Analytical Laboratory Report Title

Determination of PFOS, PFOSA, and PFOA Levels in
Surface Water Samples from Port St. Lucie, FL
Revision 1 – 06/11/01

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Report Completion Date

At signing

Performing Laboratory

3M Environmental Laboratory
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

Project Identification

Analytical Report: FACT GEN-050
3M LIMS No. E01-0096

Total Number of Pages

41

Table of Contents

List of Tables

Laboratory Personnel and Contributors

Principal Analytical Investigator
Kris Hansen, Ph.D.
3M Environmental Lab
Bldg. 2-3E-09
St. Paul, MN 55133

Laboratory Management
Dale Bacon
3M Environmental Lab
Bldg. 2-3E-09
St. Paul, MN 55133

Analytical Chemistry Laboratory

Extraction and Analyses
3M Environmental Laboratory
Building 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

3M Laboratory Contributing Personnel

Lisa A. Clemen
Harold O. Johnson
Ognjenka Krupljanin*
Thomas P. Wagner*
*Contract lab professional service employees

Location of Archives

All original raw data and analytical summary have been archived at the 3M Environmental Laboratory.

Introduction and Purpose

The purpose of the study is to determine the levels of PFOS, PFOSA, and PFOA in samples of surface water collected from three ponds in Port St. Lucie, FL. This is the third sampling event for Site I and the first sampling event for Site II and Site III.

Sampling Plan

Water samples were collected from three separate surface water bodies in Port St. Lucie,FL. Site I has been sampled on two previous occasions, in July 1999 (sample collection by Battelle) and in July 2000 (sample collection by PACE Analytical). This study presents results from the third sampling event at Site I and the first sampling events at both Site II and Site III. Please see PACE Analytical report archived with this report, for details on sample collection.

Sample Collection and Analysis

Eight water samples were collected (5 from Site I, 2 from Site II, and 1 from Site III), along with 2 field blanks, 2 field spike controls samples (FSCS), and six field matrix spikes (FMS). Additionally, a single field duplicate sample was collected at both Site I and Site II.

After collection, all samples were immediately stored on ice and were shipped to the 3M Environmental Lab on ice.

All water samples and QC were prepared for analysis using solid phase extraction. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple response mode. All analytes were quantitated by external calibration using an extracted curve. Analytical details are included in this report.

Specimen Receipt and Maintenance

On January 29, 2001, the 3M Environmental Laboratory received water specimens collected on January 24, 2001. All specimens were received cold on ice and were immediately transferred to refrigerated storage at 4°C ±5°C.

Bottled water used in the analyses performed was obtained from Kandiyohi Premium Drinking Water from Premium Waters, Inc Minneapolis MN.

Chemical Characterization of the Reference Materials

Table 1. Characterization of the Analytical Reference Materials in Study GEN-050

Material	KPFOS[a]	PFOSA	APFO[b]
Identification	Potassium Perfluorooctanesulfonate	Perfluoroctane-sulfonylamide	Ammonium Perfluorooctonoate
Cas Number	2795-39-3	754-91-6	3825-26-1
Chemical Formula	$C_8F_{17}SO_3^-K^+$	$C_8F_{17}SO_2NH_2$	$C_7F_{15}CO_2^-NH_4^+$
Molecular Weight	537.9	498.9	430.9
Source	3M Specialty Chemicals	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	08/31/01	01/01/10	10/31/01
Storage Conditions	Ambient temperature	Ambient temperature	Ambient temperature
Chemical Lot Number	Unknown	L-15709	332
Physical Description	White powder	Light Yellow Waxy Solid	White powder
Purity	97.9%	TBD	95.0

[a] The target analyte is PFOS (Perfluorooctanesulfonate), $C_8F_{17}SO_3^-$.
[b] The target analyte is PFOA (Perfluorooctanoate), $C_7F_{15}CO_2^-$.
TBD-To Be Determined

Method Summaries

Following is a brief description of the method used during this analytical study by the 3M Environmental Laboratory. A copy of the method used in this study is located in Appendix A.

3M Environmental Laboratory

PREPARATORY AND ANALYTICAL METHOD

- **ETS-8-154.0** "Determination of Perfluorooctane Sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA) and Perflurooctanoate (POAA) in Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MSMS)"

  A 40 mL aliquot of each water samples was extracted using C18 solid phase extraction (SPE) cartridges. The target analytes are eluted from the cartridge using 5 mL of methanol. Separation, identification, and quantitation are accomplished using HPLC-ESMSMS in multiple response monitoring.

ANALYTICAL EQUIPMENT

The analytical equipment settings used in this analysis are presented below:

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_8 2x50 mm (5 µm)
Column temperature: 35°C
Mobile phase components:
Component A: 2mM ammonium acetate
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 15 µL
Solvent Gradient: 14.0 minutes

Time (minutes)	*%B*
0.0	40%
0.4	40%
1.0	90%
7.0	90%
7.5	100%
9.0	100%
9.5	40%
14.0	40%

Mass Spectrometer: Micromass® API/Mass Spectrometer Ultima II™ Triple Quadrupole system
Software: Mass Lynx™ 3.4

Cone Voltage: 30–60 V
Collision Gas Energy: 25–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 2. Target Ions Monitored in GEN-050

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499.0	99.0
PFOSA	498.0	78.0
PFOA	413.0	169.0

Data Quality Objectives/Criteria and Data Integrity

The following data quality objectives (DQOs) were indicated for the methods followed in this study:

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Acceptable Spike Recoveries:** 70–130%
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and daughter ion characterization.

Data Summary, Analyses, and Results

Data quality objectives for the analyses of this study were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curve was ≥ 0.981.
- **Calibration Standards:** Quantitation of the target analytes was based on 1/X weighted linear regression analysis of two extracted matrix curves bracketing each group of samples. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Quantitation of each analyte was based on the response of one specific product ion using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within ±30% of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks.

Table 3. LOQ in the Analyses of Extracts

Analyte	Method LOQ
PFOS	10 pg/mL
PFOA	25 pg/mL
PFOSA	10 pg/mL

- **Blanks:** All field blanks (n=2) and extraction blanks (n=4) were below the lower limit of quantitation for the compounds of interest.

