Laboratory Composite Report

Analytical Reports of Data for Fluorochemical Analysis in Human Sera

LIMS No. 1623

Testing Laboratory

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1 Summary

This composite report includes ten technical reports that were issued to the 3M Medical Department btween March 2, 1998 and April 24, 1998. Each report documents the results of analysis of human sera samples collected from various sources. In most cases, each technical report includes information about the date of delivery of the data to the requester. Interpretation of the results is the responsibility of the 3M Medical Department.

Although rigorous quality control measures and 3M Environmental Laboratory Standard Operating Procedures and methods were followed when possible, the acquisition of this data was not necessarily collected according to applicable Good Laboratory Practices. Data was collected to meet immediate needs to help characterize the potential for human health concerns; it was not always possible to follow laboratory procedures that were in place at the time. Data was technically reviewed by 3M Environmental Lab technical staff, but was not reviewed by the Quality Assurance Unit in place at 3M.

Appendix A includes the analytical methods that were followed in the collection of the data supporting these technical reports.

ETS-8-4.1 Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry with the following exception: ethyl acetate was added to the aqueous extract instead of MTBE.

ETS-8-5.1 Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry. Data included in the Phase II and Phase III reports was collected using ESMS, while data for subsequent phases were collected using ESMSMS. Details can be found in each technical report, included in Appendices B through K.

Chromatographic data for each technical report is archived at the 3M Environmental Lab.

2 Signatures

Kris Hansen, Ph.D., Principal Analytical Investigator — Date: 04/28/00

3 Appendices

Appendix A: Analytical Methods
Appendix B: Phase 2
Appendix C: Phase 3
Appendix D: Phase 4
Appendix E: Phase 5
Appendix F: Phase 6
Appendix G: Phase 7
Appendix H: Phase 8
Appendix I: Phase 9, part 1
Appendix J: Phase 9, part 2
Appendix K: Summary of POAA levels for Phases 2-9

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-4.1 **Adoption Date:** 03/01/99

Revision Date:

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager Date

Group Leader Date

Technical Reviewer Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.

1.2 Applicable compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

004347

2.0 SUMMARY OF METHOD

2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see ***3.0 Definitions***). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate method.

3.0 DEFINITIONS

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 WARNINGS AND CAUTIONS

4.1 Health and safety warnings

4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

5.0 INTERFERENCES

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

004348

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Nalgene bottles, capable of holding 250 mL and 1 L

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

8.0 REAGENTS AND STANDARDS

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

004349

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557

8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

NOTE: When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.12 Surrogate stock standard preparation

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{13}SO_3H$ into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

9.0 SAMPLE HANDLING

9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method blanks and matrix blanks

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See **11.1.4**.

10.2 Matrix spikes

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

10.3 Continuing calibration checks

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.

11.1.5 Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.

11.3 Extract spiked matrix standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

004352

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
-	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.

12.3 Return unused samples to freezer after extraction amounts have been removed.

12.4 Record the initial volume on the extraction worksheet.

12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2**.

12.7 Spike each matrix with the appropriate amount of standard as described in **11.1**, or **Table 1** in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.14 Label a fresh 15 mL centrifuge tube with the same information as in **12.5**.

12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.

12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

12.18 Vortex mix for 30 seconds.

12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard x concentration of standard (µg /mL)}}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 Pollution Prevention and Waste Management

15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

004354

16.0 RECORDS

16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL.	04/02/99

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-5.1 **Adoption Date:** 03/01/99

Revision Date:

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager Date

Group Leader Date

Technical Reviewer Date

1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

004356

2.0 SUMMARY OF METHOD

2.1 This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion, m/z= 499. Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

3.0 DEFINITIONS

3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See **ETS-8-4.1**.

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted 1/x, not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

12.1.3 Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

12.2 Using the Autosampler

12.2.1 Set up sample tray according to the sample list prepared in Section **12.1.1**.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.50 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0** for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

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12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result - Background Result}}{\text{Expected Result}} \times 100$$

13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc. - Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\text{(Initial Volume of matrix (mL) + mL of Surrogate Standard)}} \times \frac{1\ \mu g}{1000\ ng}$$

Final Volume (mL)

004361

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-4.1, Attachment B,** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

14.2.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve

14.3 Calibration Curves

14.3.1 The r^2 value for the calibration curve must be 0.980 or better.

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries are must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be ± 30% of the spiked concentration.

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

14.7 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 disposed in high BTU containers, and glass pipette waste is disposed 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 runlog.

16.3 Plot the calibration curve by linear regression, weighted 1/x, 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 (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 REFERENCES

18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

004363

Laboratory Study

Study:
Test Material:
Matrix/Final Solvent:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y Intercept:
Date of Extraction/Analyst:
Date of Analysis/Analyst:

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

004364

Fluorine Analytical Chemistry Team

Contact:
K.J. Hansen
8-6018

Determination of PFOS in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:

Limit of Detection (LOD) :	1.0 ppb
Limit of Quantitation (LOQ) :	1.0 ppb
Range of Calibration Curve:	1.0ppb - 100ppb
Curve Correlation Coefficient (r^2):	0.999

Specificity:

Retention Time:	7.35 min.
Molecular Ion:	499 amu

PHASE II:Analysis of pooled sera, US 1998

Lot	Average conc. PFOS (ppb)[1]	Std. Dev.	% Recovery MS	% Recovery MSD	Lot	Average conc PFOS (ppb)[1]	Std. Dev.	% Recovery MS	% Recovery MSD
I006	44	2	-27*	73	W015	41	3	120	112
I007	44	6	-14*	35*	W016	47	8	51*	92
I008	43	2	65*	37*	W017	42	3	-45*	65*
S009	26	8	82	92					
S010	28	7	-6*	27*					
S011	45	2	100	108					
W012	54	13	78	92					
W013	50	5	90	102					
W014	41	6	106	92					

Range of Average Concentration of PFOS in Lot Sera: from 6 ppb to 54 ppb

Results of Quantitation of Method Blanks

Blank	Concentration PFOS (ppb)
Method Blank, H2O-1	<LOD
Method Blank, H2O-2	<LOD
Method Blank, sera-1 (1)	7
Method Blank, sera-1 (2)	<LOD
Method Blank, sera-1 (3)	<LOD
Method Blank, sera-2 (1)	<LOD

Sample[2]	[PFOS] recovered	[PFOS] expected	% recovery
QC1	49	49	100
QC2	49	49	100
QC3	49	49	100
QC4	50	49	102
QC5	53	49	108

System Reproducibility and Precision Checks

Reproducibility[3]:

Sample	PFOS (ppb)	RSD
I006-3 (2)	45	0.7%
S009-3 (2)	11	0.4%
W012-3 (2)	54	0.4%
W015-3 (2)	33	1.8%
W017-3 (2)	37	0.8%

Precision[4]:

Sample	PFOS (ppb)
I007-1 (1)	47
I007-1 (2)	55
I007-1 (3)	49
I007-1 (4)	47
I007-1 (5)	49
Average	49
Std. Dev.	1
RSD	2%

[1] *Reported values are the averages concentration PFOS of samples extracted in triplicate.*

[2] *QC samples are extracts of blank sera spiked with PFOS standards.*

[3] *Reproducibility samples are repeat injections of lot sera extracts, performed after every 15 samples.*

[4] *Precision samples are 5 consecutive injections of the same lot sera extract.*

*Questionable Data. Source of error currently undetermined.

3M Environmental Lab
5/7/98

Fluorine Analytical Chemistry Team

Contact:
K.J. Hansen
8-6018]

Determination of POAA in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:		Specificity:	
Limit of Detection (LOD) :	10 ppb	Retention Time:	6.90 min.
Limit of Quantitation (LOQ) :	1.0 ppb	Molecular Ion:	413 amu[1]
Range of Calibration Curve:	10ppb - 100ppb		369 amu[2]
Curve Correlation Coefficient (r^2):	0.991		

Phase II: Analysis of pooled sera (POAA)

Lot	Average conc. POAA (ppb)[3]	Std. Dev.	% Recovery MS	% Recovery MSD	Lot	Average conc POAA (ppb)[3]	Std. Dev.	% Recovery MS	% Recovery MSD
I006	<LOD	0	56*	96	W015	<LOD	0	100	101
I007	<LOD	1	53*	79	W016	<LOD	0	83	97
I008	<LOD	0	90	74	W017	<LOD	0	83	97
S009	<LOD	1	65*	68*	B001	<LOD	0	98	89
S010	<LOD	0	45*	57*	B002	<LOD	0	100	100
S011	<LOD	0	71	67*	B003	<LOD	0	108	110
W012	<LOD	1	92	94	B004	<LOD	0	60*	94
W013	<LOD	0	100	100	B005	<LOD	0	94	89
W014	<LOD	0	98	95					

Range of Average Concentration of POAA in Lot Sera: all values were below the LOD/LOQ (10ppb)

Results of Quantitation of Method Blanks

Blank	Concentration POAA (ppb)
Method Blank, H2O-1	<LOD
Method Blank, H2O-2	<LOD
Method Blank, sera-1 (1)	<LOD
Method Blank, sera-1 (2)	<LOD
Method Blank, sera-1 (3)	<LOD
Method Blank, sera-2 (1)	<LOD

Sample[4]	[POAA] recovered	[POAA] expected	% recovery
QC1	94	98	98
QC2	91	95	95
QC3	91	95	95
QC4	92	96	96
QC5	93	97	97

System Reproducibility and Precision Checks

Reproducibility[5]:

Sample	POAA (area)	RSD
I006-3 (2)	7700	0.9%
S009-3 (2)	3100	7.0%
W012-3 (2)	6500	0.0%
W015-3 (2)	4700	0.0%
W017-3 (2)	4900	5.8%
B003-3 (2)	3100	2.0%

Precision[6]:

Sample		Peak Area
I007-1 (1)		5800
I007-1 (2)		5600
I007-1 (3)		6300
I007-1 (4)		6300
I007-1 (5)		6300
	Average	6100
	Std. Dev.	300
	RSD	5%

[1] *Ion m/z=413 used for purposes of quantitation.*

[2] *Ion m/z=369 used for purposes of verfication of compound identity.*

[3] *Reported values are the averages concentration POAA of samples extracted in triplicate.*

[4] *QC samples are extracts of blank sera spiked with POAA standards.*

[5] *Reproducibility samples are repeat injections of lot sera extracts, performed after every 15 samples.*

[6] *Precision samples are 5 consecutive injections of the same lot sera extract.*

*Questionable Data. Source of error currently undetermined.

K.J. Hansen
3M Environmental Lab

Phase III
Quantitative Analysis of PFOS in Individual's Sera

3/2/98

Determination of PFOS in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:		Specificity:	
Limit of Detection (LOD) :	1.0 ppb	Retention Time:	7.35 min.
Limit of Quantitation (LOQ) :	1.0 ppb	Molecular Ion:	499 amu
Range of Calibration Curve:	1.0ppb - 100ppb		
Curve Correlation Coefficient (r^2):	1		

Results of Quantitation of Individual Sera Samples

Lot	Average conc. PFOS (ppb)	% RSD	Lot	verage conc. PFOS (ppb)	% RSD
198	58	41*	1798	46	15
298	41	14	1898	56	10
398	46	2	1998	70	3
498	32	N.A.	2098	40	2
598	67	0	2198	48	9
698	53	7	2298	60	13
798	42	N.A.	2398	72	1
898	49	35*	2498	35	20
998	39	11	2598	41	33*
1098	54	5	2698	53	11
1198	60	11	2798	57	15
1298	37	1	2898	33	N.A.
1398	45	N.A.	2998	65	7
1498	31	12	3098	67	50*
1598	96	2	3198	33	11
1698	74	1			

N.A. = No %RSD calculated as there was only enough sample for 1 analysis.

Quantitative Analysis of PFOS and POAA in Individual's Sera

4/9/98

Determination of PFOS/POAA in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:

Limit of Detection (LOD) : 1.0 ppb/10 ppb
Limit of Quantitation (LOQ) : 1.0 ppb/10 ppb
Range of Calibration Curve: 1.0ppb - 100ppb/10 ppb -100ppb
Correlation Coefficient (r^2): 0.999/ 0.999

Specificity:

Retention Time: 7.35 min./6.9 min.
Molecular Ion: 499 amu/413 amu, 369 amu

Preliminary

Results of Quantitation of Individual Sera Samples

ID#	Avg. conc. PFOS (ppb)[1]	Std. Dev.	Avg. conc. POAA (ppb)[1]	Std. Dev.
198	46	n.d.	<LOQ	n.d.
298	35	n.d.	<LOQ	n.d.
398	45	n.d.	18	3
498	32	n.d.	10	n.d.
598	67	n.d.	<LOQ	n.d.
698	53	4	<LOQ	n.d.
798	42	n.d.	<LOQ	n.d.
898	49	17	<LOQ	n.d.
998	37	n.d.	<LOQ	n.d.
1098	52	n.d.	<LOQ	n.d.
1198	39	n.d.	<LOQ	n.d.
1298	37	n.d.	<LOQ	n.d.
1398	45	n.d.	<LOQ	n.d.
1498	28	n.d.	<LOQ	n.d.
1598	96	3	10	1
1698	73	n.d.	<LOQ	n.d.

