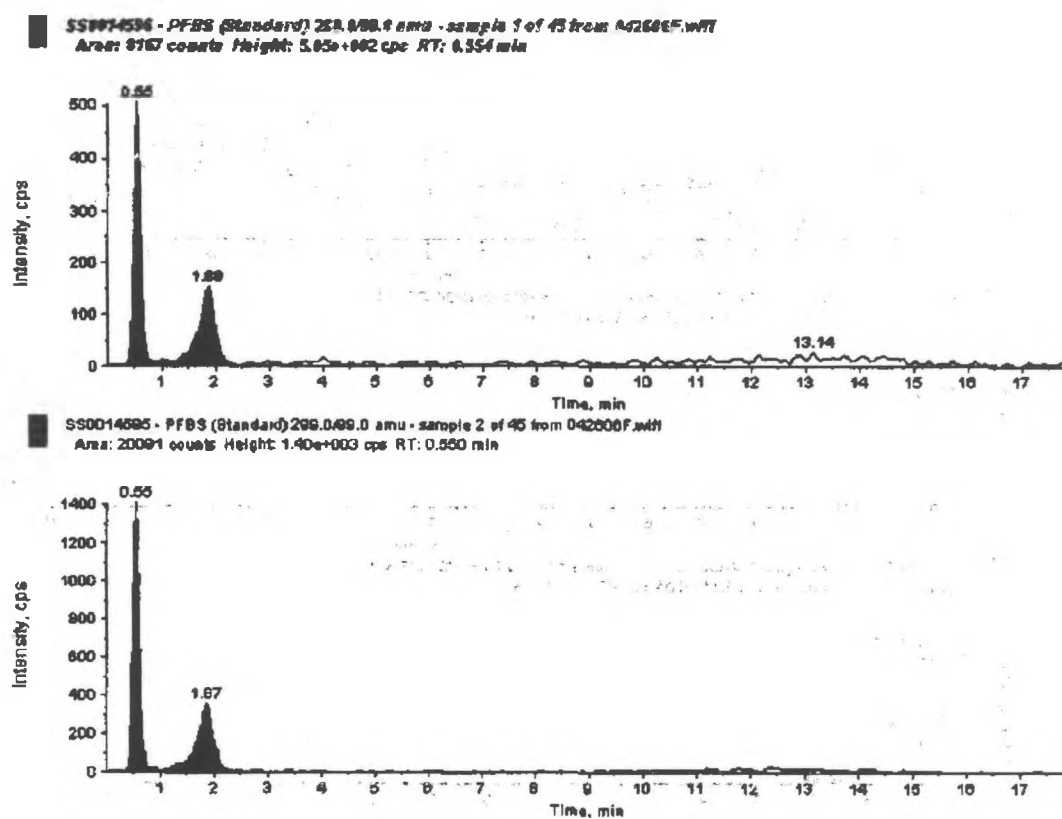


Figure 3. PFBS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

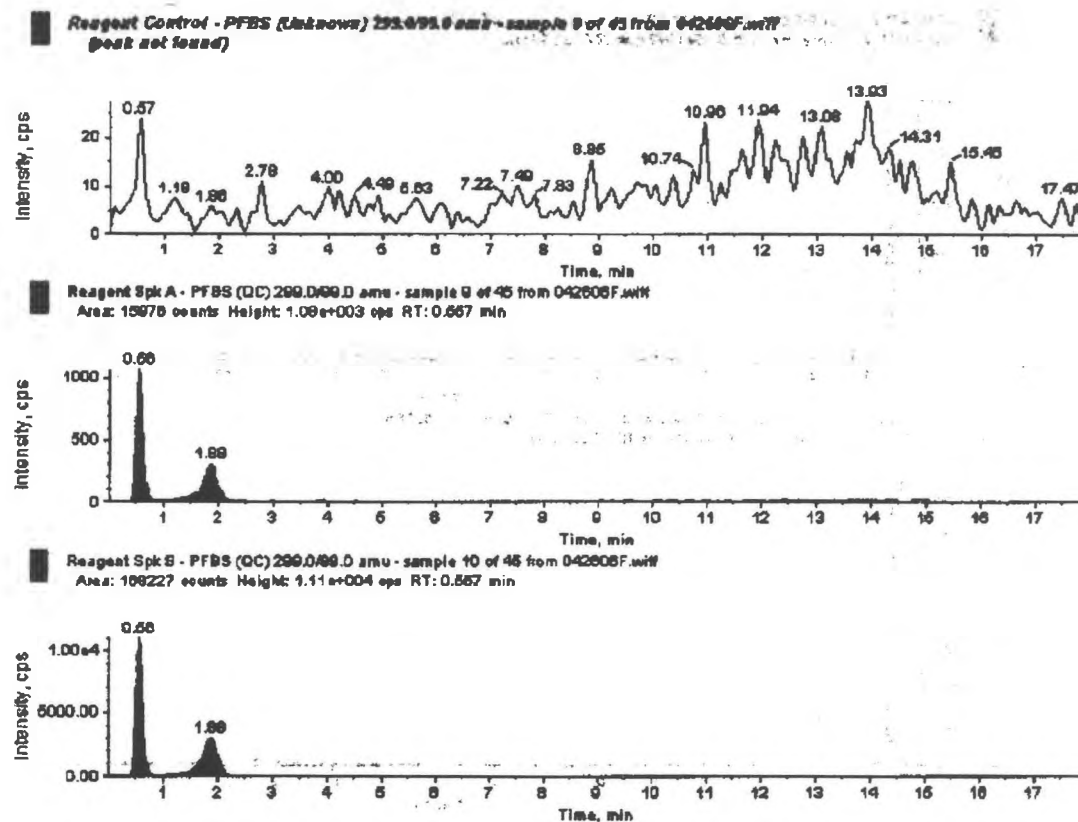


Figure 4. Chromatogram Representing a Sediment Sample Analyzed for PFBS (Exygen ID: C0172892, Data Set: 042606F)