- **Precision, Extraction:** Precision was determined by triplicate extraction and analysis of a single water sample and was reproducible to within +/-10%.
- **Precision, Collection:** Precision with respect to sample collection was determined by analysis of duplicate samples collected at Site I and at Site II; results were reproducible to within +/-20%.
- **Field Matrix Spikes:** During sample collection, six FMS were prepared by spiking water samples at concentrations ranging from 200 ppt to 5 ppb. With one exception, all FMS were within +/-30% of expected levels. One PFOS FMS at 1 ppb was recovered at only 48%. Two other PFOS FMSs prepared at this same level were recovered satisfactorily.
- **Laboratory Prepared Matrix Spikes:** In the lab, eight matrix spikes were prepared by spiking one of three water samples are either 100 ppt, 250 ppt, or 5 ppb prior to extraction. All lab-prepared matrix spikes recoveries were within +/-30% of expected values.

Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike analyses, indicates that the data are quantitative to +/-30%.

Summary of Sample Results

- **Site I:** Samples collected from different locations at Site I show close agreement with respect to PFOS levels. The average concentration of PFOS is just over 1.5 ng/mL. PFOA levels determined in samples collected form Site I showed much more dramatic variation, ranging from < 0.025 ng/mL to over 0.250 ng/mL. Four of the five samples showed close agreement with respect to PFOSA (just over 0.025 ng/mL); one sample was determined to contain PFOSA at 0.299 ng/mL.

  The relatively high levels of the target analytes in the Site I samples are consistent with results from previous sampling events. Site I is located very near a large marina and there is evidence of drainage from the marina into Site I. Additionally, there is an active culvert that feeds into Site I; the source of the water passing through the culvert is unknown. Either the marina or the culvert may be the source of the fluorinated compounds. Additional information, including a detailed description and photographs of Site I and surrounding areas can be found in the Pace Analytical report that describes the sample collection.
- **Site II:** Both PFOS and PFOA were detected in one of the two samples collected at Site II. PFOSA was not detected above the limit of quantitation in Site II samples.
- **Site III:** Only PFOA was detected above the limit of quantitation in the Site III sample. PFOA was determined to be present at 0.042 ng/mL.

  Although some fluorochemcials were detected in both Site II and Site III, the levels are more characteristic with fluorochemical levels observed in other bodies of surface water across the southeastern United States. The levels in Site II and Site III are dramatically lower than those in Site I and indicate that there is not widespread distribution of high levels of fluorochemical surfactants in this area of Port St. Lucie, FL. Detailed sample data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means, standard deviations, and spike recoveries (as detailed in the method).

Statement of Conclusion

The results of this analytical study confirm the results of previous sampling events at Site I. Relatively high levels of PFOS, PFOA, and PFOSA were found in the surface water labeled Site I. These relatively high levels were not mirrored in Site II and Site III, bodies of surface water located near to Site I, and indicate that the distribution of relatively high levels of fluorochemicals is not widespread.

Appendix A: Extraction and Analytical Method

This appendix includes the following methods:

ETS-8-154.0 "Determination of Perfluorooctane Sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA) and Perflurooctanoate (POAA) in Water by Liquid-Solid Extraction and High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC/MSMS)"

3M ENVIRONMENTAL LABORATORY

METHOD

DETERMINATION OF PERFLUOROOCTANE SULFONATE (PFOS), PERFLUOROOCTANE SULFONYLAMIDE (PFOSA), AND PERFLUOROOCTANOATE (POAA) IN WATER BY LIQUID-SOLID EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (HPLC/MS/MS)

Method Number: ETS-8-154.0 **Adoption Date:** 04/28/2000

Revision Date:

Author: Kristen J. Hansen/Harold O. Johnson

Approved By: William K. Reagen, Kent R. Lindstrom

William K. Reagen, Laboratory Management — Date: 4/28/00

Kristen J. Hansen, Ph.D., Group Leader — Date: 04/28/00

Kent R. Lindstrom, Technical Reviewer — Date: 04/28/00

1.0 SCOPE AND APPLICATION

1.1 This method provides collection, extraction, and analytical procedures for the determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane Sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in groundwater, surface water, and drinking water samples.

1.2 This method was prepared according to the EPA document, "Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141" (see Reference 18.1), and is based in part on the report "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water" (see Reference 18.2).

2.0 SUMMARY OF METHOD

2.1 Water samples are collected from a site of interest and shipped cold to an analytical facility. PFOS, PFOSA, and POAA are extracted from 40mL water samples using C_{18} solid phase extraction (SPE) cartridges. The compounds are eluted from the C_{18} cartridge, using methanol. Separation, identification, and measurement are accomplished by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analysis using multiple response monitoring (MRM).

The concentration of each identified component is measured by comparing the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the same compound in an extracted calibration standard (external standard).

3.0 DEFINITIONS

3.1 **Analytical Sample**—A portion of an extracted Laboratory sample prepared for analysis.

3.2 **Calibration Standard**—A solution prepared from the Working Standard (WS) and extracted according to this method. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 **Duplicate Sample (DS)**—A separate aliquot of a sample, taken in the analytical laboratory and analyzed separately with identical procedures. Analysis of DSs compared to that of the first aliquot give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 **Field Blank Control Sample (FB)**—Type I water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FB is to determine if test substances or other interferences are present in the field environment.

3.5 **Field Duplicate (FD)**—A sample collected in duplicate at the same time as the sample and placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of FD compared to that of the first sample gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.6 **Field Matrix Spike (FMS)**—A sample collected in duplicate to which known quantities of the target analytes are added in the field at the time of sample collection. The FMS should be spiked at approximately 50–150% of the expected analyte concentration in the sample. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.

3.7 **Field Spike Control Sample (FSCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the field at the time of sample collection (at an appropriate concentration to be determined by the project lead). The FSCS is extracted and analyzed exactly like a sample to determine whether a loss of analyte could be attributed to sample storage and/or shipment.

3.8 **Laboratory Control Sample (LCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the laboratory. Two levels are included, one at the LOQ (approx. 25Pg/mL), the other at a concentration of approx. 100–250Pg/mL or another concentration to be determined by the project lead. The LCS is extracted and analyzed exactly like a laboratory sample to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements at the required method detection limit and higher.

3.9 **Laboratory Sample**—A portion of a sample received from the field for testing.

3.10 **Limit of Detection (LOD)**—The lowest concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The LOD can be determined in several ways, including signal-to-noise ratio and statistical calculations.

3.11 **Limit of Quantitation (LOQ)**—The lowest concentration (LLOQ) or highest concentration (ULOQ) that can be reliably achieved within the specified limits of precision and accuracy during routine operating conditions.

Note: The LLOQ is generally 5–10 times the LOD. For many analytes, the LLOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve. However, it may be nominally chosen within these stated guidelines to simplify data reporting. Sample LLOQs are matrix-dependent.