ID#	Average conc. PFOS (ppb)[1]	Std. Dev.	Avg. conc. POAA (ppb)[1]	Std. Dev.
1798	41	n.d.	<LOQ	n.d.
1898	52	n.d.	<LOQ	n.d.
1998	71	n.d.	<LOQ	n.d.
2098	40	1	<LOQ	n.d.
2198	45	n.d.	<LOQ	n.d.
2298	54	n.d.	<LOQ	n.d.
2398	71	n.d.	12	n.d.
2498	28	n.d.	<LOQ	n.d.
2598	30	n.d.	<LOQ	n.d.
2698	47	1	<LOQ	n.d.
2798	51	n.d.	<LOQ	n.d.
2898*	30	n.d.	<LOQ	n.d.
2998	41	n.d.	<LOQ	n.d.
3098	41	n.d.	<LOQ	n.d.
3198	36	n.d.	<LOQ	n.d.

Results of Quantitation of Method Blanks

Blank	[PFOS] (ppb)
Method Blank, H2O-1	<LOD
Method Blank, sera-1 (1)	4
Method Blank, sera-1 (2)	<LOD

Blank	[POAA] (ppb)
Method Blank, H2O-1	<LOD
Method Blank, sera-1 (1)	<LOD
Method Blank, sera-1 (2)	<LOD

Sample[2]	[PFOS] recovered	[POAA] recovered
QC1	99%	93%
QC2	93%	87%
QC3	90%	82%

System Reproducibility Checks

Reproducibility[3]:

Sample	PFOS (ppb)	RSD	POAA (area)	RSD
0198-2 (2)	33	4.3%	2800	0.0%
1098-2 (2)	20	3.6%	1300	0.0%
2098-1 (2)	40	1.8%	1400	0.0%
2698-1 (2)	47	3.0%	2300	6.1%

[1] *Reported values are from a single analysis unless a std. dev. is indicated; in those cases, 2 alliquots of sample were analyzed and averaged.*

[2] *QC samples are extracts of blank sera spiked with PFOS/POAA standards.*

[3] *Reproducibility samples are repeat injections of lot sera extracts, performed every 15 samples.*

K.J. Hansen
3M Environmental Lab

4/9/98

Phase III
Quantitative Analysis of PFOS in Individual's Sera

Determination of PFOS in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:		Specificity:	
Limit of Detection (LOD) :	1.0 ppb	Retention Time:	7.35 min.
Limit of Quantitation (LOQ) :	1.0 ppb	Molecular Ion:	499 amu
Range of Calibration Curve:	1.0ppb - 100ppb		
Curve Correlation Coefficient (r^2):	1		

Results of Quantitation of Individual Sera Samples

Lot	Average conc. PFOS (ppb)	% RSD	Lot	verage conc. PFOS (ppb)	% RSD
198	58	41*	1798	46	15
298	41	14	1898	56	10
398	46	2	1998	70	3
498	32	N.A.	2098	40	2
598	67	0	2198	48	9
698	53	7	2298	60	13
798	42	N.A.	2398	72	1
898	49	35*	2498	35	20
998	39	11	2598	41	33*
1098	54	5	2698	53	11
1198	60	11	2798	57	15
1298	37	1	2898	33	N.A.
1398	45	N.A.	2998	65	7
1498	31	12	3098	67	50*
1598	96	2	3198	33	11
1698	74	1			

N.A. = No %RSD calculated as there was only enough sample for 1 analysis.

Fluorine Analytical Chemistry Team

contact:
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8-6018

Determination of PFOS in sera by Electrospray Mass Spectrometry (ESMS)

Quantitation:		Specificity:	
Limit of Detection (LOD) :	1.0 ppb	Retention Time:	7.35 min.
Limit of Quantitation (LOQ) :	1.0 ppb	Molecular Ion:	499 amu
Range of Calibration Curve:	1.0ppb - 100ppb		
Curve Correlation Coefficient (r^2):	1		

PHASE III: Analysis of individual sera samples, 1998 US

Lot	Average conc. PFOS (ppb)	% RSD	Lot	Average conc. PFOS (ppb)	% RSD
198	58	41*	1798	46	15
298	41	14	1898	56	10
398	46	2	1998	70	3
498	32	N.A.	2098	40	2
598	67	0	2198	48	9
698	53	7	2298	60	13
798	42	N.A.	2398	72	1
898	49	35*	2498	35	20
998	39	11	2598	41	33*
1098	54	5	2698	53	11
1198	60	11	2798	57	15
1298	37	1	2898	33	N.A.
1398	45	N.A.	2998	65	7
1498	31	12	3098	67	50*
1598	96	2	3198	33	11
1698	74	1			

N.A. = No %RSD calculated as there was only enough sample for 1 analysis.

3M Environmental Laboratory- Fluorine Analytical Chemistry Team
Kris Hansen - Sr. Analytical Chemist
Fluorine Analytical Chemistry Team
Building 2-3E-09
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Phase IV:
PFOS analysis of US human sera samples collected in the 1970s

Summary:

Twelve frozen sera samples of various volumes were supplied to the Environmental Lab by Jeff Mandel (3M Medical). One mL or no more than one half of the volume of each sample was extracted using an ion-pairing reagent. The extracts were analyzed quantitatively for PFOS by HPLC-ESMS. Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared extracted curve.

The twelve samples contained an average concentration of 27 ppb PFOS ranging from 2- 48 ppb. The PFOS levels were significantly lower than in the 31 samples collected from individuals in 02/98 and exhibited significantly greater variability in PFOS levels than those same samples. The percent abundance of the branched chain isomer was 37.3 +/- 4.8%, compared to 33.7 +/-7% in the 1998 samples. In standard solutions of PFOS, the branched chain accounts for 21.7 +/-3.1% of the total concentration. A detailed summary of the results is included.

The identity of PFOS in several randomly selected samples in this phase was confirmed using HPLC-ESMSMS. In all cases, the analyte exhibited a characteristic retention time, a characteristic primary ion, and five characteristic secondary ions.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium (TBA), is used to extract the analyte from the matrix. The cationic reagent selectively targets anionic compounds, like PFOS and POAA. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 9.1 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3$-). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

HPLC ES-MS/MS: Detection and monitoring of characteristic secondary ions

ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a characteristic ion is selected. After selection, the ES-MSMS

characterizes the ion further by smashing it apart with high energy gas. As a result of the smashing, secondary ionic fragments (daughter ions), characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the characteristic primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected at the detector.

Quality control summary:

Due to sample limitations, sera samples were not extracted in duplicate. For this same reason, matrix spikes could not be evaluated for the samples. Instead, two samples of pooled sera purchased from Sigma were used for duplicate matrix spike analysis. Methanol blanks were analyzed before and after each sample to ensure complete isolation of the sample. A mid-level quality control sample was analyzed after the sixth sera sample. The four point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves. Specific QC parameters are available on the appended results table.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/min.

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.

Injection volume: 10 µL

Injections / sample: 1

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer

MassLynx 2.1 Software

Cone voltages: -20v, -60v

Mode: electrospray negative

Source temperature: 115 °C.

Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar

Ions: 369, 413, 499

Electrode: cross-flow

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received: 3/6/98
Samples Extracted: 3/9/98
Samples Analyzed: 3/11 - 3/12/98

Analysis by: ESMS (-)
Calibration Curve Range: 1ppb - 49ppb
r^2 of Calibration Curve: 0.998
LOQ/LOD[1]: 1 ppb

PHASE IV: Analysis of historic samples, 1970-80s

Sample ID	Conc. of PFOS (ppb+B40)	Sample ID	Conc. of PFOS (ppb)
PG7-SE13	13	GRAH 0003	48
PG3-SE11	32	80-01-0089-01	15
98SE774	30	80-01-0044-01	39
98SE117	24	Will 0002	2
PG25	40	Demm 0001	44
PG5	29	80-52-0514-01	25

Average (ppb)	28
Std. Dev.	9
%RSD	32

QC Results

QC Check:	% Recovery
Calibration Check (QC1)	100%
MS	105%
MSD	105%

Branched Isomer Abundance Percentages

Sample Pool	% Abundance of Branched Isomers (Average)	% Abundance of Branched Isomers (Std. Dev.)	n (# sampled in pool)
Standard Material	22%	3%	n/a
Individuals Sampled in 1998	34%	5%	31
3M Cottage Grove Plant Workers	45%	4%	3
Individuals Sampled in 1970's	37%	5%	12
Pooled Sera Samples	31%	5%	11

1 - LOD/LOQ = Limit of Detection/Limit of Quantitation

File	Sample	Peak area	[PFOS]ppb	[PFOS]avg	St. Dev.	%RSD		
3149801	MeOH blank	3117	0.0					
3149802	Extracted human sera blank	3490	0.0					
3149803	Extracted human sera blank	3468	0.0					
3149815	MeOH blank	3547	0.0					
3149816	MeOH blank	3756	0.0					
3149817	PG7-SE13	27470	14.0					
3149818	PG7-SE13	27393	13.9					
3149819	PG7-SE13	26140	13.2	13.7	0.5	3		
3149820	MeOH blank	3654	0.0					
3149821	PG3-SE11	57153	32.2					
3149822	PG3-SE11	56044	31.5					
3149823	PG3-SE11	56116	31.5	31.7	0.4	1		
3149824	MeOH blank	3626	0.0					
3149825	98SE774	52534	29.3					
3149826	98SE774	50755	28.3					
3149827	98SE774	51951	29.0	28.9	0.6	2		
3149828	MeOH blank	3476	0.0					
3149829	98SE117	45396	25.0					
3149830	98SE117	45768	25.2					
3149831	98SE117	45698	25.2					
3149832	98SE117	44252	24.3	24.9	0.4	2		
3149833	MeOH blank	3271	0.0					
3149834	PG25	96689	56.4					
3149835	PG25	94900	55.3					
3149836	PG25	93856	54.7	55.5	0.9	2		
3149837	MeOH blank	3241	0.0				% dev. (QC)	QC recovery (%)
3149838	Std 4-1, 48.8	79521	45.9				-6	94
3149839	Std 4-1, 48.8	76841	44.3				-9	91
3149840	MeOH blank	3186	0.0					
3149841	PG5	54030	30.3					
3149842	PG5	55078	30.9					
3149843	PG5	54670	30.7					
3149844	PG5	53860	30.2	30.5	0.3	1		
3149845	MeOH blank	2907	0.0					
3149846	GRAH003	77384	44.6					
3149847	GRAH003	73104	42.0					
3149848	GRAH003	78233	45.1	43.9	1.7	4		
3149849	MeOH blank	3458	0.0					
3149850	80-01-0089-01	27217	13.8					
3149851	80-01-0089-01	27410	13.9					
3149852	80-01-0089-01	27137	13.8	13.8	0.1	1		
3149853	MeOH blank	3595	0.0					
3149854	80-01-0044-01	72475	41.6					
3149855	80-01-0044-01	71363	40.9					
3149856	80-01-0044-01	69231	39.6					
3149857	80-01-0044-01	70013	40.1	40.5	0.9	2		
3149858	MeOH blank	3351	0.0					
3149859	Std. 4-1, 48.8 ppb	78472	45.3				-7	93
3149860	MeOH blank	3534	0.0					
3149861	Will 0002	7591	1.8					
3149862	Will 0002	7385	1.6					
3149863	Will 0002	7222	1.5					
3149864	Will 0002	7589	1.8					
3149865	Will 0002	7362	1.6	1.7	0.1	6		
3149866	MeOH blank	3567	0.0					
3149867	DEMM 0001	75672	43.5					
3149868	DEMM 0001	73938	42.5					
3149869	DEMM 0001	76445	44.0	43.3	0.8	2		
3149870	MeOH blank	3420	0.0					
3149871	80-52-0514-01	41084	22.3					
3149872	80-52-0514-01	40551	22.0					
3149873	80-52-0514-01	40387	21.9	22.1	0.2	1		
3149874	MeOH blank	3822	0.0					
3149875	MeOH blank	3541	0.0					
3149876	H2O blank-1	4799	0.1					
3149877	H2O blank-2	4698	0.0					
3149878	MeOH blank	3585	0.0					
3149879	Pooled sera-1	53332	29.8					
3149880	Pooled sera-2	55640	31.3	30.5	1.0	3		
3149881	MeOH blank	4035	0.0				% dev.	Spike recovery (%)
3149882	MS 19.9 ppb	88880	51.6				6.0	106.0
3149883	MSD 19.9 ppb	86059	49.9	50.8	1.2	2	-3.0	97.0
3149884	MeOH blank	3728	0.0					
3149885	MeOH blank	3877	0.0					
3149886	Extracted human sera blank	4019	0.0					
3149887	Extracted human sera blank	4013	0.0					

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Phase V and VI: Quantitative analysis of PFOS in historic human sera samples and in human sera samples from rural Northern China

Summary:

Two sets of samples, the first with 12 samples in plastic vials (Z-set) and the second with 5 samples in glass vials (M-set), were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on March 20, 1998. For both sets, sample volume varied from 0.2 to 1.0 mL of human sera. A volume between 0.4 - 1.0 mL of each sample was extracted using an ion-pairing reagent. Six samples in the Z-set did not contain enough volume for extraction; in these cases, two samples (e.g. ZZA6 and ZZA8) were combined and extracted as one sample. The extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high-pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, five-point extracted curve ($r^2 = 0.999$). Each sample extract was analyzed twice. Calibration checks, analyzed every 5 samples, were within 6% of expected values.