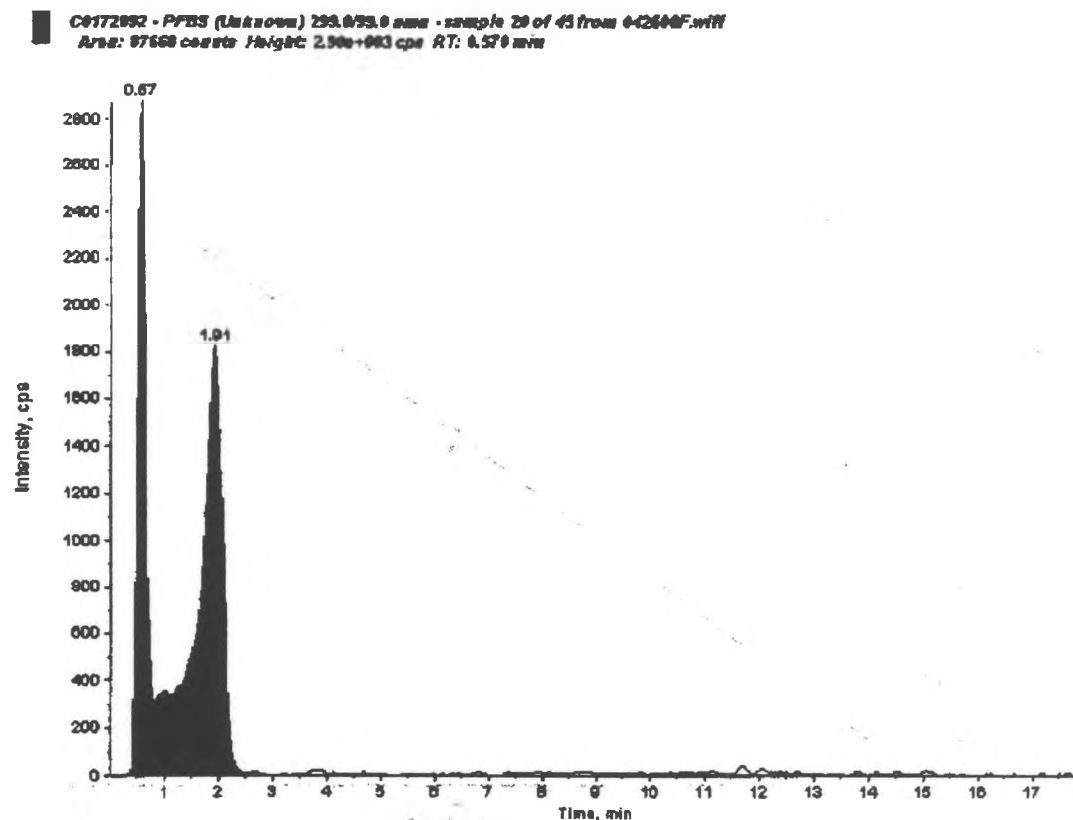


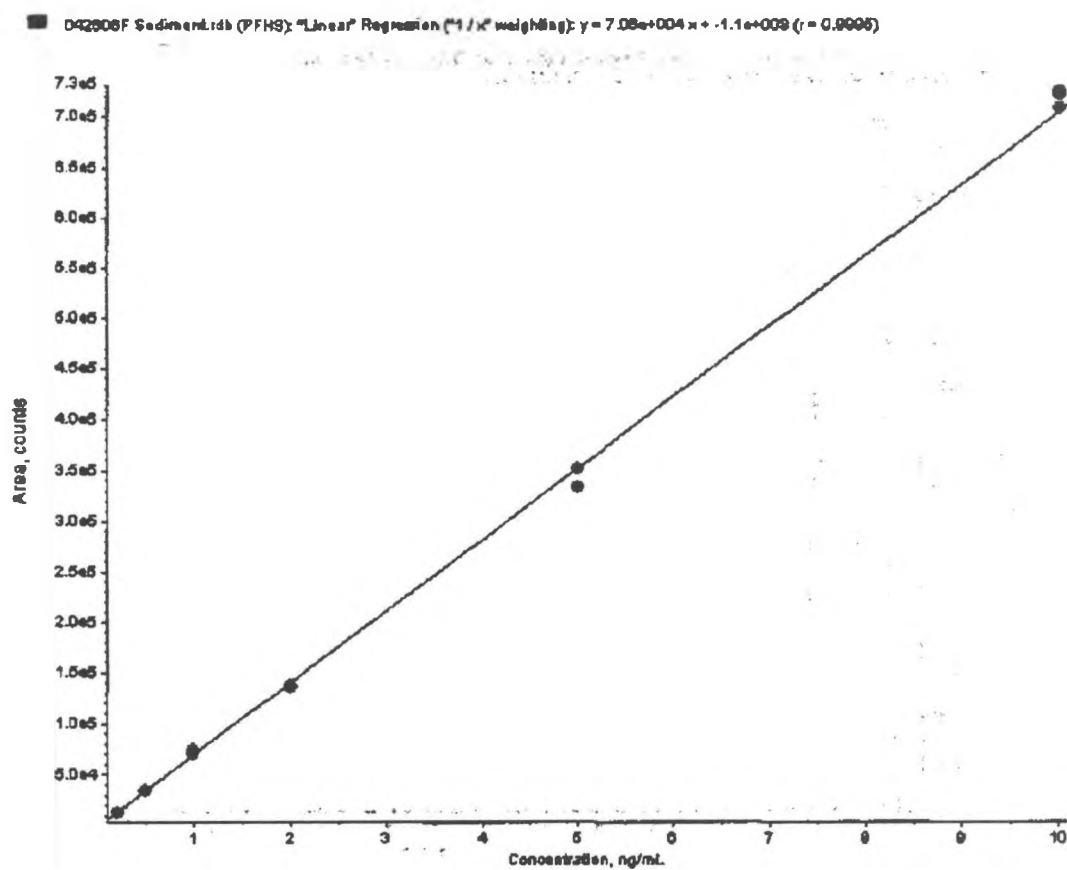
Figure 5. Typical Calibration Curve for PFHS in Methanol

Figure 6. Non-Extracted Standards of PFHS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