3.12 **Matrix Spike (MS)**—An aliquot of a sample, to which known quantities of target analytes are added in the laboratory. The MS is extracted and analyzed exactly like a laboratory sample to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations.

3.13 **Method Blank**—An aliquot of type I water that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other laboratory samples. The method blank is used to determine if test substances or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.14 **Method Detection Limit (MDL) Determination**—One of several processes that may be used to establish a LOD value. The statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero. This term is usually associated with the EPA definition in 40 CFR Part 136 Appendix B.

3.15 **Sample**—A sample is a small portion collected from a larger quantity of material intended to represent the original source material.

3.16 **Spiking Stock Standard (SSS)**—A solution prepared from stock standards used to prepare the working standard.

3.17 **Stock Standard (SS)**—A concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound.

3.18 **Working Standard (WS)**—A solution of several analytes prepared in the laboratory from SSs and diluted as needed to prepare calibration standards and other required analyte solutions.

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings**

4.1.1 The acute and chronic toxicity of the standards for this method have not been precisely determined; however, each should be treated as a potential health hazard.

4.1.2 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.

4.1.3 The laboratory is responsible for maintaining a safe work environment and a current awareness of local regulations regarding the handling of the chemicals used in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved in these analyses.

5.0 Interferences

5.1 During extraction and analysis, major potential contaminant sources are reagents and liquid-solid extraction devices.

5.2 All materials used in the analyses shall be demonstrated to be free from interferences under conditions of analysis by running method blanks.

5.3 Teflon® containing materials (e.g. caps, wash bottles) contain fluorocompounds which may cause interferences and should not be used during collection, storage, extraction, or analysis of the samples.

6.0 EQUIPMENT, SUPPLIES, AND MATERIALS

Note: Brand names, suppliers, and part numbers are for illustrative purposes only. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Sampling Equipment

6.1.1 Sample collection bottles—LDPE (e.g., Nalgene™) narrow-mouth bottles with screw cap.

Note: Do not use Teflon bottles or Teflon lined caps.

6.1.2 Coolers for sample shipment.

6.1.3 Ice for sample shipment.

6.1.4 Bottles must be lot-certified to be free of artifacts by running Method blanks according to this method.

6.2 Laboratory Equipment (Extraction and Analytical)

6.2.1 Balance, analytical (display at least 0.0001g), Mettler.

6.2.2 Vacuum pump, Büchi.

6.2.3 Visiprep vacuum manifold, Supelco.

6.2.4 Sep Pak Vac 6cc (1g) tC_{18} cartridges (part # WAT 036795),Waters.

6.2.5 50mL disposable polypropylene centrifuge tubes, VWR.

6.2.6 15mL disposable polypropylene centrifuge tubes, VWR.

6.2.7 Disposable micropipettes (50–100µL, 100–200µL), Drummond.

6.2.8 Class A pipettes and volumetric flasks, various.

6.2.9 Hypercarb drop-in guard column (4mm) (part # 844017–400), Keystone.

6.2.10 Stand-alone drop-in guard cartridge holder, Keystone.

6.2.11 125mL LDPE narrow-mouth bottles, Nalgene.

6.2.12 HPLC pump (LC10AD), Shimadzu.

6.2.13 2mL clear HPLC vial kit (cat # 5181–3400), Hewlett Packard.

6.2.14 Standard lab equipment (graduated cylinders, disposable tubes, etc.), various.

6.2.15 LC/MS/MS and HPLC systems, as described in section 10.1.

6.3 Equipment Notes

6.3.1 In order to avoid contamination, the use of disposable labware is highly recommended (tubes, pipettes, etc.).

6.3.2 Teflon or Teflon-lined containers or equipment, including Teflon-lined HPLC vials or caps for the HPLC auto sampler must **not** be used.

6.3.3 Type I water used during the sample and standard extraction should be filtered through a Hypercarb guard column using a HPLC pump. This water is referred to as "filtered type I water", hereafter in this report.

6.3.4 It is necessary to check the solvents (methanol) for the presence of contaminants (especially POAA) by LC/MS/MS prior to use. Certain lot numbers have been found to be unsuitable for use.

6.3.5 Use disposable micropipettes or pipettes to aliquot standard solutions to make calibration standards and matrix spikes.

7.0 REAGENTS AND STANDARDS

Note: Suppliers and catalog numbers are for illustrative purposes only. Equivalent performance may be achieved using chemicals obtained from other suppliers. Do not use a lesser grade of chemical than those listed.

7.1 Chemicals

- 7.1.1 Methanol (MeOH), HPLC grade, JT Baker, Catalog No. JT9093–2.
- 7.1.2 Ammonium Acetate, Reagent grade, Sigma-Aldrich, Catalog No. A–7330.
- 7.1.3 Water, type I, prepared in-house.
- 7.1.4 Sodium Thiosulfate, Reagent grade, JT Baker.

7.2 Standards

- 7.2.1 Potassium perfluorooctane sulfonate (see Attachment A, Figure 1).
- 7.2.2 Perfluorooctane sulfonylamide (see Attachment A, Figure 2).
- 7.2.3 Ammonium perfluorooctanoate (see Attachment A, Figure 3).

7.3 Reagent Preparation

- 7.3.1 **250mg/mL sodium thiosulfate solution (Extraction)**—Dissolve 25g of sodium thiosulfate in 100mL reagent water.
- 7.3.2 **40% methanol (Extraction)**—Measure 400mL methanol and adjust the volume to 1.0L with reagent water.
- 7.3.3 **100mM ammonium acetate solution (Analysis)**—Weigh 7.71g of ammonium acetate and dissolve in 1.0L of reagent water. Dilute the 100mM solution by a factor of 50 to make the 2mM ammonium acetate solution used for mobile phase A.

Note: Alternative volumes may be prepared as long as the ratios of the solvent to solute ratios are maintained.

7.4 Spiking Stock Standard (SSS) Preparation

- 7.4.1 **100µg/mL each PFOS, PFOSA, and POAA SSSs**—Weigh out 10mg of analytical standard (corrected for percent salt and purity—i.e., 10 mg $C_8F_{17}SO_3K$ purity 90% = 8.35mg $C_8F_{17}SO_3^-$) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle. Prepare a **separate** solution for each analyte. Store solutions in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.
- 7.4.2 **1µg/mL mixed SSS**—Add 1.0mL each of the 100µg/mL SSSs (from 7.4.1) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.3 **0.1µg/mL mixed SSS**—Add 10.0mL of the 1.0µg/mL-mixed solution (from 7.4.2) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.4 **0.01µg/mL mixed SSS**—Add 10.0mL of the 0.1µg/mL-mixed solution (from 7.4.3) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.4.5 **Storage Conditions**—Store all SSSs in a refrigerator in 125mL LDPE bottles at 4°±2°C for a maximum period of 3 months from the date of preparation.