The identity of PFOS in several randomly selected samples in this phase (M-set only) was confirmed using HPLC-ESMSMS. In all cases, the analyte exhibited a characteristic retention time, a characteristic primary ion, and five characteristic secondary ions.

Historic samples

The sera samples in the M-set, collected from donors in the US in the 1960s, had an average PFOS concentration of 33.4 ppb; the samples ranged in PFOS concentration from 11.8 to 59.4.

Foreign samples

The 9 samples in the Z-set (including 3 composite samples) were collected from donors in rural China and were determined to have a much lower concentration of PFOS than any human sera data set analyzed previously. Six samples did not contain PFOS above the detection limit of this analytical method (1 ppb). The remaining 3 samples evidenced PFOS concentrations less than 5 ppb, the limit of quantitation. With the exception of one human sera sample collected in the 1980s (Will 0002), these are the only human samples determined to contain less than 10 ppb PFOS.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like the cationic reagent, selectively targets PFOS and perfluorooctanoate (POAA). Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard

PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3$-). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

HPLC ES-MS/MS: Detection *and monitoring of characteristic secondary ions*

ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a characteristic ion is selected. After selection, the ES-MSMS characterizes the ion further by smashing it apart with high-energy gas. As a result of the smashing, secondary ionic fragments (daughter ions), characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the characteristic primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected at the detector.

Quality control summary:

Due to sample limitations, sera samples were not extracted in duplicate, with 1 exception. For this same reason, matrix spikes could not be evaluated for the samples. Each sample extract was analyzed in duplicate. One sample, M001278 contained enough material for two extractions. The presented results for this sample are based on duplicate analysis of each of the two extractions. Methanol blanks were analyzed periodically to ensure complete isolation of the sample. A mid-level (24 ppb) quality control sample was analyzed every 5 samples. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This five point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves (r^2 = 0.999). Specific QC parameters are available on the appended results table.

Limit of Quantitation

The current analytical method has been used to evaluate a large range of PFOS levels extracted from sera; the method is not optimized for precise low-level (< 5 ppb) quantitation, but instead for samples in the range of 20-60 ppb. Given the concentrations chosen for this optimization of the standard curve, while there is a statistical difference for samples evaluated from 5-100 ppb, there is no statistical difference between samples quantitated from 1-5 ppb. The quantitation does not become statistically defendable until 5 ppb; thus this value represents the limit of quantitation for this method. Samples designated <LOQ are estimated to contain 1-5 ppb PFOS. If further accuracy and precision in quantitation of the low-level Z-set samples (samples less than 5 ppb) is required, a special low-level quantitation system will be required.

Limit of Detection

The limit of detection for this analytical method is based on analyst recognition of the distinct peak shape resulting from PFOS analysis. PFOS standard material and PFOS extracted from sera analyzed by the conditions reported here, show near baseline resolution of 1 branched and 1 linear PFOS isomer. The apex of each peak in the HPLC-trace occurs at a specific, reproducible retention time and the ratio of the branched peak to the linear peak is fairly consistent down to the low standard, 1.0 ppb. Those samples that a) had PFOS levels evaluated at less than the limit of quantitation and b) evidenced the distinct peak shape, were designated < LOQ. Samples that a) have PFOS levels evaluated at less than the limit of quantitation and that b) do not reflect the unique PFOS peak shape, were designated < LOD. These samples are estimated to contain from 0-1 ppb PFOS.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/min.

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.

Injection volume: 10 µL

Injections / sample: 2

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software

Cone voltages: -20v, -60v

Mode: electrospray negative

Source temperature: 115 °C.

Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar

Ions: 369, 413, 499, 427

Electrode: cross-flow

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received:	3/6/98	**Analysis by:**	ESMS (-)
Samples Extracted:	3/9/98	**Calibration Curve Range:**	1ppb - 49ppb
Samples Analyzed:	3/11 - 3/12/98	**r^2 of Calibration Curve:**	0.998
		LOD[1]:	1 ppb
		LOQ2:	5 ppb

PHASE V and VI: Analysis of foreign (rural Northern China) and historic sera samples (1960)

Sample ID	Conc. of PFOS (ppb+B40)	Sample ID	Conc. of PFOS (ppb)
ZZA1	<LOD	m001205	24.4 +/- 0.4
ZZA2	<LOD	M001278	48.4 +/- 1.5
ZZA3	<LOQ	M01202	23.2 +/- 0.2
ZZA4	<LOQ	M001221	11.8 +/- 0.1
ZZA5	<LOD	M001208	59.4 +/- 0.1
ZZA6 and 8	<LOD		
ZZA7	<LOD		
ZZA9 and 10	<LOQ		
ZZA11 and 12	<LOD		

QC Results

QC Check:	% Recovery
Calibration Check (QC1)	104%
Calibration Check (QC2)	106%

Branched Isomer Abundance Percentages

Sample Pool	% Abundance of Branched Isomers (Average)	% Abundance of Branched Isomers (Std. Dev.)	n (# sampled in pool)
Standard Material	22%	3%	n/a
Individuals Sampled in 1998	34%	5%	31
3M Cottage Grove Plant Workers	45%	4%	3
Individuals Sampled in 1970's	37%	5%	12
Pooled Sera Samples	31%	5%	11

1 - LOD = Limit of Detection
2- LOQ = Limit of Quantitation

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\PHASE5.PRO\SAMPLEDB\032198
Last modified: Sun Mar 22 14:35:56 1998
Method: C:\MASSLYNX\PHASE5.PRO\METHDB\PFOS
Last modified: Sat Mar 21 19:19:42 1998
Job Code:

Printed: Sun Mar 22 14:52:37 1998

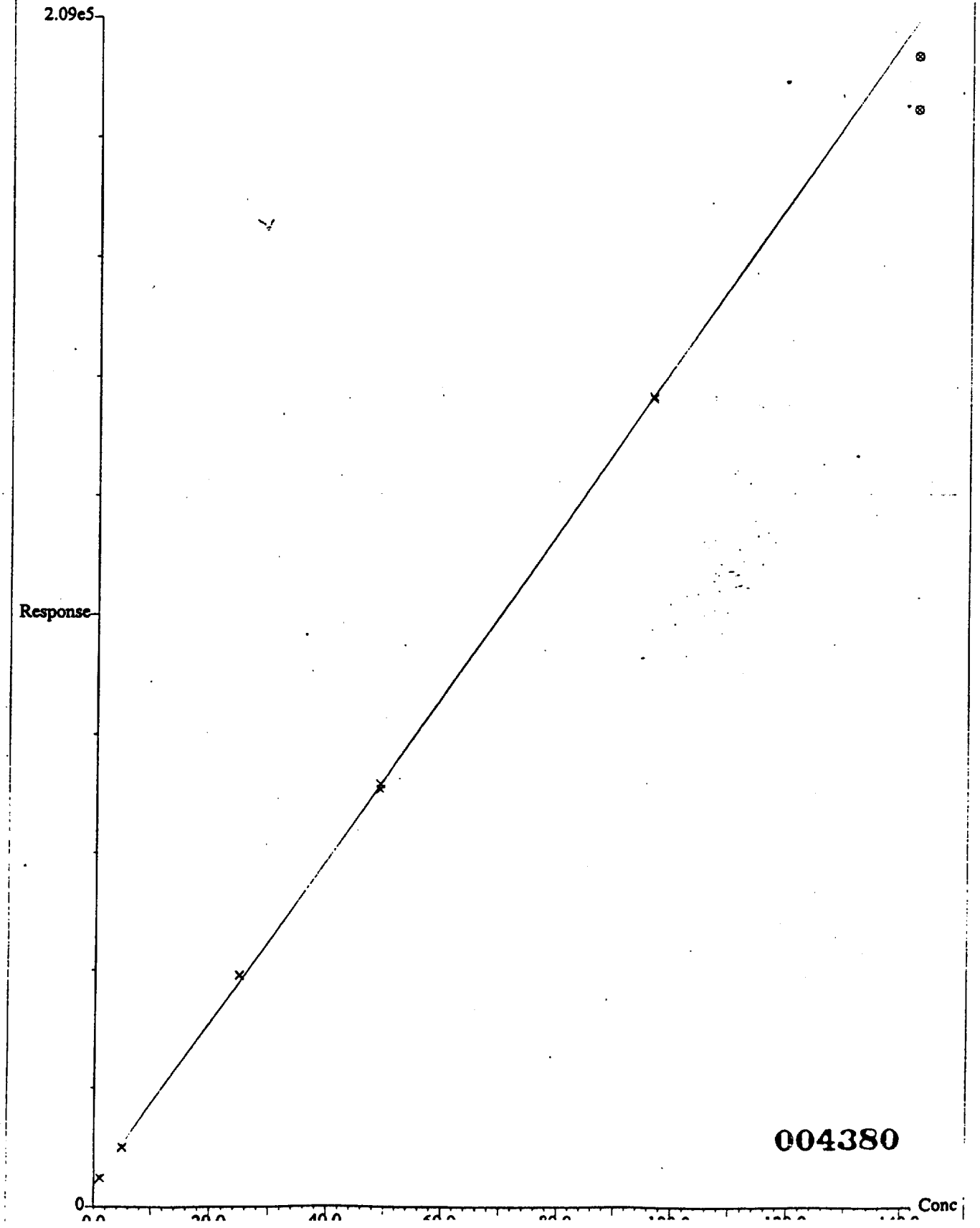
Compound 1: PFOS

#	Name	Type	Sample Text	Area	Conc.	%Dev	Flags
1	03219801	Analyte	MeOH Blank	4292	0.26		bb
2	03219802	Analyte	H2O blank-1	3239	0.00		bb
3	03219803	Analyte	Sera blank-1	2983	0.00		MM
4	03219804	Standard	0.996 ppb FC Mix	5015	0.76	-23	MM
5	03219805	Standard	4.90 ppb FC Mix	10464	4.53	-8	MM
6	03219806	Standard	24.8 ppb FC Mix	41137	25.72	4	MM
7	03219807	Standard	49.0 ppb FC Mix	74118	48.51	-1	MM
8	03219808	Standard	96.2 ppb FC Mix	142787	95.96	0	MM
9	03219809	Standard	142 ppb FC Mix	193768	131.18	-8	MMX
10	03219810	Analyte	MeOH Blank	3287	0.00		bb
11	03219811	Analyte	M001205	39673	24.71		MM
12	03219812	Analyte	M001205 (2)	38868	24.15		MM
13	03219813	Analyte	M001278-1	74992	49.11		MM
14	03219814	Analyte	M001278-1 (2)	76499	50.16		MM
15	03219815	Analyte	M001278-2	71794	46.90		MM
16	03219816	Analyte	M001278-2 (2)	72578	47.45		MM
17	03219817	Analyte	M01202	37934	23.51		MM
18	03219818	Analyte	M01202 (2)	37484	23.20		MM
19	03219819	Analyte	M001221	21224	11.96		MM
20	03219820	Analyte	M001221 (2)	21018	11.82		MM
21	03219821	Analyte	M001208	90087	59.54		MM
22	03219822	Analyte	M001208 (2)	89789	59.34		MM
23	03219823	QC	Cal check, 24.8 ppb PFOS	41304	25.84	4	MM
24	03219824	Analyte	MeOH blank	2821	0.00		bb
25	03219825	Analyte	ZZA1	3310	0.00		MM
26	03219826	Analyte	ZZA1 (2)	3397	0.00		MM
27	03219827	Analyte	ZZA2	3277	0.00		MM
28	03219828	Analyte	ZZA2 (2)	3300	0.00		MM
29	03219829	Analyte	ZZA3	5598	1.17		MM
30	03219830	Analyte	ZZA3 (2)	5721	1.25		MM
31	03219831	Analyte	ZZA4	7108	2.21		MM
32	03219832	Analyte	ZZA4 (2)	7058	2.17		MM
33	03219833	Analyte	ZZA5	5066	0.80		MM
34	03219834	Analyte	ZZA5 (2)	5340	0.99		MM
35	03219835	QC	Cal check, 24.8 ppb PFOS	41833	26.20	6	MM
36	03219836	Analyte	MeOH	2900	0.00		bb
37	03219837	Analyte	ZZA6 and 8	4883	0.67		MM
38	03219838	Analyte	ZZA6 and 8 (2)	5071	0.80		MM
39	03219839	Analyte	ZZA7	4181	0.19		MM
40	03219840	Analyte	ZZA7 (2)	4207	0.20		MM
41	03219841	Analyte	ZZA9 and 10	6631	1.88		MM
42	03219842	Analyte	ZZA9 and 10 (2)	6488	1.78		MM
43	03219843	Analyte	ZZA11 and 12	5342	0.99		MM
44	03219844	Analyte	ZZA11 and 12 (2)	5261	0.93		MM
45	03219845	Analyte	AZ26-1	44210	27.85		MM
46	03219846	Analyte	AZ26-1 (2)	44258	27.88		MM
47	03219847	Analyte	MeOH blank	2803	0.00		MM
48	03219848	Analyte	MeOH blank	2744	0.00		MM
49	03219849	Analyte	H2O blank-1	3397	0.00		bb
50	03219850	Analyte	Sera blank-1	3223	0.00		MM
51	03219851	Standard	0.996 ppb FC Mix	5081	0.81	-19	MM
52	03219852	Standard	4.90 ppb FC Mix	10482	4.54	-7	MM
53	03219853	Standard	24.8 ppb FC Mix	40948	25.59	3	MM
54	03219854	Standard	49.0 ppb FC Mix	75059	49.16	0	MM
55	03219855	Standard	96.2 ppb FC Mix	143153	96.21	0	MM
56	03219856	Standard	142 ppb FC Mix	203166	137.68	-3	MMX