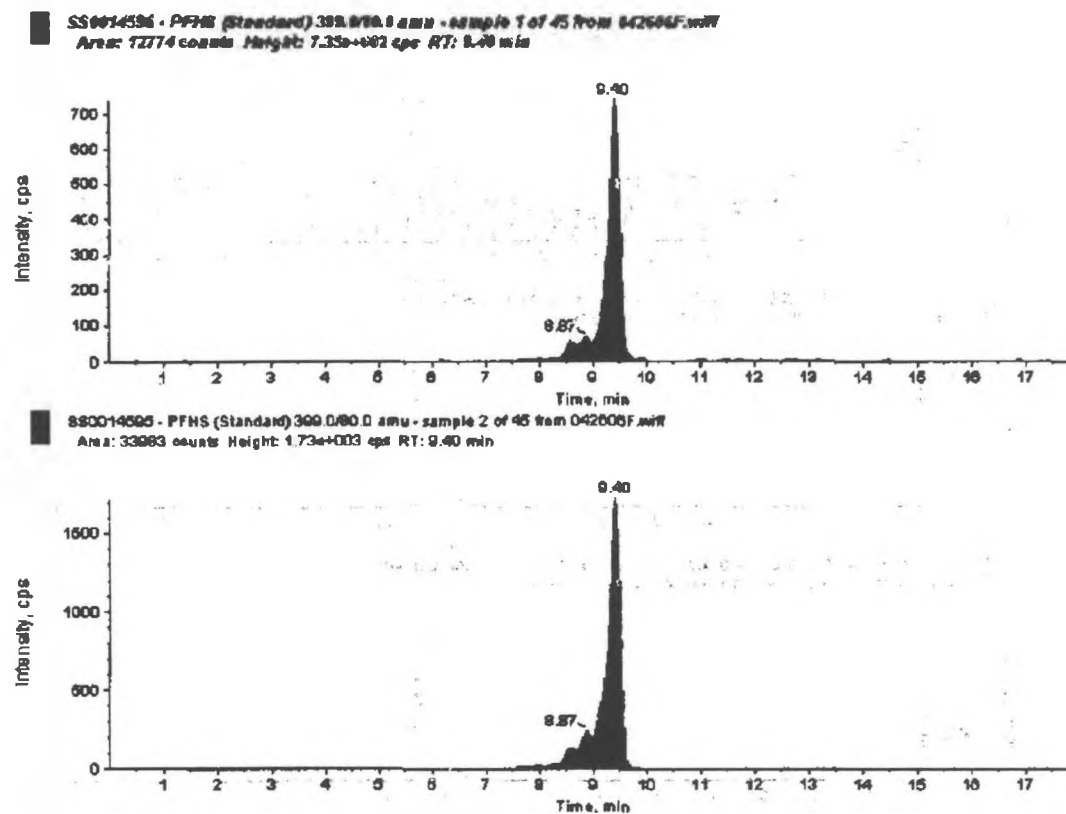


Figure 7. PFHS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

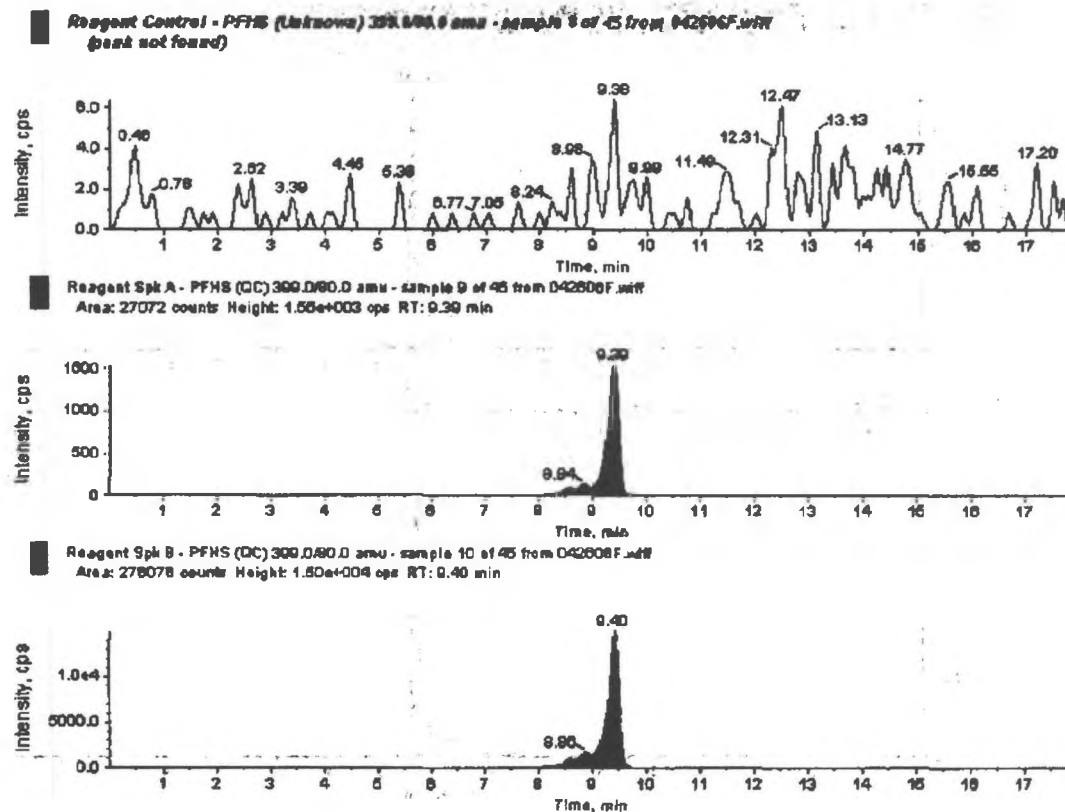


Figure 8. Chromatogram Representing a Sediment Sample Analyzed for PFHS (Exygen ID: C0172892, Data Set: 042606F)

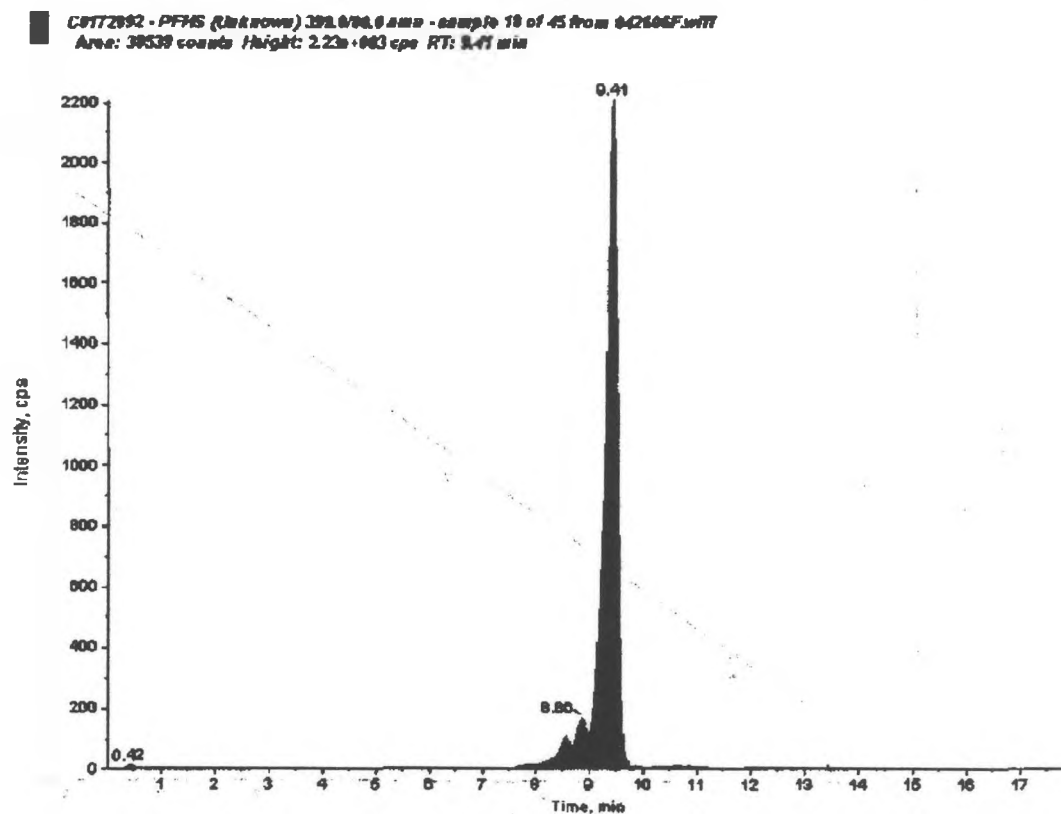


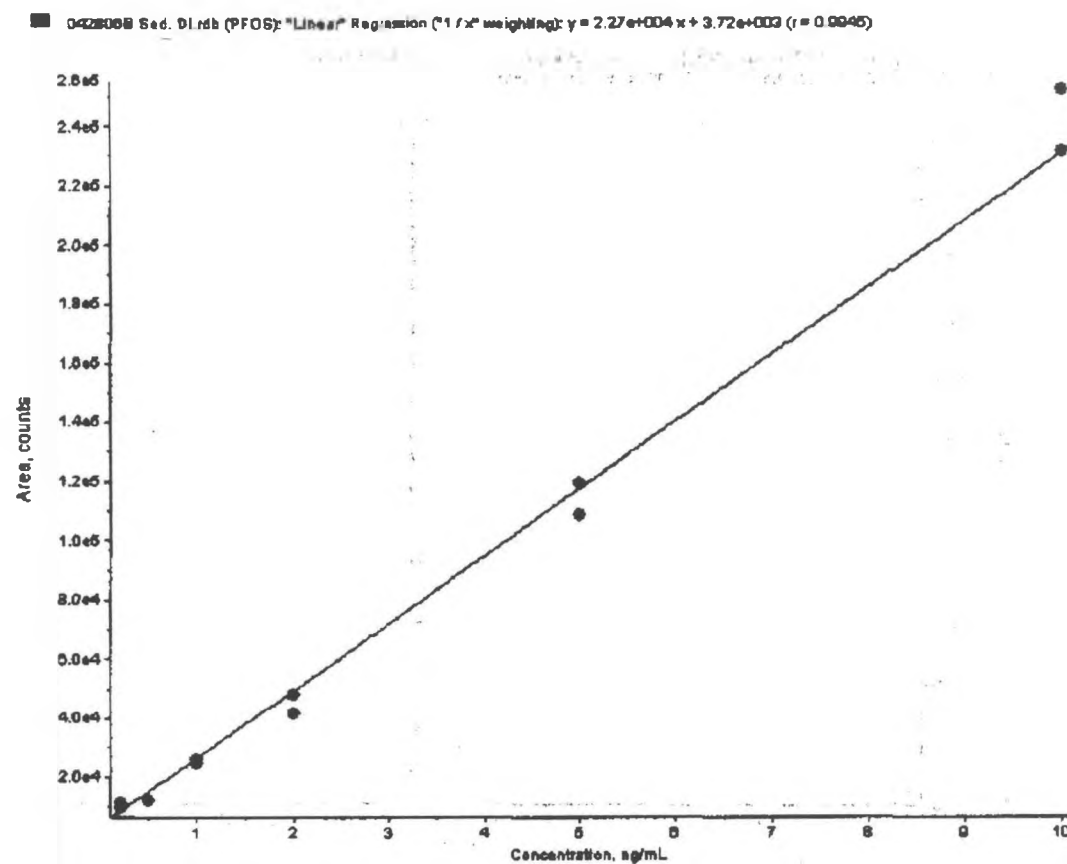
Figure 9. Typical Calibration Curve for PFOS in Methanol

Figure 10. Non-Extracted Standards of PFOS in Methanol, 0.2 ng/mL and 0.5 ng/mL, Respectively