7.5 **Calibration Standards**

7.5.1 **100µg/mL each PFOS, PFOSA, and POAA stock standard solutions**—Weigh out 10mg of analytical standard (corrected for percent salt and purity) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle. Prepare a **separate** solution for each analyte. Store solutions in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.

7.5.2 **1µg/mL Working Standard**—Add 1.0mL each of the 100µg/mL SS solutions (from 7.5.1) to a 100mL volumetric flask and bring up to volume with methanol.

7.5.3 **0.1µg/mL Working Standard** —Add 10.0mL of the 1.0µg/mL mixed solution (from 7.5.2) to a 100mL volumetric flask and bring up to volume with methanol.

7.5.4 **0.01µg/mL Working Standard** —Add 10.0mL of the 0.1µg/mL mixed solution (from 7.5.3) to a 100mL volumetric flask and bring up to volume with methanol.

7.5.5 **Storage Conditions**—Store all WSs in a refrigerator (in 125mL LDPE bottles) at 4°±2°C for a maximum period of 3 months from the date of preparation.

7.5.6 **Calibration Standard**—Prepare a minimum of five calibration solutions in filtered type I water according to the following table:

Concentration of WS, µg/mL	Volume of WS, µL	Final Calibration Standard Volume, mL	Final Concentration of Calibration Standard, Pg/mL
0.0	0	40	0
0.010	100	40	25
0.010	200	40	50
0.010	400	40	100
0.10	100	40	250
0.10	200	40	500
0.10	300	40	750[1]
0.10	400	40	1000[2]

1 May be prepared to extend the range beyond 500Pg/mL.
2 May be prepared to extend the range beyond 750Pg/mL.

Note: The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained.

7.5.7 The standards are processed through the extraction procedure (Section 9.0), identical to the laboratory samples. The extracted concentration of the calibration standard is equal to 8X the initial concentration, due to the concentration of the standard during the extraction process.

7.5.8 **Storage Conditions**—Store all extracted calibration standards in 15mL polypropylene tubes at 4°±2°C, for a maximum period of two weeks from the date of preparation.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

Note: Sampling equipment, including automatic samplers, must be free of Teflon tubing, gaskets, and other parts that may leach interfering analytes into the water sample. Automatic samplers that composite samples over time should use refrigerated polypropylene sample containers if possible. Sample bottles should not be rinsed before sample collection.

8.1 **Tap Water**—Open the tap and allow the system to flush until the water temperature (15°±10°C) has stabilized (usually about two minutes). Adjust the flow to about 500mL/min and collect samples from the flowing stream.

8.2 **Ground Water**—Purge the well of standing water using a pump or a bailer. Collect the sample directly from the pump or from the bailer.

8.3 **Surface Water**—When sampling from an open body of water, fill the sample container with water from a representative area.

8.4 **Sample Dechlorination**—All samples should be iced or refrigerated at 4°±2°C and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced by adding 200µL of a 250mg/mL sodium thiosulfate solution to each water sample, FB, and FSCS (which may be placed in each bottle before leaving for the sampling site.).

8.5 **Holding Time (HT)**— Results of the time/storage study of all target analytes showed that the three compounds are stable for 14 days in water samples when the samples are dechlorinated and stored as described in section 8.4 (see also reference 18.3). Therefore, laboratory samples must be extracted within 14 days and the extracts analyzed within 30 days of sample collection. If the HT exceeds 14 days, great care is used when evaluating field spikes to avoid misrepresentation of the sample concentration.

8.6 **Field Blanks**

8.6.1 Process a Field Blank Control Sample (FB) along with each sample set (samples collected from the same general sample site at approximately the same time). At the laboratory, prior to sample collection, fill a sample container with filtered type I water, seal, and ship the FB to the sampling site along with the empty sample containers. Return the FB to the laboratory with the filled sample bottles.

8.6.2 When sodium thiosulfate is added to samples, use the same procedure to preserve the FB.

8.7 **Field Duplicates**

8.7.1 Collect a Field Duplicate (FD) for every ten (10) samples collected or per each sampling set, if less than 10 samples are collected.

8.7.2 Separate FDs must be collected for each type of water sample (ground, tap, etc.) collected.

8.7.3 Collect the FD immediately after the sample.

8.7.4 Preserve, store and ship FD using the same procedures as used for the samples.

8.8 Field Spike Control Sample (FSCS)

8.8.1 A Field Spike Control Sample (FSCS) must be prepared for each sample shipment. If multiple coolers are used to ship a set of samples, each cooler must contain a FSCS.

8.8.2 At the laboratory, fill a sample container with 100mL of type I water. Seal and ship to the sampling site along with the empty sample containers and FBs.

8.8.3 When sodium thiosulfate is added to samples, use the same procedure to add the same amounts to the FSCS.

8.8.4 Seal and gently invert the FSCS to mix. Store and ship the FSCS using the same procedures as used for the samples.

9.0 EXTRACTION PROCEDURE

9.1 Extraction Scheme

9.1.1 Allow samples to equilibrate to room temperature. Thoroughly mix samples by gently inverting the sample bottle.

9.1.2 Measure 40mL of sample into 50mL polypropylene centrifuge tubes (Spike the QC and Matrix spikes as required*, replace lid and mix well).

Note: * Samples may need to be prescreened to determine an appropriate matrix spike level (typically 50–150% of sample concentration).

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

Note: For the following steps, maintain a ~1drop/sec flow rate. Do not allow the column to run dry at any time.

9.1.4 Load the analytical sample onto the C_{18} SPE cartridge. Discard eluate.

9.1.5 Wash with ~5mL 40% methanol in water. Discard eluate.

9.1.6 Elute with ~5mL 100% methanol. Collect 5mL of eluate into graduated 15mL polypropylene centrifuge tubes. This is the target elution fraction (final volume = 5mL).

9.1.7 Analyze a portion of the target elution fraction eluent using negative electrospray HPLC/MS/MS (Section 10.2).

Note: Samples are concentrated by a factor of eight during the extraction; Initial Vol = 40mL → Final Vol. = 5mL.

9.1.8 Samples are stable at room temperature for at least 24 hours. Analytical samples may be stored in a refrigerator at 4°±2°C until analysis.