$Y = 1446.1X + 3593.7$

Calibration: C:\MASSLYNX\PHASE5.PRO\CURVEDB\PFOS
Last modified: Sun Mar 22 14:49:58 1998
Printed: Sun Mar 22 14:52:38 1998

Compound 1 name: PFOS
Correlation coefficient: r = 0.999912, r^2 = 0.999823
Calibration curve: 1447.275720 * x + 3910.409399
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None



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Phase V and VI: Quantitative analysis of PFOS in historic human sera samples and in human sera samples from rural Northern China

Summary:

Two sets of samples, the first with 12 samples in plastic vials (Z-set) and the second with 5 samples in glass vials (M-set), were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on March 20, 1998. For both sets, sample volume varied from 0.2 to 1.0 mL of human sera. A volume between 0.4 - 1.0 mL of each sample was extracted using an ion-pairing reagent. Six samples in the Z-set did not contain enough volume for extraction; in these cases, two samples (e.g. ZZA6 and ZZA8) were combined and extracted as one sample. The extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high-pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, five-point extracted curve ($r^2 = 0.999$). Each sample extract was analyzed twice. Calibration checks, analyzed every 5 samples, were within 6% of expected values.

The identity of PFOS in several randomly selected samples in this phase (M-set only) was confirmed using HPLC-ESMSMS. In all cases, the analyte exhibited a characteristic retention time, a characteristic primary ion, and five characteristic secondary ions.

Historic samples

The sera samples in the M-set, collected from donors in the US in the 1960s, had an average PFOS concentration of 33.4 ppb; the samples ranged in PFOS concentration from 11.8 to 59.4.

Foreign samples

The 9 samples in the Z-set (including 3 composite samples) were collected from donors in rural China and were determined to have a much lower concentration of PFOS than any human sera data set analyzed previously. Six samples did not contain PFOS above the detection limit of this analytical method (1 ppb). The remaining 3 samples evidenced PFOS concentrations less than 5 ppb, the limit of quantitation. With the exception of one human sera sample collected in the 1980s (Will 0002), these are the only human samples determined to contain less than 10 ppb PFOS.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like the cationic reagent, selectively targets PFOS and perfluorooctanoate (POAA). Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard

PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3$-). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

HPLC ES-MS/MS: Detection *and monitoring of characteristic secondary ions*

ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a characteristic ion is selected. After selection, the ES-MSMS characterizes the ion further by smashing it apart with high-energy gas. As a result of the smashing, secondary ionic fragments (daughter ions), characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the characteristic primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected at the detector.

Quality control summary:

Due to sample limitations, sera samples were not extracted in duplicate, with 1 exception. For this same reason, matrix spikes could not be evaluated for the samples. Each sample extract was analyzed in duplicate. One sample, M001278 contained enough material for two extractions. The presented results for this sample are based on duplicate analysis of each of the two extractions. Methanol blanks were analyzed periodically to ensure complete isolation of the sample. A mid-level (24 ppb) quality control sample was analyzed every 5 samples. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This five point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves ($r^2 = 0.999$). Specific QC parameters are available on the appended results table.

Limit of Quantitation

The current analytical method has been used to evaluate a large range of PFOS levels extracted from sera; the method is not optimized for precise low-level (< 5 ppb) quantitation, but instead for samples in the range of 20-60 ppb. Given the concentrations chosen for this optimization of the standard curve, while there is a statistical difference for samples evaluated from 5-100 ppb, there is no statistical difference between samples quantitated from 1-5 ppb. The quantitation does not become statistically defendable until 5 ppb; thus this value represents the limit of quantitation for this method. Samples designated <LOQ are estimated to contain 1-5 ppb PFOS. If further accuracy and precision in quantitation of the low-level Z-set samples (samples less than 5 ppb) is required, a special low-level quantitation system will be required.

Limit of Detection

The limit of detection for this analytical method is based on analyst recognition of the distinct peak shape resulting from PFOS analysis. PFOS standard material and PFOS extracted from sera analyzed by the conditions reported here, show near baseline resolution of 1 branched and 1 linear PFOS isomer. The apex of each peak in the HPLC-trace occurs at a specific, reproducible retention time and the ratio of the branched peak to the linear peak is fairly consistent down to the low standard, 1.0 ppb. Those samples that a) had PFOS levels evaluated at less than the limit of quantitation and b) evidenced the distinct peak shape, were designated < LOQ. Samples that a) have PFOS levels evaluated at less than the limit of quantitation and that b) do not reflect the unique PFOS peak shape, were designated < LOD. These samples are estimated to contain from 0-1 ppb PFOS.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/min.

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.

Injection volume: 10 µL

Injections / sample: 2

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software

Cone voltages: -20v, -60v

Mode: electrospray negative

Source temperature: 115 °C.

Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar

Ions: 369, 413, 499, 427

Electrode: cross-flow

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received:	3/6/98
Samples Extracted:	3/9/98
Samples Analyzed:	3/11 - 3/12/98

Analysis by:	ESMS (-)
Calibration Curve Range:	1ppb - 49ppb
r^2 of Calibration Curve:	0.998
LOD[1]:	1 ppb
LOQ2:	5 ppb

PHASE V and VI: Analysis of foreign (rural Northern China) and historic sera samples (1960)

Sample ID	Conc. of PFOS (ppb+B40)	Sample ID	Conc. of PFOS (ppb)
ZZA1	<LOD	m001205	24.4 +/- 0.4
ZZA2	<LOD	M001278	48.4 +/- 1.5
ZZA3	<LOQ	M01202	23.2 +/- 0.2
ZZA4	<LOQ	M001221	11.8 +/- 0.1
ZZA5	<LOD	M001208	59.4 +/- 0.1
ZZA6 and 8	<LOD		
ZZA7	<LOD		
ZZA9 and 10	<LOQ		
ZZA11 and 12	<LOD		

QC Results

QC Check:	% Recovery
Calibration Check (QC1)	104%
Calibration Check (QC2)	106%

Branched Isomer Abundance Percentages

Sample Pool	% Abundance of Branched Isomers (Average)	% Abundance of Branched Isomers (Std. Dev.)	n (# sampled in pool)
Standard Material	22%	3%	n/a
Individuals Sampled in 1998	34%	5%	31
3M Cottage Grove Plant Workers	45%	4%	3
Individuals Sampled in 1970's	37%	5%	12
Pooled Sera Samples	31%	5%	11

1 - LOD = Limit of Detection
2- LOQ = Limit of Quantitation

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\PHASE5.PRO\SAMPLEDB\032198
Last modified: Sun Mar 22 14:35:56 1998
Method: C:\MASSLYNX\PHASE5.PRO\METHDB\PFOS
Last modified: Sat Mar 21 19:19:42 1998
Job Code:

Printed: Sun Mar 22 14:52:37 1998

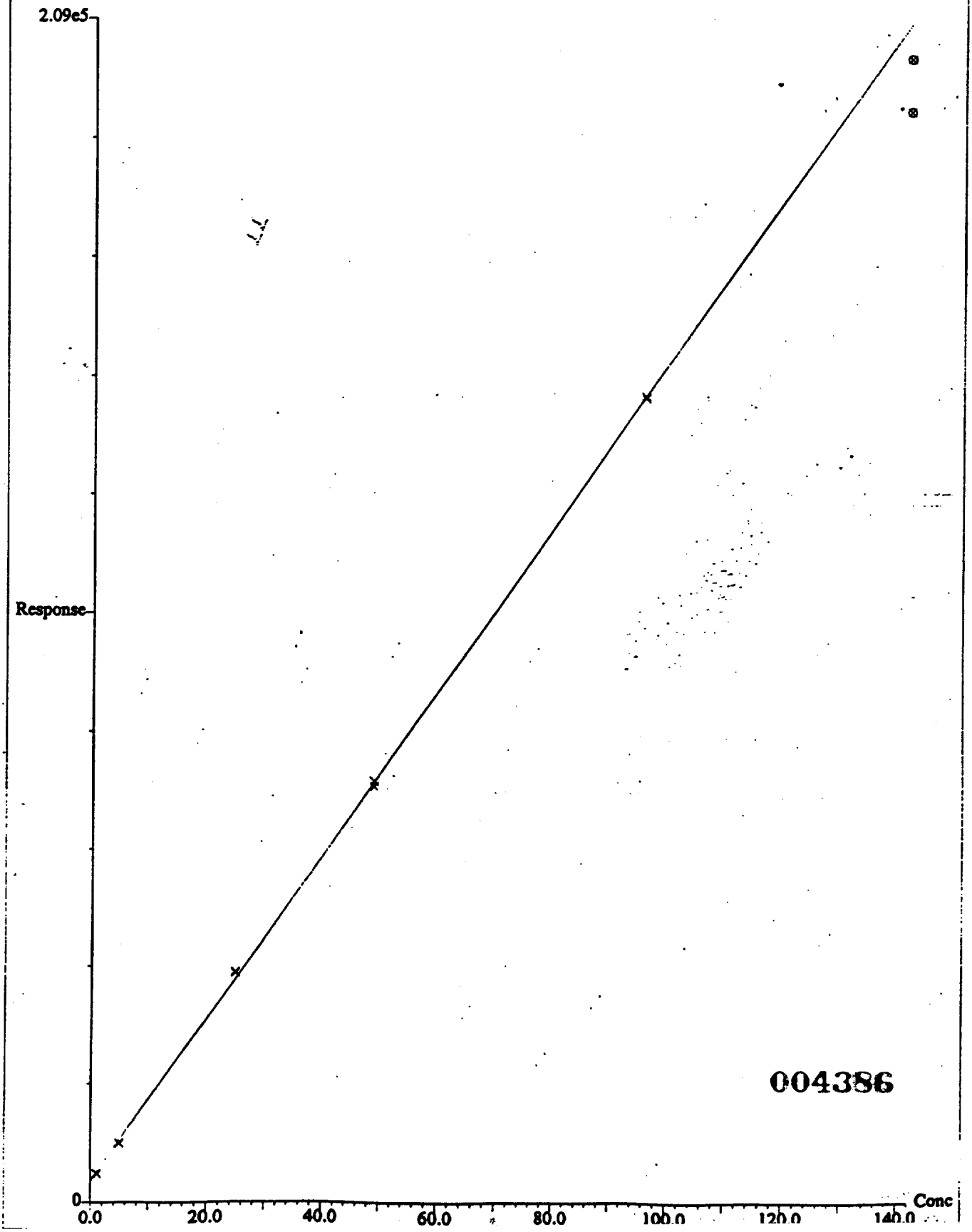
Compound 1: PFOS

#	Name	Type	Sample Text	Area	Conc.	%Dev	Flags
1	03219801	Analyte	MeOH Blank	4292	0.26		bb
2	03219802	Analyte	H20 blank-1	3239	0.00		bb
3	03219803	Analyte	Sera blank-1	2983	0.00		MM
4	03219804	Standard	0.996 ppb FC Mix	5015	0.76	-23	MM
5	03219805	Standard	4.90 ppb FC Mix	10464	4.53	-8	MM
6	03219806	Standard	24.8 ppb FC Mix	41137	25.72	4	MM
7	03219807	Standard	49.0 ppb FC Mix	74118	48.51	-1	MM
8	03219808	Standard	96.2 ppb FC Mix	142787	95.96	0	MM
9	03219809	Standard	142 ppb FC Mix	193768	131.18	-8	MMX
10	03219810	Analyte	MeOH Blank	3287	0.00		bb
11	03219811	Analyte	M001205	39673	24.71		MM
12	03219812	Analyte	M001205 (2)	38868	24.15		MM
13	03219813	Analyte	M001278-1	74992	49.11		MM
14	03219814	Analyte	M001278-1 (2)	76499	50.16		MM
15	03219815	Analyte	M001278-2	71794	46.90		MM
16	03219816	Analyte	M001278-2 (2)	72578	47.45		MM
17	03219817	Analyte	M01202	37934	23.51		MM
18	03219818	Analyte	M01202 (2)	37484	23.20		MM
19	03219819	Analyte	M001221	21224	11.96		MM
20	03219820	Analyte	M001221 (2)	21018	11.82		MM
21	03219821	Analyte	M001208	90087	59.54		MM
22	03219822	Analyte	M001208 (2)	89789	59.34		MM
23	03219823	QC	Cal check, 24.8 ppb PFOS	41304	25.84	4	MM
24	03219824	Analyte	MeOH blank	2821	0.00		bb
25	03219825	Analyte	ZZA1	3310	0.00		MM
26	03219826	Analyte	ZZA1 (2)	3397	0.00		MM
27	03219827	Analyte	ZZA2	3277	0.00		MM
28	03219828	Analyte	ZZA2 (2)	3300	0.00		MM
29	03219829	Analyte	ZZA3	5598	1.17		MM
30	03219830	Analyte	ZZA3 (2)	5721	1.25		MM
31	03219831	Analyte	ZZA4	7108	2.21		MM
32	03219832	Analyte	ZZA4 (2)	7058	2.17		MM
33	03219833	Analyte	ZZA5	5066	0.80		MM
34	03219834	Analyte	ZZA5 (2)	5340	0.99		MM
35	03219835	QC	Cal check, 24.8 ppb PFOS	41033	26.20	6	MM
36	03219836	Analyte	MeOH	2900	0.00		bb
37	03219837	Analyte	ZZA6 and 8	4883	0.67		MM
38	03219838	Analyte	ZZA6 and 8 (2)	5071	0.80		MM
39	03219839	Analyte	ZZA7	4181	0.19		MM
40	03219840	Analyte	ZZA7 (2)	4207	0.20		MM
41	03219841	Analyte	ZZA9 and 10	6631	1.88		MM
42	03219842	Analyte	ZZA9 and 10 (2)	6488	1.78		MM
43	03219843	Analyte	ZZA11 and 12	5342	0.99		MM
44	03219844	Analyte	ZZA11 and 12 (2)	5261	0.93		MM
45	03219845	Analyte	AZ26-1	44210	27.85		MM
46	03219846	Analyte	AZ26-1 (2)	44258	27.88		MM
47	03219847	Analyte	MeOH blank	2803	0.00		MM
48	03219848	Analyte	MeOH blank	2744	0.00		MM
49	03219849	Analyte	H2O blank-1	3397	0.00		bb
50	03219850	Analyte	Sera blank-1	3223	0.00		MM
51	03219851	Standard	0.996 ppb FC Mix	5081	0.81	-19	MM
52	03219852	Standard	4.90 ppb FC Mix	10482	4.54	-7	MM
53	03219853	Standard	24.8 ppb FC Mix	40948	25.59	3	MM
54	03219854	Standard	49.0 ppb FC Mix	75059	49.16	0	MM
55	03219855	Standard	96.2 ppb FC Mix	143153	96.21	0	MM
56	03219856	Standard	142 ppb FC Mix	203166	137.68	-3	MMX

$y = 1446.1x + 3493.7$

Calibration: C:\MASSLYNX\PHASE5.PRO\CURVEDB\PFOS
Last modified: Sun Mar 22 14:49:58 1998
Printed: Sun Mar 22 14:52:38 1998

Compound 1 name: PFOS
Correlation coefficient: r = 0.999912, r^2 = 0.999823
Calibration curve: 1447.275720 * x + 3910.409399
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None



004386

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Phase VII:
US Geographical Distribution Study - PFOS levels in human sera

Summary:

Fifty-five samples of cold human sera in plastic centrifuge tubes were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on March 19 and March 20, 1998. The samples, collected from sites around the United States, contained several mLs of sera. One mL aliquots of sera were removed from each sample and extracted with an ion-pairing reagent; nearly all of the samples were extracted in duplicate. The sera extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, five-point extracted curve ($r^2 = 0.996$). A matrix spike calibration check, analyzed in the middle of the analysis sequence, was within 1% of expected values.

The sera samples in the geographic distribution study had an average PFOS concentration of 28 ppb; the samples ranged in PFOS concentration from 9 ppb to 59 ppb. The percent abundance of the branched chain isomer was 41 +/- 4%. In standard solutions of PFOS, the branched chain accounts for 21.7 +/-3.1% of the total concentration. A detailed summary of the results is attached.

The identity of PFOS in several randomly selected samples in this phase (M-set only) was confirmed using HPLC-ESMSMS. In all cases, the analyte exhibited a characteristic retention time, a characteristic primary ion, and five characteristic secondary ions.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for HPLC-ESMS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3$-). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

HPLC ES-MS/MS: Detection *and monitoring of characteristic secondary ions*

ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a characteristic ion is selected. After selection, the ES-MSMS characterizes the ion further by smashing it apart with high-energy gas. As a result of the smashing, secondary ionic fragments (daughter ions), characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the characteristic primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected at the detector.

Quality control summary:

In most cases, the sera samples were extracted in duplicate; for those samples that were, standard deviation values are included in the table of results. Matrix spikes were prepared for several samples, too. If available, the matrix spike recoveries are included in the results table. A mid-level (49 ppb) quality control sample was analyzed; recovery for the matrix spike was determined to be 101%. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This five point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves ($r^2 = 0.996$). Specific QC parameters are available on the appended results table.

Limit of Quantitation

The current analytical method has been used to evaluate a large range of PFOS levels extracted from sera; the method is not optimized for precise low-level (< 5 ppb) quantitation, but instead for samples in the range of 20-60 ppb. Given the concentrations chosen for this optimization of the standard curve, while there is a statistical difference for samples evaluated from 5-100 ppb, there is no statistical difference between samples quantitated from 1-5 ppb. The quantitation does not become statistically defensible until 5 ppb, thus this value represents the limit of quantitation for this method. Samples designated <LOQ are estimated to contain 1-5 ppb PFOS. If further accuracy and precision in quantitation of the low level Z-set samples (samples less than 5 ppb) is required, a special low-level quantitation system will be required.

Limit of Detection

The limit of detection for this analytical method is based on analyst recognition of the distinct peak shape resulting from PFOS analysis. PFOS standard material and PFOS extracted from sera analyzed by the conditions reported here, show near baseline resolution of 1 branched and 1 linear PFOS isomer. The apex of each peak in the HPLC-trace occurs at a specific, reproducible retention time and the ratio of the branched peak to the linear peak is fairly consistent down to the low standard, 1.0 ppb. Those samples that a) had PFOS levels evaluated at less than the limit of quantitation and b) evidenced the distinct peak shape, were designated < LOQ. Samples that a) have PFOS levels evaluated at less than the limit of quantitation and that b) do not reflect the unique PFOS peak shape, were designated < LOD. These samples are estimated to contain from 0-1 ppb PFOS.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}

2 x 100 mm

5 µm particle size

Flow rate: 300 µL/min.
Solvent A: 2.0 mM Ammonium Acetate
Solvent B: Methanol
Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.
Injection volume: 10 µL
Injections / sample: 1

Electrospray mass spectrometer
Micromass Platform II API Mass Spectrometer, "Chick"
MassLynx 2.4 Software
Cone voltages: -60v
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Ions: 499, 427
Electrode: cross-flow

3M Environmental Lab

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received: 3/19/98
Samples Extracted: 3/19/98
Samples Analyzed: 3/20-3/25/98

Analysis by: ESMS (-)
Calibration Curve Range: 1ppb to 248 ppb
r^2 of Calibration Curve: 0.996
LOD[1]: 1 ppb
LOQ[2]: 5 ppb

PHASE VII Results: US geographical distribution study - PFOS levels in human sera

Sample ID	[PFOS] (ppb)	Std. Dev.	Sample ID	[PFOS] (ppb)	Std. Dev.
AK01	21	0.4	MS51	56	5.1
AK02	31	1.2	MS52	27	9.5
AK03	25	NA	MT41	26	4.1
AK04	19	NA	MT42	24	3.5
AZ026	28	2.5	MT43	18	3.2
AZ027	37	6.4	NE21	20	1.3
AZ028	47	5.8	NE22	12	1.0
CA029	23	4.1	NE23	27	6.7
CA030	23	0.7	NE24	9	2.0
CA031	26	0.0	NE25	19	0.8
DE016	24	4.5	NJ010	27	5.9
DE017	32	2.2	NJ011	20	0.8
DE018	21	0.1	NJ012	24	2.0
DE019	21	NA	NV44	28	0.8
DE020	23	0.3	NV45	25	0.1
IA013	40	0.1	NV46	27	6.6
IA014	29	4.2	SB38	13	0.2
IA015	28	3.0	SB39	14	0.7
LA47	49	7.4	SB040	16	0.6
LA048	39	1.9	SC032	56	1.8
LA049	50	7.9	SC033	47	2.8
MI03	31	NA	SC034	52	2.1
MI04	18	NA	TX035	32	11.2
MI05	22	0.9	TX036	26	1.2
MI06	31	1.1	TX037	29	3.6
MO07	24	4.9	WY53	35	0.7
MO08	31	6.6	WY54	13	0.4
MO09	35	0.6	WY55	20	1.3
MS50	35	5.3			

QC Results

QC Check:	% Recovery
Calibration Check (QC1)	101%

Branched Isomer Abundance Percentages

Sample Pool	% Abundance of Branched Isomers (Average)	% Abundance of Branched Isomers (Std. Dev.)	n (# samples)
Standard Material	22%	3%	n/a
Individuals Sampled in 1998	34%	5%	31
3M Cottage Grove Plant Workers, 1998	45%	4%	3
Individuals Sampled in 1970s and 80s	37%	5%	12
Pooled Sera Samples from 1997-98	31%	5%	11
Foreign Sera Samples, 1984 and 1994	na	na	na
Individuals Sampled in late 1950s	38%	4%	5
US Geographical Study Samples, 1998	41%	4%	55

1 - LOD = Limit of Detection
2 - LOQ = Limit of Quantitation

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\PHASE8.PRO\SAMPLEDB\PHASE8
Last modified: Thu Apr 09 09:59:18 1998
Method: C:\MASSLYNX\PHASE8.PRO\METHDB\PFOS
Last modified: Thu Apr 09 10:00:36 1998
Job Code:

Printed: Thu Apr 09 10:11:38 1998

Compound 1: PFOS

#	Name	Type	Sample Text	RT	Area	Conc.	%Dev	Flags
1	04079801	Analyte	Meoh Blank	9.988	58376	38.04		bb
2	04079802	Analyte	Water blank inj 1	10.471	5263	0.00		bb
3	04079803	Analyte	Water blank inj 2	10.479	4594	0.00		bb
4	04079804	Analyte	HS Blank inj 1	10.487	5092	0.00		bb
5	04079805	Analyte	HS Blank inj 2	10.489	4828	0.00		bb
6	04079806	Standard	0.996 ppb inj-1	10.411	5970	0.00	-100	MM
7	04079807	Standard	0.996 ppb inj-2	10.430	5750	0.00	-100	MM
8	04079808	Standard	4.90 ppb inj-1	10.423	11454	3.29	-33	MM
9	04079809	Standard	4.90 ppb inj-2	10.415	11188	3.09	-37	MM
10	04079810	Standard	24.8 ppbinj-1	10.405	41237	25.35	2	MM
11	04079811	Standard	24.8 ppb inj-2	10.411	41564	25.59	3	MM
12	04079812	Standard	49.0 ppb inj-1	10.419	75645	50.83	4	MM
13	04079813	Standard	49.0 ppb inj-2	10.373	74906	50.29	3	MM
14	04079814	Standard	96.2 ppb inj-1	10.421	132595	93.01	-3	MM
15	04079815	Standard	96.2 ppb inj-2	10.418	127286	89.08	-7	MM
16	04079816	Standard	142 ppbinj-2	10.434	192332	137.26	-3	bbX
17	04079817	Standard	142 ppbinj-1	10.425	196237	140.15	-1	bbX
18	04079818	Analyte	meoh blank	10.496	4489	0.00		bb
19	04079819	Analyte	SE-11 HS04078 inj 1	10.503	8860	1.37		MM
20	04079820	Analyte	SE-11 HS04078 inj 2	10.481	9246	1.66		MM
21	04079821	Analyte	SE-12 HS04078 inj 1	10.485	7869	0.64		MM
22	04079822	Analyte	SE-12 HS04078 inj 2	10.478	7340	0.24		MM
23	04079823	Analyte	SE-13 HS04078 inj 1	10.481	6139	0.00		MM
24	04079824	Analyte	SE-13 HS04078 inj 2	10.485	6473	0.00		MM
25	04079825	Analyte	SE-14 HS04078 inj 1	10.486	7496	0.36		MM
26	04079826	Analyte	SE-14 HS04078 inj 2	10.485	7116	0.08		MM
27	04079827	Analyte	SE-15 HS04078 inj 1	10.479	7746	0.54		MM
28	04079828	Analyte	SE-15 HS04078 inj 2	10.477	7431	0.31		MM
29	04079829	QC	24.8 ppb cal check	10.365	12549	4.10	-83	MM
30	04079830	Analyte	meoh blank	10.482	3708	0.00		MM
31	04079831	Analyte	SE-16 HS04078 inj 1	10.486	7723	0.53		MM
32	04079832	Analyte	SE-16 HS04078 inj 2	10.488	7611	0.44		MM
33	04079833	Analyte	SE-17 HS04078 inj 1	10.424	5624	0.00		MM
34	04079834	Analyte	SE-17 HS04078 inj 2	10.479	5633	0.00		MM
35	04079835	Analyte	SE-18 HS04078 inj 1	10.487	5265	0.00		MM
36	04079836	Analyte	SE-18 HS04078 inj 2	10.477	5330	0.00		MM
37	04079837	Analyte	SE-19 HS04078 inj 1	10.437	4707	0.00		MM
38	04079838	Analyte	SE-19 HS04078 inj 2	10.420	4569	0.00		MM
39	04079839	Analyte	SE-20 HS04078 inj 1	10.418	3712	0.00		bb
40	04079840	Analyte	SE-20 HS04078 inj 2	10.435	4205	0.00		bb
41	04079841	QC	24.8ppb cal check	10.358	12009	3.70	-85	MM
42	04079842	Analyte	meoh blank	10.438	3757	0.00		MM
43	04079843	Analyte	SE-1 HS04078 inj 1	10.353	9577	1.90		MM
44	04079844	Analyte	SE-1 HS04078 inj 2	10.357	9822	2.08		MM
45	04079845	Analyte	SE-2 HS04078 inj 1	10.352	9759	2.04		MM
46	04079846	Analyte	SE-2 HS04078 inj 2	10.340	9610	1.92		MM
47	04079847	Analyte	SE-3 HS04078 inj 1	10.470	10125	2.31		MM
48	04079848	Analyte	SE-3 HS04078 inj 2	10.467	10474	2.56		MM
49	04079849	Analyte	SE-4 HS04078 inj 1	10.336	8107	0.81		MM
50	04079850	Analyte	SE-4 HS04078 inj 2	10.337	7985	0.72		MM
51	04079851	Analyte	SE-5 HS04078 inj 1	10.434	9294	1.69		MM
52	04079852	Analyte	SE-5 HS04078 inj 2	10.403	9300	1.70		MM
53	04079853	QC	24.8 ppb cal check	10.309	11515	3.34	-87	MM
54	04079854	Analyte	meoh blank	10.388	4037	0.00		bb
55	04079855	Analyte	SE-6 HS04078 inj 1	10.305	8249	0.92		MM
56	04079856	Analyte	SE-6 HS04078 inj 2	10.281	7947	0.69		MM
57	04079857	Analyte	SE-7 HS04078 inj 1	10.410	18581	8.57		MM
58	04079858	Analyte	SE-7 HS04078 inj 2	10.470	17578	7.83		MM
59	04079859	Analyte	SE-8 HS04078 inj 1	10.463	7772	0.56		MM
60	04079860	Analyte	SE-8 HS04078 inj 2	10.455	7839	0.61		MM
61	04079861	Analyte	SE-9 HS04078 inj 1	10.336	8225	0.90		MM
62	04079862	Analyte	SE-9 HS04078 inj 2	10.343	9132	1.57		bb
63	04079863	Analyte	SE-10 HS04078 inj 1	10.461	20521	10.01		MM
64	04079864	Analyte	SE-10 HS04078 inj 2	10.404	20053	9.66		MM
65	04079865	Analyte	meoh blank	10.406	4387	0.00		MM
66	04079866	Analyte	Water blank inj 1	10.402	4524	0.00		bb
67	04079867	Analyte	Water blank inj 2	10.407	4379	0.00		MM
68	04079868	Analyte	Sera blank inj 1	10.398	4342	0.00		MM
69	04079869	Analyte	Sera blank inj 2	10.404	4493	0.00		bb
70	04079870	Analyte	0.996 ppb inj-1	10.336	5875	0.00	-100	bb
71	04079871	Analyte	0.996 ppb inj-2	10.334	5794	0.00	-100	bb
72	04079872	Analyte	4.90 ppb inj-1	10.339	11191	3.10	-37	bb
73	04079873	Analyte	4.90 ppb inj-2	10.340	11298	3.17	-35	bb
74	04079874	Analyte	24.8 ppbinj-1	10.340	40768	25.00	1	bb
75	04079875	Analyte	24.8 ppbinj-2	10.340	41257	25.36	2	bb
76	04079876	Analyte	49.0 ppb inj-1	10.340	68332	45.42	-7	MM
77	04079877	Analyte	49.0 ppb inj-2	10.344	68993	45.91	-6	MM
78	04079878	Analyte	96.2 ppb inj-1	10.339	116061	80.77	-16	MM
79	04079879	Analyte	96.2 ppb inj-2	10.342	115887	80.64	-16	MM
80	04079880	Analyte	142 ppbinj-1	10.346	162115	114.88	-19	MM
81	04079881	Analyte	142 ppbinj-2	10.342	165549	117.42	-17	MM

PFOS preliminary Smeon

Quantify Compound Summary Report

Sample List: C:\MASSLYNX\PHASE5.PRO\SAMPLES\032398
Last modified: Wed Mar 25 12:51:24 1998
Method: C:\MASSLYNX\PHASE5.PRO\METHODS\POAA
Last modified: Tue Apr 07 14:09:38 1998
Job Code:

Printed: Tue Apr 07 14:33:32 1998

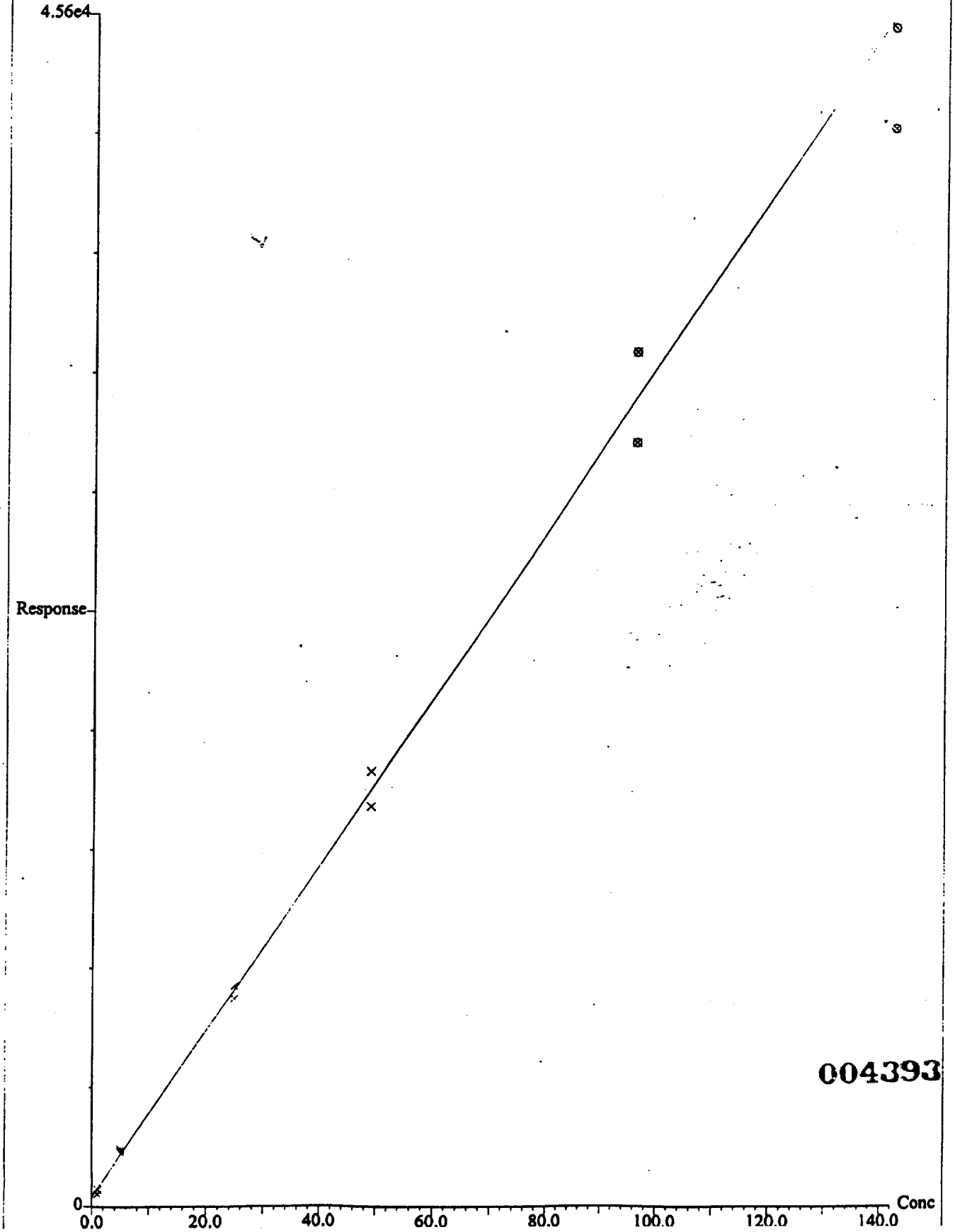
Compound 1: POAA

#	Name	Type	Sample Text	RT	Area	Conc.	%Dev	Flags
1	03239801	Analyte	MeOH Blank	9.864	208	0.00		bb
2	03239802	Analyte	Sera blank-1	9.921	445	0.16		bb
3	03239803	Analyte	H2O blank-1	9.915	536	0.45		bb
4	03239804	Standard	0.996 ppb FC Mix	9.922	658	0.83	-17	bb
5	03239805	Standard	4.90 ppb FC Mix	9.737	2214	5.72	17	bb
6	03239806	Standard	24.8 ppb FC Mix	9.742	8430	25.25	2	bb
7	03239807	Standard	49.0 ppb FC Mix	9.678	16694	51.20	4	bb
8	03239808	Standard	96.2 ppb FC Mix	9.621	32746	101.63	6	bbX
9	03239809	Standard	142 ppb FC Mix	9.620	45197	140.74	-1	bbX
10	03239810	Analyte	MeOH Blank	9.735	224	0.00		bb
11	03239811	Analyte	SB38-1	9.620	1546	3.62		bb
12	03239812	Analyte	SB38-2	9.621	2041	5.17		bb
13	03239813	Analyte	SB38-MS	9.621	2422	6.37		bb
14	03239814	Analyte	MeOH Blank	9.681	213	0.00		bb
15	03239815	Analyte	SB39-1	9.632	1764	4.30		bb
16	03239816	Analyte	SB39-2	9.620	1564	3.68		bb
17	03239817	Analyte	SB40-1	9.616	1635	3.90		bb
18	03239818	Analyte	SB40-2	9.618	1596	3.78		bb
19	03239819	Analyte	MT41-1	9.622	2159	5.55		bb
20	03239820	Analyte	MT41-2	9.622	1992	5.02		bb
21	03239821	Analyte	MT41-MS	9.617	2078	5.29		bb
22	03239822	Analyte	MeOH Blank	9.677	274	0.00		bb
23	03239823	Analyte	MT42-1	9.616	1888	4.70		bb
24	03239824	Analyte	MT42-2	9.624	1709	4.13		bb
25	03239825	Analyte	MT43-1	9.619	1459	3.35		bb
26	03239826	Analyte	MT43-2	9.616	1509	3.50		bb
27	03239827	Analyte	MV44-1	9.622	2347	6.14		bb
28	03239828	Analyte	MV44-2	9.616	2209	5.70		bb
29	03239829	Analyte	MV44-MS	9.624	2174	5.59		bb
30	03239830	Analyte	MeOH Blank	9.677	225	0.00		bb
31	03239831	Analyte	MV45-1	9.616	1953	4.90		bb
32	03239832	Analyte	MV45-2	9.623	2360	6.10		bb
33	03239833	Analyte	MV46-1	9.563	2242	5.80		bb
34	03239834	Analyte	MV46-2	9.620	2168	5.57		bb
35	03239835	Analyte	LA47-1	9.614	3025	8.26		bb
36	03239836	Analyte	LA47-2	9.621	3296	9.12		bb
37	03239837	Analyte	LA47-MS	9.617	2695	7.23		bb
38	03239838	Analyte	MeOH Blank	9.682	246	0.00		bb
39	03239839	Analyte	LA48-1	9.615	2204	5.69		bb
40	03239840	Analyte	LA48-2	9.616	2151	5.52		bb
41	03239841	Analyte	LA49-1	9.609	2726	7.33		bb
42	03239842	Analyte	LA49-2	9.789	2484	6.57		bb
43	03239843	Analyte	MS50-1	9.823	1514	3.52		bb
44	03239844	Analyte	MS50-2	9.832	1591	3.76		bb
45	03239845	Analyte	MS50-MS	9.735	2199	5.67		bb
46	03239846	Analyte	MeOH Blank	9.680	242	0.00		bb
47	03239847	Analyte	MS51-1	9.620	2670	7.15		bb
48	03239848	Analyte	MS51-2	9.619	2137	5.48		bb
49	03239849	Analyte	MS52-1	9.615	2088	5.32		bb
50	03239850	Analyte	MS52-2	9.557	1923	4.80		bb
51	03239851	Analyte	WY53-1	9.576	2183	5.62		bb
52	03239852	Analyte	WY53-2	9.556	1583	3.74		bb
53	03239853	Analyte	WY53-MS	9.555	2111	5.40		bb
54	03239854	Analyte	MeOH Blank	10.157	59	0.00		bb
55	03239855	QC	49.0 ppb FC Mix	9.557	15496	47.44	-3	bb
56	03239856	Analyte	MeOH Blank	10.220	35	0.00		bb
57	03239857	Analyte	WY54-1	9.554	1210	2.57		bb
58	03239858	Analyte	WY54-2	9.562	1397	3.15		bb
59	03239859	Analyte	WY55-1	9.554	1698	4.10		bb
60	03239860	Analyte	WY55-2	9.558	1536	3.59		bb
61	03239861	Analyte	AK03-1	9.556	1906	4.75		bb
62	03239862	Analyte	AK04-1	9.555	1809	4.45		bb
63	03239863	Analyte	MI03-2	9.556	2488	6.58		bb
64	03239864	Analyte	MI04-2	9.563	2289	5.95		bb
65	03239865	Analyte	MI04-MS	9.558	2240	5.80		bb
66	03239866	Analyte	MeOH Blank	9.676	301	0.00		bb
67	03239867	Analyte	MO09-1	9.557	2747	7.39		bb
68	03239868	Analyte	MO09-2	9.557	2474	6.53		bb
69	03239869	Analyte	NJ012-1	9.555	2228	5.76		bb
70	03239870	Analyte	NJ012-2	9.555	2007	5.07		bb
71	03239871	Analyte	IA015-1	9.561	7521	22.39		bb
72	03239872	Analyte	IA015-2	9.559	7260	21.57		bb
73	03239873	Analyte	DE018-1	9.560	2240	5.80		bb
74	03239874	Analyte	DE018-2	9.562	2206	5.69		bb
75	03239875	Analyte	DE019-2	9.561	2738	7.36		bb
76	03239876	Analyte	DE020-1	9.563	1959	4.92		bb
77	03239877	Analyte	DE020-2	9.555	2158	5.54		bb
78	03239878	Analyte	NE021-MS	9.556	1675	4.02		bb
79	03239879	Analyte	MeOH Blank	10.094	32	0.00		bb
80	03239880	Analyte	NE023-1	9.563	1218	2.89		bb
81	03239881	Analyte	NE023-2	9.559	1228	2.62		bb
82	03239882	Analyte	NE024-1	9.559	889	1.56		bb
83	03239883	Analyte	NE024-2	9.562	1042	2.03		bb
84	03239884	Analyte	NE025-1	9.554	1743	4.24		bb
85	03239885	Analyte	NE025-2	9.558	1964	4.93		bb
86	03239886	Analyte	AZ026-1	9.560	2602	6.94		bb
87	03239887	Analyte	AZ026-2	9.568	2543	6.75		bb
88	03239888	Analyte	CA031-1	9.569	1716	4.15		bb
89	03239889	Analyte	CA031-2	9.557	1716	4.15		bb
90	03239890	Analyte	SC034-1	9.554	4500	12.90		bb
91	03239891	Analyte	SC034-2	9.556	4899	14.15		bb
92	03239892	Analyte	TX035-1	9.565	2674	7.16		bb
93	03239893	Analyte	TX035-2	9.562	2039	5.17		bb
94	03239894	Analyte	TX035-MS	9.563	2209	5.70		bb
95	03239895	Analyte	MeOH Blank	9.675	246	0.00		bb
96	03239896	Analyte	TX036-1	9.555	2179	5.61		bb
97	03239897	Analyte	TX036-2	9.558	1715	4.15		bb
98	03239898	Analyte	TX037-1	9.558	1911	4.77		bb
99	03239899	Analyte	TX037-2	9.554	1706	4.12		bb
100	03239900	Analyte	SC032-MS	9.557	2861	7.75		bb
101	03239901	Analyte	MeOH Blank	9.681	269	0.00		bb
102	03239902	Analyte	SC033-1	9.554	2297	5.98		bb
103	03239903	Analyte	SC033-2	9.555	2461	6.49		bb
104	03239904	Analyte	MeOH Blank	9.623	178	0.00		bb
105	03239905	Analyte	Sera Blank-1	9.671	489	0.30		bb
106	03239906	Analyte	H2O Blank-1	9.563	633	0.75		bb
107	03239907	Standard	0.996 ppb FC Mix	9.555	476	0.26	-74	bb
108	03239908	Standard	4.90 ppb FC Mix	9.555	2110	5.39	10	bb
109	03239909	Standard	24.8 ppb FC Mix	9.560	7958	23.76	-4	bb
110	03239910	Standard	49.0 ppb FC Mix	9.561	15350	46.98	-4	bb
111	03239911	Standard	96.2 ppb FC Mix	9.562	29297	90.79	-6	bbX
112	03239912	Standard	142 ppb FC Mix	9.556	41343	128.63	-9	bbX

POAA
Geographical
Phase 7

Calibration: C:\MASSLYNX\DEMOSTOR.PRO\CURVES0\POAA
Last modified: Tue Apr 07 14:32:42 1998
Printed: Tue Apr 07 14:33:33 1998

Compound 1 name: POAA
Correlation coefficient: r = 0.997996, r^2 = 0.995995
Calibration curve: 318.335230 * x + 393.755503
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: Null, Axis trans: None



004393

3M Environmental Laboratory- Fluorine Analytical Chemistry Team

Kris Hansen - Sr. Analytical Chemist
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Quantitative analysis of PFOS in historic human sera samples from Sweden

Summary:

Two groups of ten sera samples in plastic vials were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on March 20, 1998. The two groups (samples SE-1 to SE-10 and SE11-SE-20) represent two distinct time periods. Both groups of samples originated in Sweden and in all cases, sample volume varied from 0.5 to 1.0 mL of human sera. An exact volume, usually the entire sample was extracted using an ion-pairing reagent. The extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, four-point extracted curve ($r^2 = 0.992$). Each sample extract was analyzed twice. Calibration checks, analyzed every 5 samples, were between 88-106% of expected values.

Description of samples groups: SE-1 to SE-10 and SE-11 to 20

Sera samples SE-1 through SE-10 were collected from Swedish donors more recently than samples SE-11 through SE-20. The average PFOS concentration in the first groups was determined to be 1.3 ppb; there were three samples below the limit of detection. The second set of samples had an average concentration of 2.0 ppb. In two of these samples, PFOS concentration was determined to be less than the detection limit of the method. All samples contained PFOS at less than 5 ppb. It was not possible to determine the isomer ratio of these low level samples.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3^-$). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected. In those samples

that were determined to contain PFOS at concentrations greater than 10 ppb, is possible to verify the identity of the analyte using HPLC-ESMSMS to monitor four characteristic fragment daughter ions.

Quality control summary:

Due to sample limitations, sera samples were not extracted in duplicate. For this same reason, matrix spikes could not be evaluated for the samples. Each sample extract was analyzed in duplicate. Methanol blanks were analyzed periodically to ensure complete isolation of the sample. Two mid-level (2.98 ppb and 4.95 ppb) quality control samples were analyzed between every 5 samples. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This four point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves ($r^2 = 0.992$). Specific QC parameters are available on the appended results table.

Limit of Quantitation

These low level samples were analyzed against a specially prepared low level curve. As a results, confidence limits calculated around the curve values indicate a quantifiable difference between samples evaluated at 0 and 1 ppb. For this analysis, the limit of quantitation and the limit of detection are equivalent.

Limit of Detection

The limit of detection for this analytical method is based on analyst recognition of the distinct peak shape resulting from PFOS analysis. PFOS standard material and PFOS extracted from sera analyzed by the conditions reported here, show near baseline resolution of 1 branched and 1 linear PFOS isomer. The apex of each peak in the HPLC-trace occurs at a specific, reproducible retention time and the ratio of the branched peak to the linear peak is fairly consistent down to the low standard, 1.0 ppb. Those samples that a) have PFOS levels evaluated at less than the limit of quantitation and that b) do not reflect the unique PFOS peak shape, were designated b.d.l. (below detection limit. These samples are estimated to contain less than 1 ppb PFOS.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size
Flow rate: 300 µL/min.
Solvent A: 2.0 mM Ammonium Acetate
Solvent B: Methanol
Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.
Injection volume: 10 µL
Injections / sample: 2

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software
Cone voltages: -20v, -60v
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Ions: 369, 413, 499, 427
Electrode: cross-flow

Electrospray MS/MS

Micromass Quattro II API MSMS, "Madeline"

MassLynx 3.1 Software
Cone voltages: -60v
Collision gas energy: 40
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Primary Ion: 499
Daughter ions: 80, 99, 130, 180, 230
Electrode: Z-source

004396

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received:	4/6/98	**Analysis by:**	ESMS (-)
Samples Extracted:	4/7-4/08/98	**Calibration Curve Range:**	1ppb to 10 ppb
Samples Analyzed:	4/09-4/13/98	**r^2 of Calibration Curve:**	0.992
		LOD[1]:	1 ppb
		LOQ[2]:	1 ppb

Study of PFOS levels in historical Swedish human sera samples

Sample ID	[PFOS] (ppb)	Sample ID	[PFOS] (ppb)
SE-1	1.2 +/- 0.1	SE-11	4.1 +/- 0.2
SE-2	1.5+/-0.1	SE-12	2.9 +/- 0.0
SE-3	1.0 +/- 0.1	SE-13	2.0 +/- 0.1
SE-4	1.0 +/- 0.1	SE-14	2.8 +/- 0.0
SE-5	b.d.l.	SE-15	2.9 +/- 0.0
SE-6	1.2 +/- 0.0	SE-16	2.7 +/- 0.2
SE-7	2.8 +/- 0.0	SE-17	1.2 +/- 0.0
SE-8	b.d.l.	SE-18	1.2 +/- 0.0
SE-9	2.6 +/- 0.9	SE-19	b.d.l.
SE-10	b.d.l.	SE-20	b.d.l.

QC Results

QC Check:	% Recovery
Calibration Check, 2.98 ppb	106%
Calibration Check, 4.95 ppb	100%
Calibration Check, 2.98 ppb	100%
Calibration Check, 4.95 ppb	96%
Calibration Check, 2.98 ppb	89%
Calibration Check, 4.95 ppb	88%

Branched Isomer Abundance Percentages

Sample Pool	% Abundance of Branched Isomers (Average)	% Abundance of Branched Isomers (Std. Dev.)	n (# samples)
Standard Material	22%	3%	n/a
Individuals Sampled in 1998	40%	5%	31
3M Cottage Grove Plant Workers, 1998	45%	4%	3
Individuals Sampled in 1970s and 80s	37%	5%	12
Pooled Sera Samples from 1997-98	31%	5%	11
Foreign Sera Samples, 1984 and 1994	na	na	na
Individuals Sampled in late 1950s	38%	4%	5
US Geographical Study Samples, 1998	41%	4%	55

1 - LOD = Limit of Detection
2 - LOQ = Limit of Quantitation

004397

file number	sample description	peak area	[analyte]	[PFOS]avg	St. Dev.	
4099807	0.998 ppb inj 1	4654	0.6			
4099808	2.98 ppb inj 1	8096	3.1			
4099809	2.98 ppb inj 2	8247	3.2			
4099810	4.95 ppb inj 1	11239	5.4			
4099811	4.95 ppb inj 2	11381	5.5			
4099812	9.8 ppb inj 1	17949	10.2			
4099813	9.8 ppb inj 2	17582	9.9			
4099818	Meoh blank	4466	0.5			
4099819	SE-11 HS04078 inj 1	9659	4.2			
4099820	SE-11 HS04078 inj 2	9265	3.9	4.1	0.2	
4099821	SE-12 HS04078 inj 1	7886	3.0			
4099822	SE-12 HS04078 inj 2	7794	2.9	2.9	0.0	
4099823	SE-13 HS04078 inj 1	6507	2.0			
4099824	SE-13 HS04078 inj 2	6735	2.1	2.0	0.1	
4099825	SE 14 HS04078 inj 1	7684	2.8			
4099826	SE-14 HS04078 inj 2	7674	2.8	2.8	0.0	
4099827	SE-15 HS04078 inj 1	7808	2.9			
4099828	SE-15 HS04078 inj 2	7803	2.9	2.9	0.0	%Rec, QC
4099829	Meoh blank	4133	0.3			
4099830	2.98 ppb cal check	8173	3.2			106
4099831	4.95 ppb cal check	10721	5.0			100
4099832	Meoh blank	3844	0.0			
4099833	SE-16 HS04078 inj 1	7766	2.9			
4099834	SE-16 HS04078 inj 2	7411	2.6	2.7	0.2	
4099835	SE-17 HS04078 inj 1	5539	1.3			
4099836	SE-17 HS04078 inj 2	5450	1.2	1.2	0.0	
4099837	SE-18 HS04078 inj 1	5380	1.2			
4099838	SE-18 HS04078 inj 2	5459	1.2	1.2	0.0	
4099839	SE-19 HS04078 inj 1	4579	0.6			
4099840	SE-19 HS04078 inj 2	4335	0.4	0.5	0.1	
4099841	SE-20 HS04078 inj 1	4018	0.2			
4099842	SE-20 HS04078 inj 2	3971	0.1	0.2	0.0	
4099843	Meoh blank	3569	-0.1			
4099844	2.98 ppb cal check	7932	3.0			100
4099845	4.95 ppb cal check	10483	4.8			96
4099846	Meoh blank	3377	-0.3			
4099847	SE-1 HS04078 inj 1	5330	1.1			
4099848	SE-1 HS04078 inj 2	5436	1.2	1.2	0.1	
4099849	SE-2 HS04078 inj 1	5773	1.4			
4099850	SE-2 HS04078 inj 2	5878	1.5	1.5	0.1	
4099851	SE-3 HS04078 inj 1	5197	1.0			
4099852	SE-3 HS04078 inj 2	5114	1.0	1.0	0.0	
4099853	SE-4 HS04078 inj 1	5337	1.1			
4099854	SE-4 HS04078 inj 2	5127	1.0	1.0	0.1	
4099855	SE-5 HS04078 inj 1	4807	0.7			
4099856	SE-5 HS04078 inj 2	4937	0.8	0.8	0.1	
4099857	Meoh blank	3416	-0.3			
4099858	2.98 ppb cal check	7479	2.7			89
4099859	4.95 ppb cal check	9904	4.4			88
4099860	Meoh blank	3219	-0.4			