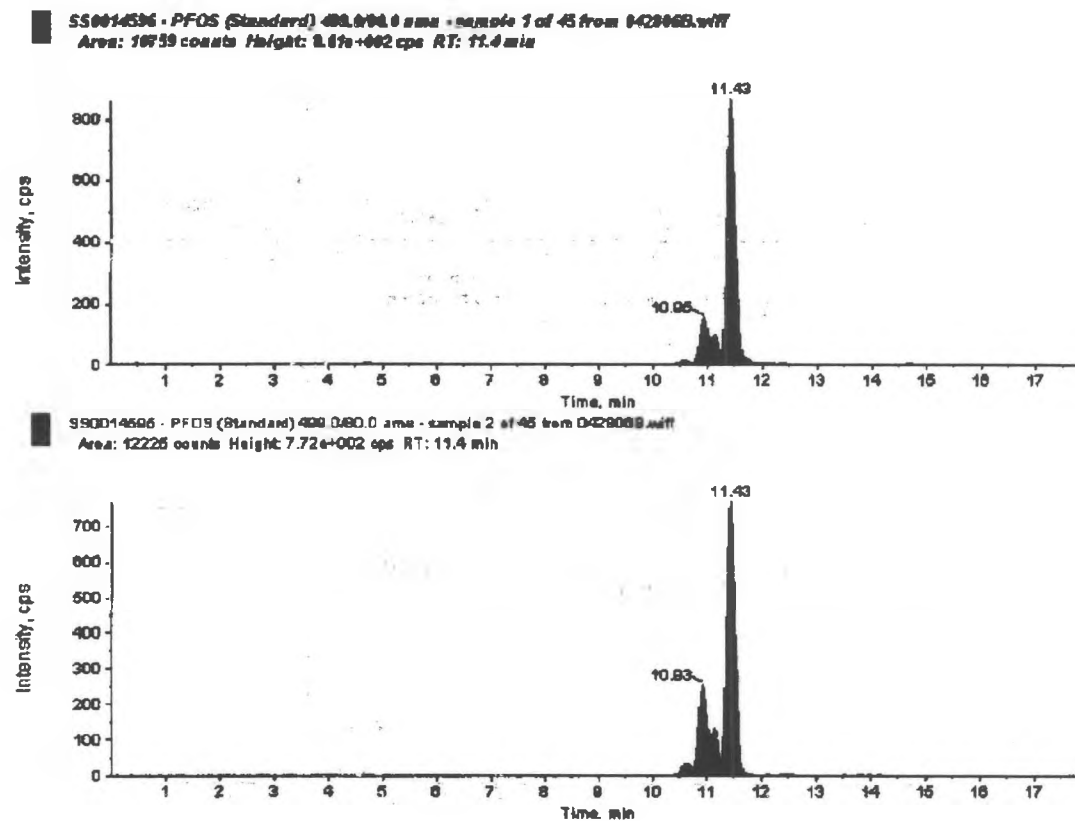


Figure 11. PFOS in Reagent Blank, 0.4 ng/mL Fortified Reagent Spk A, and 4.0 ng/mL Fortified Reagent Spk B, Respectively

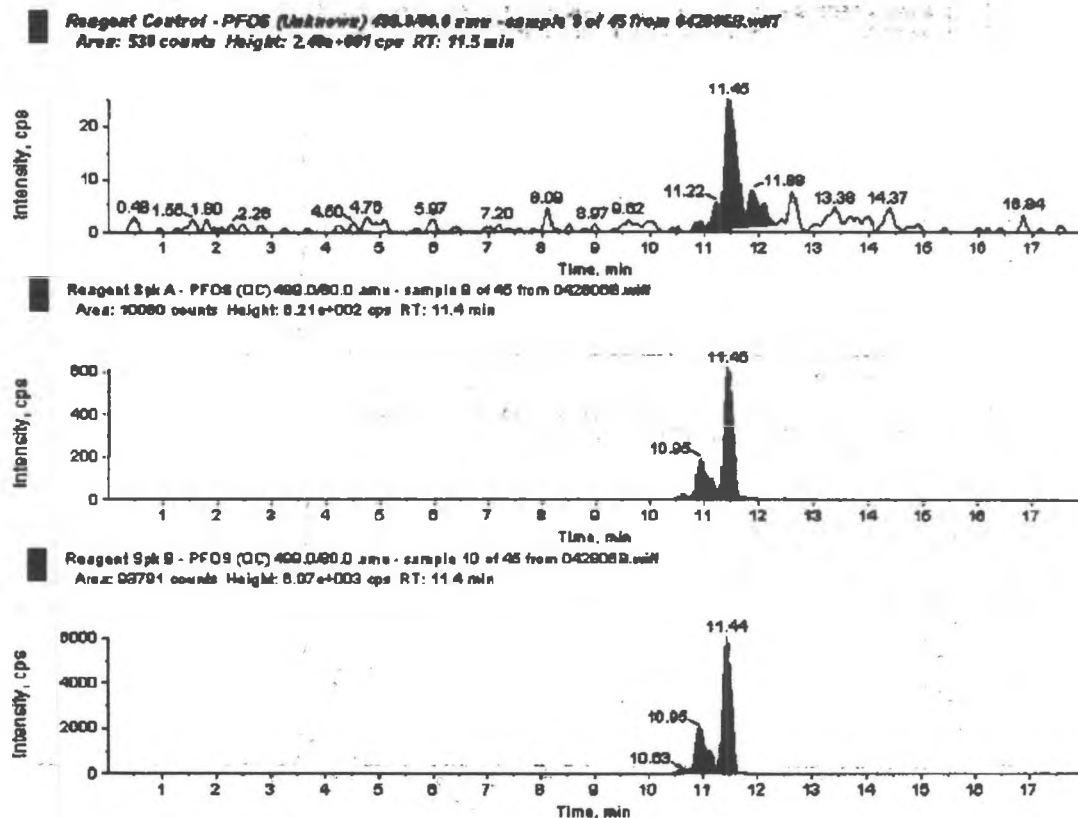
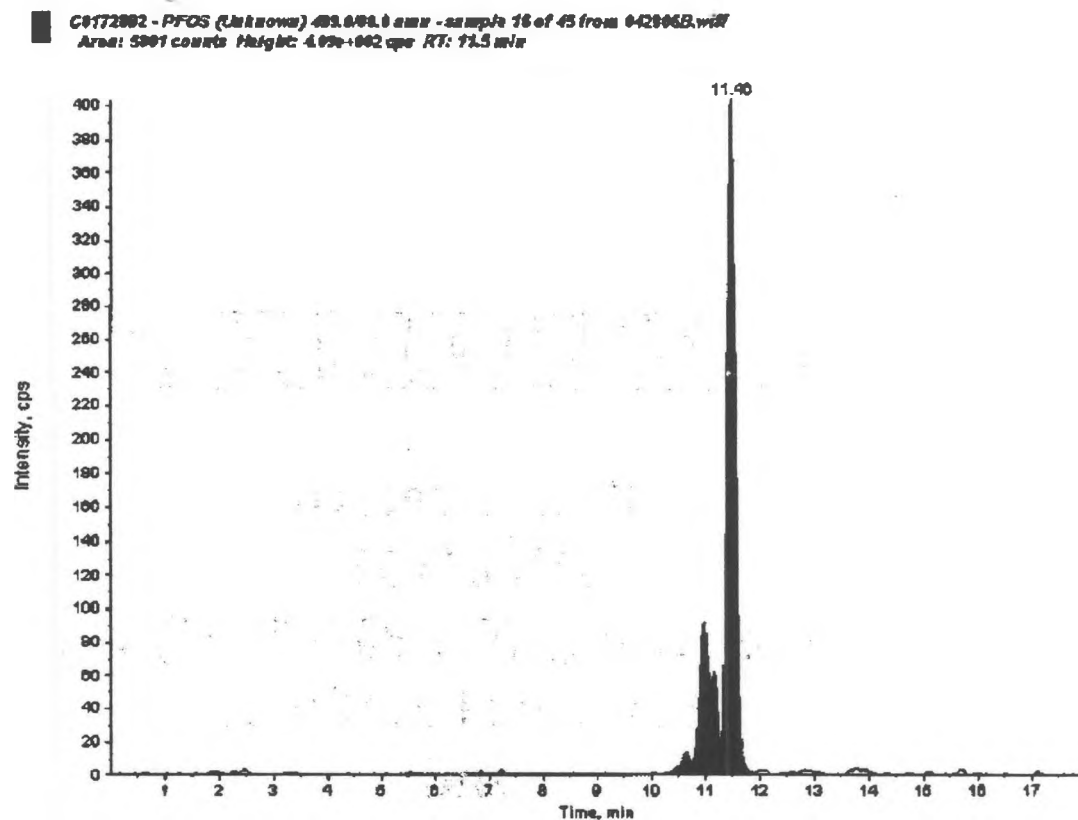


Figure 12. Chromatogram Representing a Sediment Sample Analyzed for PFOS (Exygen ID: C0172892, Data Set:042806B)



APPENDIX A

Study Protocol P0001131 (Exygen Study No. P0001131) with Analytical Methods and Protocol Amendments

Exygen Protocol Number: P0001131

STUDY PROTOCOL

Study Title:

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

Exygen Protocol Number: P0001131

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

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

Page 1 of 63

Oxygen Protocol Number: P0001131

DISTRIBUTION:

- 1) Jaiminba 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

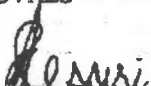
Exygen Protocol Number: P0001131

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 LCMS/MS for the 3M Decatur Monitoring Program

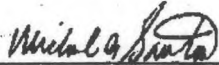
Exygen Protocol Number: P0001131

APPROVALS



Jaisunha Klean, Study Director
Weston Solutions

11/5/04
Date



Michael A. Santoro, Sponsor Representative
3M Company

11/11/04
Date



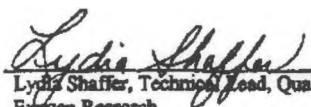
John M. Flaherty, Principal Investigator
Exygen Research

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

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Exygen Protocol Number: P0001131

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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.