9.1.9 **Standardization of C_{18} SPE columns**—If poor recoveries are observed, it may be necessary to standardize the C_{18} SPE columns in the following manner before analyzing samples.

9.1.9.1 Use a standard with an analyte concentration between 1000 and 4000 Pg/mL. Follow the extraction scheme as outlined from steps 9.1.1 to 9.1.6, except, collect the eluate fraction separately (approx. 5mL), as well as the target elution fraction.

9.1.9.2 After step 9.1.6, collect a post-elution fraction by, eluting with an additional 5mL of 100% methanol.

9.1.9.3 Analyze all three fractions by HPLC/MS/MS. If the target fraction contains a minimum of 85% of the respective analytes, it may be considered acceptable.

9.1.9.4 If the wash contains significant standard (>15%), either the wash volume or percentage of MeOH should be decreased.

9.1.9.5 If the post-elution fraction contains significant standard (>15%), the target elution volume should be increased.

10.0 CALIBRATION AND STANDARDIZATION (ANALYTICAL SETUP)

Note: Other instruments may be used and the equipment and conditions may be very different as long as the method criteria are met. The operator must optimize and document the equipment and settings used.

10.1 Establish the LC/MS/MS system and operating conditions equivalent to the following:

Mass Spec: Micromass Quattro Ultima (Micromass)
Interface: Electrospray (Micromass)
Mode: Electrospray Negative, Multiple Response Monitoring (MRM)
Harvard infusion pump (Harvard Instruments), for tuning
Computer: COMPAQ Professional Workstation AP200
Software: Windows NT, MassLynx 3.3
HPLC: Hewlett Packard (HP) Series 1100
HP Quat Pump
HP Vacuum Degasser
HP Autosampler
HP Column Oven

Note: A 4 x 10mm Hypercarb drop-in guard cartridge (Keystone, part # 844017–400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_8 (Jones Chromatography), 2.1mm x 50mm, 4µm
Column Temperature: 35°C
Injection Volume: 15µL
Mobile Phase (A): 2mM Ammonium Acetate in filtered type I water (See 7.3.1)
Mobile Phase (B): Methanol

HPLC Gradient Program:

Time, min	Percent Mobile Phase A	Percent Mobile Phase B	Flow Rate, mL/min
0.0	60	40	0.3
0.4	60	40	0.3
1.0	10	90	0.3
7.0	10	90	0.3
7.5	0	100	0.3
9.0	0	100	0.4
9.5	60	40	0.4
13.5	60	40	0.4
14.0	60	40	0.3

Note: Other HPLC gradients may be used as long as the method criteria are met.

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1mm x 30mm) and columns from different manufacturers (Keystone Betasil C_{18} etc.) may be used.

Ions Monitored:

Analyte	Primary Ion	Product Ion	Approximate Retention Time
POAA	413.0	169.0	5.0
PFOS	499.0	99.0	5.2
PFOSA	498.0	78.0	5.8

Other product ions may be chosen at the discretion of the analyst, although m/z 99 is suggested for PFOS. Use of the suggested primary ion is recommended. Retention times may vary slightly, on a day-to-day basis, depending on the batch of mobile phase etc. Drift in retention times is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are interspersed throughout the analytical run.

10.2 Tune File Parameters

10.2.1 The following values are provided as an example. Actual values may vary from instrument to instrument. Also, these values may be changed from time to time in order to optimize for greatest sensitivity.

Analyte	Dwell, sec	Collision Energy, eV	Cone, V
POAA	0.2–0.4	10–25	20–30
PFOS	0.2–0.4	30–60	50–80
PFOSA	0.2–0.4	20–50	30–60

Source	Set
Capillary	2.56–3.5kV
Hexapole 1	0.5V
Aperture 1	0.2V
Hexapole 2	0.8V
Source Block Temp.	100–150°C
Desolvation Temp.	250–400°C

Analyzer	Set
LM Res 1	12.0–15.0V
HM Res 1	12.0–15.0V
IEnergy 1	0.7V
Entrance	–2V
Exit	1V
LM Res 2	11.0V
HM Res 2	11.0V
IEnergy 2	1.0V
Multiplier	650V

Gas Flows	Set
Cone Gas	150L/hr
Desolvation	700L/hr

Pressures	Set
Gas Cell	3.0e–3mbar

11.0 ANALYTICAL QUALITY CONTROL

11.1 Analytical results of the FB, FMS, FD, and FSCS should be evaluated at the conclusion of the study to help interpret the data quality of samples data. Analytical results for these control/duplicate samples must be reported with the sample data.

12.0 ANALYTICAL PROCEDURE

12.1 Sample Analysis

12.1.1 Set up analysis sample queue.

12.1.2 Inject the same aliquot (between 5–25µL) of each standard, analytical sample, recovery, control etc. into the LC/MS/MS system.

12.1.3 All samples showing a response for one or more analytes above the response of the highest, active calibration curve level must be diluted and reanalyzed.

12.2 Calibration Curve

12.2.1 Starting with the standard of lowest concentration, inject the same size aliquot (between 10–25µL) of each extracted calibration standard according to Section 12.1 and tabulate the response (peak height or area) versus the concentration in the standard. Use linear standard curves for quantitation generated for each analyte by linear regression with 1/x weighting of peak area versus calibration standard concentration. The correlation coefficient (r) for the calibration curves must be ≥0.990 (r^2≥0.980). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation and the standards reanalyzed.

12.2.2 **Curve**—The measured value for each curve point must be within ± 30% of theoretical values when curve is evaluated over a range appropriate to the data. High or low points may be deactivated to achieve these criteria, but an acceptable curve must contain at least five active curve points.

12.2.3 **Continuing Curve Verification (CCV)**—Mid- and low-level calibration checks should be analyzed every 5–10 injections. The analyte level measured in the CCVs should be within ± 30% of theoretical values. If CCVs fall outside of this range, data collected subsequent to the last passing CCV should not be used. Only data collected between acceptable CCVs or the initial curve can be used.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculate the analytical sample (extract) concentration from the standard curve using the following equation:

$$\text{Extract Concentration, pg/mL} = \frac{(\text{Peak area} - \text{intercept})}{(\text{slope})}$$

13.2 Calculate the percent recovery of the FSCS using the following equation:

$$\text{FSCS \% rec.} = \frac{(\text{FSCS conc., Pg/mL})}{(\text{Conc. added, Pg/mL})} \times 100$$

13.3 Calculate the percent recovery of the MSs using the following equation:

$$\text{MS \% rec.} = \frac{(\text{MS conc., Pg/mL} - \text{Sample Conc., Pg/mL})}{(\text{Conc. added, Pg/mL})} \times 100$$

14.0 METHOD PERFORMANCE PARAMETERS

Note: Any method performance parameters that are not achieved must be considered in the evaluation of the data. Nonconformance to any specified parameters must be described and discussed in any reporting of the data.