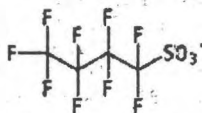
The study will be audited for compliance with EPA TSCA Good Laboratory Practice Standards 40 CFR 792 by the Quality Assurance Unit of Exygen Research.

TEST MATERIALS

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

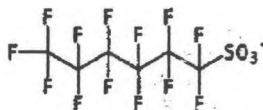
PFBS

Chemical Name: Perfluorobutanesulfonate
Molecular Weight: 338 supplied as the potassium salt ($C_4F_9SO_3K^+$)
Lot Number: 101
Purity: 96.7%
Transitions Monitored: 299 → 99
Structure:



PFHS

Chemical Name: Perfluorohexanesulfonate
Molecular Weight: 438 supplied as the potassium salt ($C_6F_{13}SO_3K^+$)
Lot Number: SB036
Purity: 98.6%
Transitions Monitored: 399 → 80
Structure:

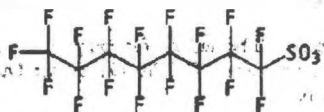


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PFO8

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 (PFO8) 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

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Exygen Protocol Number: P0001131

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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.

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IDENTIFICATION AND JUSTIFICATION OF THE TEST SYSTEM

The following are the test systems for this study:

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

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

The test systems were chosen to assess the environmental impact of PFBS, PFHS and PPOS 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 on 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 $\leq -10^{\circ}\text{C}$. 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 $\leq -10^{\circ}\text{C}$.

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

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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

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

For soil, sediment, clams, and vegetation, Exygen will supply one 500 mL precleaned SciSpec 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

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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).

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- 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).

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AMENDMENTS TO PROTOCOL

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

DATA RECORD KEEPING

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

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

Chromatograms- All chromatograms will contain the following:

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

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- 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.

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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"

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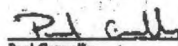
Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0801780

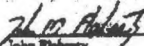
Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Water
by LC/MS/MS

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

Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research

10/26/04
Date


John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0861789

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 Requirements

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

4.0 Reagents and Standards

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

5.0 Instrument and Equipment

- 5.1 A high performance liquid chromatograph capable of pumping up to 2 mL/min equipped with a variable volume injector capable of injecting 5-200 µL connected to a tandem Mass Spectrometer (LC/MS/MS).
- 5.2 A device to collect raw data for peak integration and quantitation.
- 5.3 Analytical balance capable of reading to 0.00001 g.
- 5.4 50 mL disposable polystyrene centrifuge tubes.
- 5.5 15 mL disposable polystyrene centrifuge tubes.
- 5.6 Disposable micro pipets (50-100 µL, 100-200 µL).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.
- 5.9 Disposable pipettes.
- 5.10 Autopipette (100-1000 µL and 10-100 µL), with disposable tips.
- 5.11 Waters Sep Pak Vac 6 cc (1g) C18 SPE cartridges.

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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 SPS vacuum maintained.

5.13 Centrifuge capable of spinning 50 mL polycarbonate tubes at 3000 rpm.

6.0 Chromatographic System

6.1 Analytical Column: Phenomenex RP (Keyence Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82305-853130)

6.2 Temperature: 30°C

6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water

6.4 Mobile Phase (B): 1 (ethanol)

6.5 Gradient Program:

Time (min)	%A	%B	Flow Rate mL/min
0.8	65	35	0.3
1.8	65	35	0.3
8.8	25	75	0.3
20.0	25	75	0.3
22.3	65	35	0.3

6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).

6.7 Quantitation: Peak Area - external standard calibration curve.

6.8 Run Time: ~ 25 minutes

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

7.0 MS/MS System

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

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 mixtures may be prepared.

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Exygen Protocol Number: P0001131

Exygen Network

Method Number V0001788

ANALYTICAL METHODS**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 (extracted 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 (µg/L)	Fortification Volume (mL)	Volume of Fortified Control Sample (mL)	Final Concentration of Calibration Standard (µg/L) ^a	Calibration Standard ID (see table)
0	0	40	0	XCalibdry-0
10	100	40	25	XCalibdry-1
10	200	40	50	XCalibdry-2
10	400	40	100	XCalibdry-3
100	100	40	250	XCalibdry-4
100	200	40	500	XCalibdry-5
100	400	40	1000	XCalibdry-6

^a The extraction concentration of the calibration standard is equal to 1/4 its initial concentration, C₀ is the concentration of the standard during the extraction (SPE). XC = extracted calibration standard.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001780

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

- 9.2.3 A new 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 (reagent 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 duplication and spiking 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 C₁₈ SPE cartridge (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 C₁₈ 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 injected 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 (in support of a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantification. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

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Exygen Protocol Number: P0001131

Exygen Research

Method # 131001700

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

verify calibration standard concentration using Method 1.3 (or equivalent) solvent system.

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

13.0 Assay Criteria

- 13.1 Chromatogram must show a peak of a daughter ion at 360 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (360 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOD. 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 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-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 Page 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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:

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

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001780

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

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

Recovery (%) =

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

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Oxygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001781

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

Analytical Testing Facility:

Oxygen Research
3858 Research Drive
State College, PA 16801

Approved By:

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

10/26/04
Date

John Finkley
John Finkley
Vice President, Operations, Oxygen Research

10/26/04
Date

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Exygen Protocol Number: P0001131

Exygen Research	Method Number V0001761
ANALYTICAL METHOD	
Method of Analysis for the Detection and Quantitation of Perfluorooctanoic Acid (PFOA) in Soil by LC/MS/MS	
1.0	Scope
This method is to be employed for the isolation and quantitation of perfluorooctanoic acid by High Performance Liquid Chromatography coupled to a tandem Mass Spectrometer Detector (LC/MS/MS) in soil.	
2.0	Safety
2.1	Always observe 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 mL/min equipped with a variable volume injector capable of injecting 3-200 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 0.0001 g.
5.4	50 mL disposable polypropylene centrifuge tubes.
5.5	15 mL disposable polypropylene centrifuge tubes.
5.6	Disposable microplates (50-100µL, 100-200µL).
5.7	125-mL LOPS narrow-mouth bottles.
5.8	2 mL clear HPLC vial kit.
5.9	Disposable pipettes.
5.10	Autopipettes (100-1000 µL and 10-100 µL), with disposable tips.
5.11	Waters Sep Pak Vac 6 or (1g) C18 SPE cartridges.
5.12	SPE vacuum manifold.
5.13	Ultrasonic bath.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V8001781

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 Containers capable of spinning 50 mL polypropylene tubes at 5800 rpm.

6.5 Chromatography System

- 6.1 Analytical Column: Pluraphase RP (Krytox Scientific), 2.1 mm x 50 mm, 3µ (P/N: 82245-832130)
 6.2 Temperature: 30°C
 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
 6.4 Mobile Phase (B): Methanol
 6.5 Gradient Program:

Time (min)	%A	%B	Flow Rate (mL/min)
0.0	65	35	0.3
1.0	65	35	0.3
5.0	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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0081751

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 (certified 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 vials. The calibration standards are processed through the extraction procedure, identical to samples.
- 9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

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

* The extracted concentration of the calibration standard is equal to 6x its initial concentration, due to the concentration of the standard during the extraction (SPE).
 XL = extracted concentration standard.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001791

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0004701

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

- extracted calibration standards may be injected at the beginning of a set followed by extracted calibration standards interspersed every 5-10 samples (to account for a second set of extracted standards). In either case, extracted calibration standards must be the first and last injection in a sample set.
- 12.4 Use linear standard curves for quantitation. Linear standard curves are generated for the analyte by linear regression using the weighting of peak area versus calibration standard concentration using Masslynx 3.3 (or equivalent) software system.
- 12.5 Sample response should not exceed standard response. 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 mass from a parent of 413 mass. The 413 mass parent corresponds to the PFOA anion, while the daughter ion (369 mass) 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 Hughes Box 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 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standard in the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001731

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in So. by
LCMS/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:

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

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.

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 ug/g (ppb).

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

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

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

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Oxygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0081782

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

Analytical Testing Facility:

Oxygen Research
3831 Research Drive
State College, PA 16801

Approved By:

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

10/26/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Oxygen Research

10/26/04
Date

Total Pages: 7

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Exygen Protocol Number: P0001131

Exygen Research	Method Number V908172
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 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 Instruments 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 polystyrene centrifuge tubes. 5.5 15 mL disposable polystyrene centrifuge tubes. 5.6 Disposable micropipets (50-100µL, 100-200µL). 5.7 25-µL LDPE narrow-mouth bottles. 5.8 3 mL clear HPLC vial kit. 5.9 Disposable pipettes. 5.10 Autopipettes (100-1000 µL and 10-100 µL) with disposable tips. 5.11 Waters Sep Pak Vac 6 or (1g) C18 SPE cartridges. 5.12 SPE vacuum manifold.

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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 Vial: 2 mL
 5.14- Vial-seal: rubber
 5.15- Cist (10g) capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Phenomenex RP (Kymene Scientific), 2.1 mm x 50 cm, 5µ (P/N: 03905-052139)
 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
22.3	65	35	0.3

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number VM017E2

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/Verification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (certified for purity) and dilute to 100 mL with methanol in a 125 mL LDPE bottle.
- 9.1.2 A 10 µg/mL verification 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 verification 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 verification 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 verification 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 verification 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 verification solution.
- 9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

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

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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**

9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 8°C, up to six months.

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

10.0 Batch Set Up

10.1 Each batch of samples submitted (typically 25 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (all control spikes) to verify procedural recovery for the batch.

10.2 Requirements for field and laboratory dry masses 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~2 drops/sec). Do not let column run dry.

11.5 Load (decant) the sample on the conditioned C₁₈ 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 20 mL of HPLC water (~2 drops/sec). Do not let column run dry.

11.11 Add the methanol to ~300 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 pre-weighed 15 mL polypropylene centrifuge tubes (final volume = 5 mL).

11.13 Analyze samples using electrospray LC/MS/MS.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001702

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 unknown 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 3-10 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 3-10 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 replicates should not exceed standard responses. Any samples that exceed standard responses should be further diluted and reanalyzed.

13.0 Acceptance Criteria

- 13.1 Chromatogram must show a peak of a daughter ion at 369 m/z from a parent of 413 m/z. The 413 m/z peak corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.2 ng/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 Iyengar Test, may be excluded from the calculation of the calibration curve. However, the total number of extracted calibration standards that could be excluded must not exceed 20% of the total number of extracted standards injected.
- 13.5 The correlation coefficient (R^2) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001702

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

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

14.0 Calculations:

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

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

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.

Recovery (%) =

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

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

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

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

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

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Exygen Protocol Number: P0001131

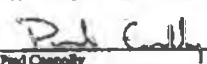
ANALYTICAL METHOD
Method Number: V0001783

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

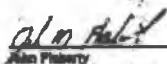
Analytical Testing Facility:

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Approved By:


Paul Connolly
Technical Leader, LC-MS, Exygen Research


Date


John Flaherty
Vice President, Operations, Exygen Research


Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V000170

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Fish and Clams by LC/MS/MS****1.0 Scope**

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

2.0 Safety

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

3.0 Sample Requirements

- 3.1 At least 20 g of 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 to fumes 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 (60-100 mesh) – Reagent grade
- 4.6 Florisil (50-100 mesh) – Reagent grade
- 4.7 Supercolumn LC-NH₂ – Reagent grade
- 4.8 1-Octanol – HPLC grade
- 4.9 L-Ascorbic acid – Reagent grade
- 4.10 Dimethylchlorosilane – Reagent grade
- 4.11 Toluene – Reagent grade
- 4.12 Acetic Acid – 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 0.0001 g.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001783

ANALYTICAL METHOD

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

- 5.4 Rotary evaporator.
- 5.5 Titronmeter.
- 5.6 2.5 mL pear-shaped flask.
- 5.7 50 mL disposable polypropylene centrifuge tubes.
- 5.8 15 mL disposable polypropylene centrifuge tubes.
- 5.9 Disposable vials/pipets (50-100µL, 100-200µL).
- 5.10 125-µL LDPE narrow-mouth bottles.
- 5.11 1 mL clear HPLC vial kit.
- 5.12 Disposable pipettes.
- 5.13 Aspirations (125-1000 µL and 10-100 µL), with disposable tips.
- 5.14 SPE tubes (20mL) (Supelco cat. no. NS57177).
- 5.15 Vial action shaker.
- 5.16 Centrifuge capable of spinning 50 mL polypropylene tubes at 2000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Fluorophase RP (Keystone Scientific), 2.1 mm x 50 mm, 5µ (P/N: 42583-852138)
- 6.2 Temperature: 30°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

- 6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL)
- 6.7 Quantitation: Peak Area - external standard calibration curve.
- 6.8 Run Time: ~ 25 minutes.

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0081743

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% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Fortification Solution**

- 9.1.1** Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (assessed for purity) and dilute to 100 mL with methanol in a 125 mL LDPE bottle.
- 9.1.2** A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3** A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4** A 0.01 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 0.1 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.5** The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001753

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
0.01	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 unextracted control and two unextracted controls fortified at known concentrations (lab control spikes) to verify procedural recovery for the batch.**10.2** Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.**11.0 Sample Extractions****11.1** Weigh 5 g of frozen sample into 50 mL polypropylene centrifuge tubes (sanitize as needed, spin 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 stabilize the pear-shaped flasks.**11.5** Pack the 20 mL SPE tubes in sequence with 2 g florisil, 2 g silica gel, 2 g octasil, 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 columns to dry.**11.6** Stabilize the 125 mL pear-shaped flasks by rinsing with the 30% acetonitrile:methanol in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flasks completely before use, either by air-drying or with a stream of nitrogen.

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Exygen Protocol Number: PC001131

Exygen Research

Method Number: V0801783

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFDA) 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 nitrogenized pear-shaped flask.
- 11.9 Add 10 mL of acetonitrile to the sample in the 50 mL centrifuge tube. Homogenize the flask fit phase using a tumbler for ~30 seconds and rinse the tumbler 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 flask 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 PFDA 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 3-10 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 MetaLynx 3.3 (or equivalent) software system.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0004783

ANALYTICAL METHODMethod 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.

13.8 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-analyzed.
- 13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-analyzed.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hugh Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
- 13.6 Retention times between standards and samples must not drift more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the 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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Name: V0801783

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

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

Recovery (%) =

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001784

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

Analytical Testing Facility:

Exygen Research
3058 Research Drive
State College, PA 16801

Approved By:

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

10/22/11
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/22/11
Date

Total Pages: 7

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Exygen Protocol Number: P0001131

Exygen Control

Method Number: V0001794

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Vegetation by LC/MS/MS****1.0 Scope**

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

2.0 Safety

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

3.0 Sample Requirement

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

4.0 Reagents and Standards

- 4.1 Water – HPLC grade
- 4.2 Acetonitrile – HPLC grade
- 4.3 Carbon (120-400 mesh) – Reagent grade
- 4.4 Methanol – HPLC grade
- 4.5 Silica gel (60-200 mesh) – Reagent grade
- 4.6 Florisil (60-100 mesh) – Reagent grade
- 4.7 Supraclean LC-MH₂ – Reagent grade
- 4.8 1-Octanol – HPLC grade
- 4.9 L-Ascorbic acid – Reagent grade
- 4.10 Diethylstilbestrol – Reagent grade
- 4.11 Telomers – 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 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.