14.1 Linearity—Linear standard curves for quantitation generated for each analyte by linear regression with 1/x weighting of peak area versus calibration standard concentration. The correlation coefficient (r) for the calibration curves must be ≥0.990 (r^2≥0.980).

14.2 Calibration Curve Standards—The measured value for each curve point must be within ± 30% of theoretical values when curve is evaluated over a range appropriate to the data. High or low points may be deactivated to achieve these criteria, but an acceptable curve must contain at least five active curve points.

14.3 CCV Performance—Mid and low level calibration checks to be analyzed every 5–10 injections. The analyte level measured in the CCVs should be within ± 30% of theoretical values. If CCVs fall outside of this range, data collected subsequent to the last passing CCV should not be used. Only data collected between acceptable CCVs can be used.

14.4 Limit of Detection (LOD)—The lowest calibration standard with a peak area at least 2X the peak area of the extraction blank that can be measured at a concentration greater than zero.

14.5 Limits of Quantitation (LOQ)—The lower LOQ (LLOQ) is the lowest non-zero active standard in the calibration curve; the peak area of the LLOQ must be at least 2X that of the extraction blank. By definition, the measured value of the LLOQ must be within 30% of the theoretical value.

14.6 Matrix Spikes—Matrix spike percent recoveries must be within ± 30% of the spiked concentration.

14.7 Solvent Blanks, Method Blanks, and Matrix Blanks—Values must be below the lowest non-zero active standard in the calibration curve. Matrix blanks are considered compliant if no test substance is detected above the LOD for that analyte.

14.8 Reproducibility—Reproducibility of the method is defined by the results of the matrix spikes and matrix spike duplicates. The MS/MSD should be reproducible to within 20%.

14.9 Use of Confirmatory Methods—None

14.10 Demonstration of Specificity—Specificity is demonstrated by chromatographic retention time (within 3% of standard) and the mass spectral response of unique product ions generated from a characteristic primary ion.

14.11 Documentation

14.11.1 If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed, or other actions taken as determined by the analyst. Document all actions in the appropriate logbook.

14.11.2 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

15.1 Sample extract waste and flammable solvent is discarded in high BTU containers, and glass pipette waste is discarded in broken glass containers located in the laboratory.

16.0 RECORDS

16.1 Each page generated for a study must have the following information included, either in the header or hand-written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument run log.

16.3 Plot the calibration curves as described in this method, then print these graphs and store in the study folder.

16.4 Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

16.5 Summarize data using suitable software (MS Excel 97) and store in the study folder.

16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 ATTACHMENTS

17.1 **Attachment A:** Figures—Fluorochemical Compounds

18.0 REFERENCES

18.1 "Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141", U.S. Environmental Protection Agency, Office of Science and Technology Office of Water, Washington, D.C. Draft 1996.

18.2 "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, January 2000.

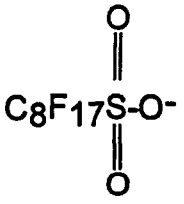
18.3 Validation report for the "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water", E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, (Approval pending)

19.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

Figure 1: PFOS

Chemical Name = Perfluorooctane sulfonate
Molecular ion = 499 ($C_8F_{17}SO_3^-$)

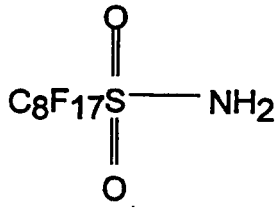


PFOS

Note: Standards are made from the salt, potassium perfluorooctane sulfonate [$C_8F_{17}SO_3K$], m/w 538.

Figure 2: PFOSA

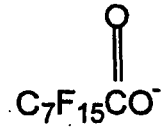
Chemical Name = Perfluorooctane sulfonylamide
Molecular ion = 498 ($C_8F_{17}SO_2NH_2$)



PFOSA

Figure 3: POAA

Chemical Name = Perfluorooctanoate
Molecular ion = 413 ($C_7F_{15}COO^-$)



POAA

Note: Standards are made from the salt, ammonium perfluorooctanoate [$C_7F_{15}COONH_4$], m/w 431

Appendix B: Data Summary Tables

Table 4. Port St. Lucie Resample January 24, 2001 Sample Results for GEN-050

Sample Number and Description			PFOS ng/mL	PFOA ng/mL	PFOSA ng/mL
Site I	E00-0096-19527	East	1.60	<0.0250A	0.0283
	E00-0096-19541	South	1.54	0.123	0.0238
	E00-0096-19545	West	1.59	0.107	0.0258
	E00-0096-19549	North	1.78	0.397	0.299*
	E00-0096-19553	Culvert Discharge	1.38	0.081	0.0363
		Average	1.58	0.180	0.029
		Std. Dev.	0.15	0.15	0.005
		RSD (%)	9.2	83.5	19.2
Site II	E00-0096-19555	Site II	<0.0100A	<0.0250A	<0.0100A
	E00-0096-19557	Site II, Dupe	0.0148	0.0290	<0.0100A
		Average	0.0099	0.0208	<0.0100A
Site III	E00-0096-19565	Site III	<0.0100A	0.0420	<0.0100A

It is not possible to verify true recovery of endogenous analyte from samples without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to ±30%.

Averages are calculated with the value of ½ LOQ assigned to samples reported at <LOQ.

A = No Limit of Detection (LOD) determined for this analysis. LOQ/LOD is the same concentration and is equal to the following:

LOQ = 0.0100 ng/ml for PFOS & PFOSA

LOQ = 0.0250 ng/mL for PFOA.

* Not included in calculation of site average.