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Exygen Protocol Number: P0001131

Exygen Summary

Method Number: V0001781

ANALYTICAL METHOD

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

- 1.4 Rotary evaporator.
- 1.5 125 mL pear-shaped flask.
- 1.6 50 mL disposable polypropylene centrifuge tubes.
- 1.7 15 mL disposable polypropylene centrifuge tubes.
- 1.8 Disposable micropipets (50-100 µL, 100-200 µL).
- 1.9 125-mL LDPE screw-mouth bottles.
- 1.10 5 µL clear HPLC vial kit.
- 1.11 Disposable pipettes.
- 1.12 Autopipette (100-1000 µL and 10-100 µL), with disposable tips.
- 1.13 PPS tubes (20mL) (Beckman cat. no. N057177).
- 1.14 Vial action station.
- 1.15 Centrifuge capable of spinning 50 mL polypropylene tubes at 3000 rpm.

6.0 Chromatographic System

- 6.1 Analytical Column: Phenomena RP (Kymene Scientific), 2.1 mm x 50 mm, 5 µm (P/N: 82508-051130)
- 6.2 Temp. oven: 30°C
- 6.3 Mobile Phase (A): 2 mM Ammonium Acetate in Water
- 6.4 35% Phosphate (B): Methanol
- 6.5 Gradient Program:

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

- 6.6 Injection Volume: 15 µL (can be increased to as much as 50 µL).
- 6.7 Quantitation: Peak Area – external standard calibration curve.
- 6.8 Run Time: ~ 25 minutes.

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

7.0 MS/MS System

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0801784

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

8.2 Extraction Solutions

- 8.2.1 2% ascorbic acid in methanol is prepared by dissolving 2 g of ascorbic acid in 100 mL of methanol.
- 8.2.2 30% Dimethylchlorosilane in toluene is prepared by bringing 3 mL of dimethylchlorosilane to a final volume of 10 mL with toluene.

Alternate volumes may be prepared.

9.0 Standard Preparation**9.1 Standard Stock/Fertilization 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 fertilization 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 fertilization 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 fertilization 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 fertilization 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 fertilization solution.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V8081784

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

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

Concentration of Perfluorooctanoic Acid Solution (µg/mL)	Volume (µL)	Diluted to (mL)	Final Concentration (µg/mL)
1.0	2.5	100	0.025
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	0.01
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 untreated control and two untreated controls fortified at known concentrations (spike control spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

11.0 Sample Extraction

- 11.1 Weigh 5 g of frozen sample into 50 mL polystyrene centrifuge tubes (fluffy 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 polystyrene tubes containing sample at ~1000 rpm for ~10 minutes.
- 11.4 Push and sonicate the SPE tubes and eliminate the pear-shaped flask.
- 11.5 Push 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 30 mL of acetonitrile. Discard all wastes. Do not allow the column to dry.
- 11.6 Silanize the 125 mL pear-shaped flask by rinsing with the 30% dimethylsiloxane in toluene solution. Rinse the flask with toluene once, followed by methanol (three times). Dry the flask completely before use, either by air-drying or with a stream of nitrogen.
- 11.7 Elute the extract on to a conditioned SPE column fitted inside the mouth of the pear-shaped flask. Collect the eluate in the 125 mL silanized pear-shaped flask.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001768

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 polycarbonate tubes containing sample at ~2000 rpm for ~5 minutes.
- 11.11 Decant the extract into the auto 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% acetic 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 method 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 quantification. 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 Acceptor Criteria

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0081794

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

- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 0.5 ppb, then a new blank sample must be obtained and the entire set must be re-extracted.
- 13.3 Recoveries of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hogg-Bray 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 30% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 (R² ≥ 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:

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

- 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.

Recovery (%) =

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

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Exygen Protocol Number: P0001131

ANALYTICAL METHOD
Method Number: V0001713

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

Analytical Testing Facility:

Exygen Research
1455 Research Drive
State College, PA 16801

Approved By:

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

10/24/04
Date

John Flaherty
John Flaherty
Vice President, Operations, Exygen Research

10/26/04
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001785

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Liver by LC/MS/MS****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 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 frozen storage overnight to allow for carbon dioxide sublimation. Seal and place the samples in frozen storage until time of analysis. Alternatively, 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. 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 Methyl - HPLC grade
- 4.3 Acetonitrile - HPLC grade
- 4.4 Acetonitrile 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 conditioned 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 Drying tube in nitrogen (50-100mL, 100-200mL).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial kit.

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Exygen Protocol Number: PC001131

Exygen Research

Method Number: V0001793

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 or (1g) K12 SPE cartridge.
- 5.12 SPE vacuum manifold.
- 5.13 Tintometer.
- 5.14 Wrist-action shaker.
- 5.15 Centrifuge capable of spinning 15 mL polycarbonate tubes at 3600 rpm.

6.0 Chromatographic System:

- 6.1 Analytical Column: Phenomena RP (Kymene Scientific), 2.1 mm x 50 mm, 5µ (P/N: 82565-652130)
- 6.2 Temperature: 50°C
- 6.3 Mobile Phase (A): 1 mM Ammonium Acetate in Water
- 6.4 Mobile Phase (B): Methanol
- 6.5 Gradient Program:

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

- 6.6 Injection Volume: 15 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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: P0001131

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small
Marine Liver by LC/MS/MS****8.0 Preparation of Solutions****8.1 Mobile Phase**

- 8.1.1 3 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/Verification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (certified for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 1.0 µg/mL verification 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 verification 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 verification 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 by methanol via dilution of the 0.1 µg/mL verification solution.
- 9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

Concentration of Verification Solution (µg/mL)	Volume (µL)	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 8°C, up to six months.

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

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Exygen Protocol Number: P0001131

Exygen Research Method Number V0001783

ANALYTICAL METHOD

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

10.0 Blank Set Up

- 10.1 Blank batch of samples extracted (typically 20 or less) must include at least one unspiked control and two unspiked controls fortified at known concentrations (spike control spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicate(s) 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 (verify as needed, replace lid and mix well). Note that alternate weights of liver may be required 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 tissueacher 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 C₁₈ SPE cartridge (1 g, 6 mL) by passing 10 mL methanol followed by 5 mL of HPLC water (~2 h at 4 mL). Do not let column run dry.
- 11.9 Load the sample on conditioned C₁₈ 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 blank 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 quantification. Linear standard curves are generated for the analyte by linear regression using 1/x weighting of peak area.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0041725

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 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA anion, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOD. 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 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spike outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hugh Baxter Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R^2) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). 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 differ more than $\pm 4\%$ within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

14.0 Calculations

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

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

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

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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.

Recovery (%) =

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

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

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

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Oxygen 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:

Oxygen Research
1000 Research Drive
State College, PA 16801

Approved By:


Paul Connolly
Technical Leader, LC MS, Oxygen Research

10/24/01
Date


John Pichot
Vice President, Operations, Oxygen Research

11/6/01
Date

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001766

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 Detection (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 Requirements

- 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 Acetic Acid – 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 microspigots (50-100µL, 100-200µL).
- 5.7 125-mL LDPE narrow-mouth bottles.
- 5.8 2 mL clear HPLC vial lid.
- 5.9 Disposable pipettes.
- 5.10 Syringettes (100-1000 µL and 10-100 µL), with disposable tips.
- 5.11 Waters Bay Port Vac 6 or (1A) C18 SPE cartridges.
- 5.12 SPE vacuum manifold.
- 5.13 Vortexer.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V000179b

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

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

6.0 Chromatographic System

- 6.1. Analytical Column: Phenomenex RP (Kymene Scientific), 2.1 mm x 50 mm, 5µ (P/N: 12545-032130)
6.2. Temperature: 30°C
6.3. Mobile Phase (A): 2 mM Ammonium Acetate in Water
6.4. Mobile Phase (B): Methanol
6.5. Gradient Program:

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

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

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

7.0 MS/MS System

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

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

8.0 Preparation of Solutions**8.1 Mobile Phase**

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

Alternate volumes may be prepared.