Appendix C: Data Spreadsheets

PORT ST. LUCIE RESAMPLE: JANUARY 24, 2001

Extraction 020201 OK
Analysis 020601 HOJ, 020801 HOJ (Ruby)

Sample number			PFOS extract (pg/mL)	PFOS sample (pg/mL)	PFOS Sample (ng/mL)	Notes	File name	PFOA extract (pg/mL)	PFOA sample (pg/mL)	PFOA Sample (ng/mL)*	Notes	File name	PFOSA extract (pg/mL)	PFOSA sample (pg/mL)	PFOSA Sample (pg/mL)	Notes	File name
Blanks																	
	water, ext blank-1	Extraction Blank	0.00	0.00	<10 pg/mL		r010206019	0	0.00	<25 pg/mL		r010206019	0	0	<10 pg/mL		r010206019
	water, ext blank-2	Extraction Blank	0.00	0.00	<10 pg/mL		r010206020	182	22.75	<25 pg/mL		r010206020	0	0	<10 pg/mL		r010206020
	water, ext blank-3	Extraction Blank	0.00	0.00	<10 pg/mL		r010206021	47	5.88	<25 pg/mL		r010206021	0	0	<10 pg/mL		r010206021
	water, ext blank-4	Extraction Blank	0.00	0.00	<10 pg/mL		r010206022	0	0.00	<25 pg/mL		r010206022	0	0	<10 pg/mL		r010206022
	E01-0096-19523	Field Blank	0.00	0.00	<10 pg/mL		r010206025	0	0	<25 pg/mL		r010206025	0	0	<10 pg/mL		r010206025
	E01-0096-19524	Field Blank	0.00	0.00	<10 pg/mL		r010206026	137	19.6	<25 pg/mL		r010206026	0	0	<10 pg/mL		r010206026
*LOQ criteria stated in the method were not met for PFOA for reported values <2.5 ng/mL.																	
Samples																	
SITE I	E01-0096-19527	Site I, east	12797	1599.6	1.60		R010206045	158	19.8	<25 pg/mL		R010208045	226	28.25	0.0283		R010206045
	E01-0096-19541	Site I, south	12326	1540.8	1.54		r010206056	981	123	0.123		r010208056	190	23.75	0.0238		r010206056
	E01-0096-19545	Site I, west	12746	1593.3	1.59		r010206059	857	107	0.107		r010208059	206	25.75	0.0258		r010206059
	E01-0096-19549	Site I, north	14253	1781.6	1.78		r010206060	3173	397	0.397		r010208060	2391	298.9	0.299*		r010206060
	E01-0096-19553	Site I, culvert discharge	11010	1376.3	1.38		r010206061	645	81	0.081		r010208061	290	36.3	0.0363		r010206061
					Average	1.58				Average	0.18				Average	0.029	
					Std. Dev.	0.15				Std. Dev.	0.15				Std. Dev.	0.005	
					RSD (%)	9.2				RSD (%)	83.5				RSD (%)	19.2	
SITE II	E01-0096-19555	Site II	0	0.0	<10 pg/mL		r010206029	28	3.5	<25 pg/mL		r010208029	0	0.0	<10 pg/mL		r010206029
	E01-0096-19557	Site II, dupe	118	14.8	0.0148		r010206032	232	29.0	0.0290		r010208032	0	0.0	<10 pg/mL		r010206032
					Average	0.0099			Average	0.0208				Average	<10 pg/mL		
					Std. Dev.	NA			Std. Dev.	NA				Std. Dev.	NA		
					RSD (%)	NA			RSD (%)	NA				RSD (%)	NA		
Averages are calculated with the value of 1/2LOQ asigned to samples reported at <LOQ.															* outlier not included in calculation of site average		
	E00-0096-19369	Blind	11983	1498	1.498		r010206036	664	83.0	0.083		r010208036	166	20.8	0.021		r010206036

PORT ST. LUCIE RESAMPLE: JANUARY 24, 2001

Extraction 020201 OK
Analysis 020401 HOJ, 020801 HOJ (Ruby)

						%REC					%REC				%REC	
Field Spike Control Samples																
	E01-0096-19525	FSCS-200 ppt	1287	161	0.161	80.4	r010206027	1107	138	0.138	69.2	r010208027	1445	181	0.181	90.3	r010206027
	E01-0096-19526	FSCS-1 ppb	5768	721	0.721	72.1	r010206028	6907	863	0.863	86.3	r010208028	5625	703	0.703	70.3	r010206028
Field Matrix Spikes																	
	E01-0096-19531	Site I, east FMS 1 ppb	16542	2068	2.07	48.9	r010206052	7274	909	0.91	73.3	r010208052	7864	983	0.983	95.5	r010206052
	E01-0096-19532	Site I, east FMS 25 ppb	1,760	22000	22	81.7	r010206053	1428	17850	18	70.7	r010208053	1857	23213	23	92.7	r010206053
	E01-0096-19535	Site I, east FMS 1 ppb	19908	2489	2.49	91.0	r010206054	8320	1040	1.04	86.3	r010208054	8437	1055	1.05	103	r010206054
	E01-0096-19536	Site I, east FMS 25 ppb	1,586	19825	20	73.0	r010206055	1649	20613	21	81.7	r010208055	1980	24750	25	98.9	r010206055
	E01-0096-19559	Site II, FMS 200 ppt	1260	157.5	0.158	73.8	r010206033	1504	188	0.188	94.0	r010208033	1412	176.5	0.177	88.3	r010206033
	E01-0096-19560	Site II, FMS 1 ppb	5669	709	0.709	69.9	r010206034	7797	975	0.975	97.5	r010208034	6796	850	0.850	85.0	r010206034
Laboratory Matrix Spike																	
	E01-0096-19555 MS-100 ppt	Site II, MS	810		0.101	91.4	r010206039	1353		0.169	148	r010208039	859		0.107	107.4	r010206039
	E01-0096-19555 MSD-100 ppt	Site II, MSD	822		0.103	92.9	r010206040	985		0.123	102	r010208040	874		0.109	109.3	r010206040
	E01-0096-19565 MS-100 ppt	Site III, MS	1149		0.144	139	r010206041	1357		0.170	128	r010208041	699		0.087	87.4	r010206041
	E01-0096-19565 MSD-100 ppt	Site III, MSD	957		0.120	115	r010206042	1339		0.167	126	r010208042	799		0.100	99.9	r010206042
	E01-0096-19541-MS-250 ppt	Site I, MS	14359		1.795	102	r010206062	2382		0.298	70.1	r010208062	2290		0.286	105	r010206062
	E01-0096-19541-MSD-250 ppt	Site I, MSD	14917		1.865	150	r010206063	2943		0.368	104	r010208063	2524		0.316	117	r010206063
	E01-0096-19541-MS-5 ppb 1:10	Site I, MS	48180		6.023	89.6	r010206066	37760		4.720	91.9	r010208066	41890		5.236	104	r010206066
	E01-0096-19541-MSD-5 ppbt 1:10	Site I, MSD	52330		6.541	100	r010206067	44670		5.584	109	r010208067	44440		5.555	111	r010206067
Precision																	
	E01-0096-19527	Site I, east	12797	1599.6	1.60		R010206045	158	19.8	<25 pg.mL		R010208045	226	28.3	0.0283		R010206045
	E00-0096-19527-1	Site I dupe	12562	1570	1.57		r010206046	182	22.8	<25 pg/mL		r010208046	202	25.3	0.0253		r010206046
	E01-0096-19527-2	Site I dupe	12647	1581	1.58		r010206047	533	66.6	0.0666		r010208047	235	29.4	0.0294		r010206047
	E01-0096-19527-3	Site I dupe	12436	1555	1.55		r010206048	34	4.25	<25 pg/mL		r010208048	207	25.9	0.0259		r010206048
	E01-0096-19529	Site I, east FD	14699	1837	1.84		r010206049	648	81.0	0.0810		r010208049	235	29.4	0.0294		r010206049
				Precision, extraction (%RSD)		1.20			Precision, extraction (%RSD)		NA			Precision, extraction (%RSD)		7.17	
				Precision, collection (%)		117			Precision, collection (%)		122			Precision, collection (%)		108	