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Exygen Protocol Number: P0001786

Exygen Research

Method Number Y0001786

ANALYTICAL METHOD**Method of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Small Mammal Serum by LC/MS/MS****9.0 Standard Preparation****9.1 Standard Stock/Fortification Solution**

- 9.1.1 Prepare a stock solution of ~100 µg/mL of PFOA by weighing 10 mg of analytical standard (corrected for purity) and dilute to 100 mL with methanol in a 125-mL LDPE bottle.
- 9.1.2 A 1.0 µg/mL fortification solution of PFOA is prepared by bringing 1 mL of the 100 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.3 A 0.1 µg/mL fortification solution of PFOA is prepared by bringing 10 mL of the 1.0 µg/mL solution to a final volume of 100 with methanol in a 125 mL LDPE bottle.
- 9.1.4 The stock and fortification solutions are to be stored in a refrigerator at approximately 4°C and are stable for a maximum period of 6 months from the date of preparation.

9.2 Standard Calibration Solutions

- 9.2.1 LC/MS/MS calibration standards are prepared in methanol via dilution of the 0.1 µg/mL fortification solution.
- 9.2.2 The following is a typical example; additional concentrations may be prepared as needed.

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

- 9.2.3 Store all calibration standards in 125-mL LDPE narrow-mouth bottles at 2°C to 8°C, up to six months.

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

10.0 Batch Set Up

- 10.1 Each batch of samples extracted (typically 20 or less) must include at least one untreated control and two untreated controls fortified at known concentrations (all control spikes) to verify procedural recovery for the batch.
- 10.2 Requirements for field and laboratory duplicates and spikes will be specified in the quality assurance plan for this project.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number Y0801786

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 drape tube; 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 ~3600 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 C₁₈ 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 C₁₈ 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 sample matrix of each standard, sample and fortified sample into the LC/MS/MS system. A calibration standard must precede and follow all sample injections.
- 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 included 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/n 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.

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Exygen Protocol Number: P0001131

Exygen Research

Method Number V0001736

ANALYTICAL METHODMethod of Analysis for the Determination of Perfluorooctanoic Acid (PFOA) in Solid
Material Samples by LC/MS/MS**13.0 Acceptance Criteria:**

- 13.1 Chromatograms must show a peak of a daughter ion at 369 m/z from a parent of 413 m/z. The 413 m/z parent corresponds to the PFOA mass, while the daughter ion (369 m/z) represents the loss of carbon dioxide.
- 13.2 Method blanks must not contain PFOA at levels greater than the LOQ. If a blank contains PFOA at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.
- 13.3 Recovery of control spikes and matrix spikes must be between 70-130% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spikes outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.
- 13.4 Any calibration standard found to be a statistical outlier by using the Hughes River Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded cannot exceed 20% of the total number of standards injected.
- 13.5 The correlation coefficient (R) for calibration curves generated must be ≥ 0.992 ($R^2 \geq 0.985$). 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.

Recovery (%) =

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

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Exygen Protocol Number: P0001131

Exygen Research

Method Number: V0001704

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

1.1.3 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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3058 Research Drive
State College, PA 16801Phone: 814-272-1039
Fax: 814-231-1580PROTOCOL AMENDMENTAmendment Number: 1Effective Date: 01/19/05Exygen Study Number: P0001131 Client Study Number: None

Page 1 of 1

DESCRIPTION OF AMENDED SECTION

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

AMENDED TO

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

RATIONALE

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

IMPACT ON STUDY

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

Study Director Signature

Principal Investigator Signature

Safety Director/Management Signature

Sponsor Signature (if requested)

Date

Date

Date

Date

Exygen QAU Review 1/25/05/1/18

LIBRARY ID: VV0001228-6

ADMINISTRATIVE FORM



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PROTOCOL AMENDMENT

Amendment Number:

2

Page 1 of 1

Effective Date:

03/07/05

Oxygen Study Number

P0001131

Client Study Number:

None

DESCRIPTION OF AMENDED SECTION

- 2) Test Materials, page 6 of 85: PFOS transition monitored 480 -> 80.

AMENDED TO

- 1) Instead of one final report, interim reports will be issued.
- 2) PFOS transition monitored may also be 499 → 50.

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 timely manner. *Interim orders sent*
- 2) The API 4000 LC/MS/MS systems detect the 499 → 80 PFO8 transition with greater sensitivity than the 499 → 80 transition.

IMPACT ON STUDY

- 1) The client will be able to receive and review the data more quickly.
- 2) The 499 → 500 transition can be detected with greater sensitivity, therefore, giving better chromatography.

Study Director Signature _____

Dagbladet

Principal Investigator Signature _____

Index

Study Director Management Signature _____

Dentist

Expen Management Signature

Diphenyl

Sponsor Signature (if required)

Dental

Enygon QAU Review LTJ 03/05/15

LIBRARY ID: V0001228-8

ADMINISTRATIVE FORM


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DEVIATION FORM

Page 1 of 2

General:	
☒ 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:	Deviation Type: (Include V# for methods and SOPs)
☒ GMP ☐ GLP ☐ Other ☐ None	☒ 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 ¹³C 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 ¹³C PFOA were still poor. Another alternative method was then used for PFOA and ¹³C 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 C₁₈ 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 ☒ Other	
Impact:	
More usable data was obtained.	

LIBRARY ID: V0001640-6

ADMINISTRATIVE FORM

CHEM EHSR 236 1B 651 733 1958
 05-01-2008 09:00am From: WESTON SOLUTIONS

05/01 '06 13:46 NO. 106 03/03
 T-004 P. 003/003 P-010



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DEVIATION FORM

Revision:		Page 2 of 2	
☒ Project Specific Deviation	☐ Facility Deviation	Date of Occurrence: 5/1/06	
Exygen Project #: P770/P1131		Deviation #: 2	
Client Project #: NA		Reference #:	
Description of Deviation:			
Deviation Issued:			
Principal Investigator: <i>John H. Baker</i>		Exygen Manager: <i>William J. Baker</i>	
Quality Director: <i>John H. Baker</i>		Exygen Representative: <i>William J. Baker</i>	
Quality Assurance: <i>John H. Baker</i>		Exygen Representative: <i>William J. Baker</i>	
Date: 5/1/06		Date: 5/1/06	
Signature: <i>John H. Baker</i>		Signature: <i>William J. Baker</i>	
Date: 5/1/06		Date: 5/1/06	
Signature: <i>John H. Baker</i>		Signature: <i>William J. Baker</i>	
Date: 5/1/06		Date: 5/1/06	

LIBRARY ID: V0001643-8

ADMINISTRATIVE FORM

CHEM EHSR 236 1B 651 733 1958
05-01-2000 09:08pm From: TESTON SOLUTIONS

05/01 '06 13:46 NO.106 03/03
T-6M P.003/003 F-218



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DEVIATION FORM

Section 1: ☒ Project Specific Deviation ☐ Facility Deviation Date of Occurrence: 5/1/06							
Exygen Project #: P7001131	Deviation #: 5						
Client Project #: NA	Reference #:						
Section 2: Description of Deviation Issued:							
Section 3: <table border="0"> <tr> <td> <i>[Signature]</i> Principal Investigator Date: 5/1/06 </td> <td> <i>[Signature]</i> Exygen Manager Date: 5/1/06 </td> </tr> <tr> <td> <i>[Signature]</i> Study Director Date: 5/1/06 </td> <td> <i>[Signature]</i> Special Representative Date: </td> </tr> <tr> <td> <i>[Signature]</i> Quality Assurance Date: </td> <td> NA Operator Management Date: </td> </tr> </table>		<i>[Signature]</i> Principal Investigator Date: 5/1/06	<i>[Signature]</i> Exygen Manager Date: 5/1/06	<i>[Signature]</i> Study Director Date: 5/1/06	<i>[Signature]</i> Special Representative Date:	<i>[Signature]</i> Quality Assurance Date:	NA Operator Management Date:
<i>[Signature]</i> Principal Investigator Date: 5/1/06	<i>[Signature]</i> Exygen Manager Date: 5/1/06						
<i>[Signature]</i> Study Director Date: 5/1/06	<i>[Signature]</i> Special Representative Date:						
<i>[Signature]</i> Quality Assurance Date:	NA Operator Management Date:						

LIBRARY ID: V0001440-0

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