Appendix D: Example Calculations

Formula Used for Water Analyses in Study FACT GEN-050

$$\text{AR (pg/mL)} \times \frac{\text{FV (mL)}}{\text{IV (mL)}} \times \frac{1.0\text{ ng}}{1000\text{ pg}} = \textbf{Reported Concentration (ng/mL)}$$

Calculation Used for PFOS Concentration in EOO-0096-19527

$$12797\text{ pg/mL} \times \frac{5\text{ mL}}{40\text{ mL}} \times \frac{1.0\text{ ng}}{1000\text{ pg}} = \textbf{1.60 ng/mL}$$

AR— Analytical result from MassLynx summary
IV— Initial Volume of H2O Extracted
FV— Final Volume of Extract

Formula Used for Spike Recovery Analyses in Study FACT GEN-050

$$\frac{(RC_{spiked}\text{ (ng/mL)} - RC_{unspiked}\text{ (ng/mL)})}{SL\text{ (ng/mL)}} \times 100\% = \textbf{\% Recovery}$$

Calculation Used for EOO-0096-19535[a] (Site I)

$$\frac{(2.49\text{ ng/mL} - 1.58\text{ ng/mL})}{1.0\text{ ng/mL}} \times 100\% = 91\%$$

[a] Average concentration of all Site I samples used for $RC_{unspiked}$.

RC— Reported Concentration
SL— Spike Level

Appendix E: Interim Certificates of Analysis

Centre Analytical Laboratories, Inc.
3048 Research Drive, State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

Centre Analytical Laboratories COA Reference #: 023-021
3M Product: PFOS, Primary Standard
Test Control Reference #: TCR00017-46
Purity: 97.9%

Test Name	Specifications	Result
Purity[1]		**97.9%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		 1. 0.006 wt./wt.% 2. 0.002 wt./wt.% 3. 1.216 wt./wt.% 4. 6.685 wt./wt.% 5. <0.001 wt./wt.% 6. 0.002 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.13 wt./wt.%
Total % Impurity (LC/MS)		0.14 wt./wt.%
Total % Impurity (GC/MS)		None Detected
Related Compounds – POAA		<0.01 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		97.5 Mol. %.
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[3]		 1. <0.015 wt./wt.% 2. 0.60 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 7.88 wt./wt.%
Organic Acids [4] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[5]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	 1. 11.39 wt./wt.% 2. 0.456 wt./wt.% 3. 1.51 wt./wt.% 4. 7.80 wt./wt.% 5. 58.0 wt./wt.%

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-021

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.226% +LC/MS impurities, 0.14%+Inorganic Fluoride, 0.60%+NMR Impurity, 0.13%).
Total impurity from all tests = 2.09%
Purity = 100% - 2.09% = 97.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Sulfur in the sample appears to be converted to SO_4 and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the SO_4 is not considered an impurity.

[4]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonofluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[5]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3K^+$ (MW=538).

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-021

LC/MS Purity Profile:

Impurity	wt./wt. %
C4	ND
C5	ND
C6	ND
C7	0.14
Total	**0.14**

Note: The C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ 9/10/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 9/11/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Revision 1 (11/6/00)

Centre Analytical Laboratories COA Reference #: 023-034
3M Product: PFOA, Primary Standard
Test Control Reference #: TCR-99030-30
Purity: 95.0%

Test Name	Specifications	Result
Purity[1]		**95.0%**
Appearance	White, crystalline solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		 1. 0.001 wt./wt.% 2. <0.001 wt./wt.% 3. 0.001 wt./wt.% 4. <0.001 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.36 wt./wt.%
Total % Impurity (LC/MS)		4.68wt./wt.%
Total % Impurity (GC/MS)		None Quantified.%
Residual Solvents (TGA)		None Detected
Purity by DSC		99.8%
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. <0.015 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.006 wt./wt.% 7. <0.040 wt./wt.%
Organic Acids[2] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		 1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[3]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 22.3% 2. Theoretical Value = 0.935% 3. Theoretical Value = 3.25% 4. Theoretical Value = 0% 5. Theoretical Value = 66.1%	 1. 18.9 wt./wt.% 2. 1.27 wt./wt.% 3. 3.76 wt./wt.% 4. 4.38 wt./wt.% 5. 62.1 wt./wt.%
Ammonium Analysis[3] Ion Selective Electrode	Theoretical Value = 4.18%	2.94 wt./wt. %

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-034

Date of Last Analysis: 10/31/00

Expiration Date: 10/31/01

Storage Conditions: < -10 °C

Re-assessment Date: 10/31/01

[1]Purity = 100% - (total metal impurities, 0.002% + Total NMR impurities, 0.36 + Total LC/MS impurities, 4.68 %)

Total impurity from all tests = 5.042%
Purity = 100% - 5.042% = 95.0%

[2] TFA Trifluoroacetic acid
HFBA Heptafluorobutyric acid
NFPA Nonafluoropentanoic acid
PFPA Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_8F_{15}O_2^{(-)}NH_4^{(+)}$ (MW=431.1)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

LC/MS Purity Profile:

Peak #	Retention Time (min)	Mass(s)	Identity	Area	% Area
1	12.144	269.0	C6	205859	0.87
2	13.533	331, 319	F_{13}/C7	903329	3.81
3	14.238	369	PFOA	22630600	-
Total	-	-	-	23739788	4.68

Prepared By: David S. Bell 11/6/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: John M Flaherty 11/6/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

Appendix F: Report Signature Page

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator*

06/12/01
Date