004398

4099861	SE-6 HS04078 inj 1	5421	1.2		
4099862	SE-6 HS04078 inj 2	5480	1.2	1.2	0.0
4099863	SE-7 HS04078 inj 1	7673	2.8		
4099864	SE-7 HS04078 inj 2	7729	2.8	2.8	0.0
4099865	SE-8 HS04078 inj 1	4556	0.6		
4099866	SE-8 HS04078 inj 2	4682	0.7	0.6	0.1
4099867	SE-9 HS04078 inj 1	6497	2.0		
4099868	SE-9 HS04078 inj 2	8183	3.2	2.6	0.9
4099869	SE-10 HS04078 inj 1	4312	0.4		
4099870	SE-10 HS04078 inj 2	4710	0.7	0.5	0.2
4099871	Meoh blank	1555	0.0		
4099872	water blank inj 1	3813	0.0		
4099873	water blank inj 2	3891	0.1		
4099874	sera blank inj 1	4273	0.4		
4099875	sera blank inj 2	4498	0.5		
4099876	0.998 ppb inj 1	5199	1.0		
4099877	0.998 ppb inj 2	5054	0.9		
4099878	2.98 ppb inj 1	7712	2.8		
4099879	2.98 ppb inj 2	7792	2.9		
4099880	4.95 ppb inj 1	10297	4.7		
4099881	4.95 ppb inj 2	10556	4.9		
4099882	9.80 ppb inj 1	16807	9.4		
4099883	9.80 ppb inj 2	16888	9.4		

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Quantitative analysis of PFOS in historic human sera samples from the University of Minnesota

Summary:

Ten samples were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on April 14, 1998. The ten samples were part of a group of samples that, after extraction, were pre-screened for PFOS levels. These ten samples were classified as "low-level" (<10 ppb); they are numbered 1, 2A, 3A, 4, 5, 6A, 7A, 8, 9A, and 10A. Two-one mL portions of the samples were extracted using an ion-pairing reagent. The extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) using high pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, five-point extracted curve ($r^2 = 0.987$). Calibration checks, analyzed every 5 samples, were between 80-108% of expected values.

Description of samples:

In all ten of these pre-screened samples, PFOS concentration was determined to be less than the detection limit of the method, 1 ppb.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3^-$). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

Quality control summary:

Sera samples were extracted in duplicate. Methanol blanks were analyzed periodically to ensure complete isolation of the sample and two mid-level (2.98 ppb and 4.95 ppb) quality control samples were analyzed between every 5 samples. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This five

point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves (r^2 = 0.987). Specific QC parameters are available on the appended results table.

Limit of Quantitation/ Limit of Detection

These low level samples were analyzed against a specially prepared low level curve. As a results, confidence limits calculated around the curve values indicate a quantifiable difference between samples evaluated at 0 and 1 ppb. For this analysis, the limit of quantitation and the limit of detection are equivalent.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size
Flow rate: 300 µL/min.
Solvent A: 2.0 mM Ammonium Acetate
Solvent B: Methanol
Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.
Injection volume: 10 µL
Injections / sample: 2

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software
Cone voltages: -20v, -60v
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Ions: 369, 413, 499
Electrode: cross-flow

004401

Fluorine Analytical Chemistry Team

K.J.Hansen
778-6018

Samples Received:	4/14/98	**Analysis by:**	ESMS (-)
Samples Extracted:	4/15/98	**Calibration Curve Range:**	1ppb to 24.9 ppb
Samples Analyzed:	4/16-4/21/98	**r^2 of Calibration Curve:**	0.987
		LOD[1]:	1 ppb
		LOQ[2]:	1 ppb

Analysis of PFOS in historic samples of human sera (U of M)

Sample ID	[PFOS] (ppb)	Sample ID	[PFOS] (ppb)
1	< LOD	6A	< LOD
2A	< LOD	7A	< LOD
3A	< LOD	8	< LOD
4	< LOD	9A	< LOD
5	< LOD	10A	< LOD

QC Results

QC Check:	% Recovery
Calibration Check (QC1 at 2.98 ppb)	80%
Calibration Check (QC2 at 4.95 ppb)	108%

1 - LOD = Limit of Detection
2 - LOQ = Limit of Quantitation

004402

3M Environmental Laboratory- Fluorine Analytical Chemistry Team

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Phase XI, Part 2:

PFOS levels in individual's human sera samples, a blind repeatability experiment

Summary:

Fifteen samples of human sera in plastic centrifuge tubes were supplied to the Environmental Lab by Jeff Mandel (3M Medical) on April 15, 1998. The fifteen samples were part of a group of samples that, after extraction, were pre-screened for PFOS levels. These fifteen samples were classified as "mid-level" (>10 ppb and <100 ppb); they are numbered 1A, 2B, 2C, 4A, 5A, 5B, 6B, 7B, 8A, 9B, 9C, 10B, 11, 12, and 13. Sample of sera from these donors have been analyzed previously by the FACT. One mL aliquots of sera were removed from each sample and extracted with an ion-pairing reagent; all of the samples were extracted in duplicate.

The sera extracts were analyzed quantitatively for perfluorooctane sulfonate (PFOS) by high pressure liquid chromatography-electrospray mass spectrometry (HPLC-ESMS). Analyte identification was verified by comparison of molecular ion-HPLC retention time of the extracted analyte and standard material. Samples were quantitatively evaluated against a specially prepared, five-point extracted curve ($r^2 = 0.996$). Matrix spike calibration checks, analyzed in the middle of the analysis sequence, were within 10% of expected values.

The presence of PFOS in several of the samples (1A through 6B) was verified using HPLC-ESMSMS. This technique provides an additional degree of certainty to the analyte identification by specifically monitoring fragments daughter ions (m/z=80, 99, 130, 180, 230) characteristic of the PFOS primary ion (m/z = 499).

The PFOS values in this blind reproducibility analysis are within 11% of values collected previously. Specific results are attached to this report.

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for HPLC-ESMS analysis.

HPLC: Characteristic retention times for PFOS

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

004403

ES/MS: Detection and monitoring of the molecular ion

Analysis of PFOS standards indicates that the primary ion characteristic of PFOS is m/z = 499 amu, corresponding to the mass of the anionic surfactant ($C_8F_{17}SO_3$-). This ion was monitored selectively to maximize sensitivity. A scan of m/z=100 to 1210 (negative only) was also collected.

ES/MSMS: Confirmation of analyte identification

Several samples in this set were analyzed by ESMSMS to verify the identity of the PFOS analyte ion. ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a compound specific ion is selected. After selection, the selected ion is characterized further by smashing it apart with high energy gas. As a result of the smashing, ionic fragments, characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the compound specific primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). If PFOS is present in the samples, each of these secondary fragments is detected at the detector.

Quality control summary:

Each sera sample was extracted in duplicate; standard deviation values are included in the table of results. Two mid-level (25 ppb and 49 ppb) quality control sample were analyzed between every five samples; recovery for the six matrix spike analysis were determined to be within 10% of expected values. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. This five point extracted curve was analyzed before and after the samples. Quantitation of the samples is based on the linear regression equation derived from the average of the two bracketing curves (r^2 = 0.996). Specific QC parameters are available on the appended results table.

Limit of Quantitation

As these samples were pre-screened, it was determined that optimizing instrumental detection limits was not necessary for accurate analysis of these "mid-level" samples. As a result, the limit of quantitation was 10 ppb. All samples were quantitated well above this level.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/min.

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.

Injection volume: 10 µL

Injections / sample: 1

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software

004404

Cone voltages: -60v
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Ions: 499, 413, 369
Electrode: cross-flow

Electrospray MSMS
Micromass Quattro II API Mass Spectrometer, "Madeline"
MassLynx 3.1 Software
Cone voltages: -60v
Collision gas energy: 45 V
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Primary Ion: 499
Daughter ions: 80, 99, 130, 180, 230
Electrode: Z-spray

Fluorine Analytical Chemistry Team

Contact:
K.J. Hansen
8-6018

Samples Received: 4/14/98
Samples Extracted: 4/15/98
Samples Analyzed: 4/16-4/22/98

Analysis by: ESMS (-)
Calibration Curve Rang 10 ppb to 96 ppb
r^2 of Calibration Curve: 0.996
LOD[1]: 10 ppb
LOQ[2]: 10 ppb

PHASE IX, part 2: Blind repeatability experiment

Sample ID	[PFOS] (ppb)	Reported Previously	Sample ID	[PFOS] (ppb)	Reported Previously
1A	28 +/- 0.3	28	9B	34 +/- 1	30
2B	50 +/- 3	41	9C	47 +/- 9	39
2C	38 +/- 0.2	37	10B	51 +/- 1	41
4A	47 +/- 0.4	45	11	44 +/- 4	n.a.
5A	62 +/- 1	54	12	79 +/- 4	n.a.
5B	54 +/- 2	49	13	70 +/- 8	n.a.
6B	44 +/- 4	42	14	79 +/- 26	n.a.
7B	54 +/- 4	45	16	37 +/- 2	32
8A	78 +/- 5	67	17	80 +/- 14	73

QC Results

QC Check:	% Recovery
Calibration Check, 24.9 ppb	96%
Calibration Check, 49 ppb	100%
Calibration Check, 24.9 ppb	92%
Calibration Check, 49 ppb	95%
Calibration Check, 24.9 ppb	90%
Calibration Check, 49 ppb	98%

004406

1 - LOD = Limit of Detection
2 - LOQ = Limit of Quantitation

3M Environmental Laboratory- Fluorine Analytical Chemistry Team

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Preliminary results: Analysis of POAA in human sera samples

Summary:

To date, data from nine phases of a study of fluorochemicals in human sera has been collected.

PHASE I: Semi-quantitative investigation of PFOS in samples of pooled human sera
PHASE II: Quantitative analysis of PFOS and POAA in samples of pooled human US sera samples, 1997-1998
PHASE III: Quantitative analysis of PFOS and POAA in samples of individual's sera, 1998
PHASE IV: Quantitative analysis of PFOS in historical US sera samples, 1970s-80s
PHASE V: Quantitative analysis of PFOS in historical US sera samples, 1960s
PHASE VI: Quantitative analysis of PFOS in samples of sera from individuals in rural northern China, 1985-1997
PHASE VII: Geographical distribution: a quantitative analysis of PFOS in sera collected around the United States, 1998
PHASE VIII: Quantitative analysis of PFOS in historical samples from Sweden, specific date not supplied to Environmental Lab
PHASE IX, part 1: Quantitative analysis of PFOS in historical US samples, 1948-1951
PHASE IX, part 2: PFOS levels in individual's human sera samples; Blind Repeatability Experiment

The analysis of perfluorooctanesulfonate (PFOS) has been the primary focus in these phases; quantitative data for perfluorooctonoate (POAA) has also been collected for several phases of the study. Due to time constraints, we have not devoted the same attention to the POAA as we have to the PFOS analysis. The following report describes observations of POAA levels in some of the samples analyzed as part of this ongoing study. Reports detailing the PFOS analysis listed above have been issued to Jeff Mandel in the 3M Medical Department. LOQ for POAA is 10 ppb.

PHASE II: Quantitative analysis of PFOS and POAA in samples of pooled human sera
Number of samples with POAA > LOQ = 0
Number of samples with POAA > LOD = 4
Total number of samples = 12
Comments:

PHASE III: Quantitative analysis of PFOS and POAA in samples of individual's sera
Number of samples with POAA > LOQ = 4
Total number of samples = 31
Comments: Data has not been examined carefully

PHASE IV: Quantitative analysis of PFOS in historical sera samples, 1970s-80s
Number of samples with POAA > LOQ = 0
Total number of samples = 10
Comments: Data has not been precisely quantitated

004407

PHASE V: Quantitative analysis of PFOS in historical sera samples, 1960s
Number of samples with POAA > LOQ = 0
Number of samples with POAA > LOD = 6
Total number of samples = 6
Comments: Data has not been precisely quantitated

PHASE VI: Quantitative analysis of PFOS in samples of sera from individuals in rural northern China
Number of samples with POAA > LOQ = 0
Number of samples with POAA > LOD = 5
Total number of samples = 9
Comments: Data has not been precisely quantitated

PHASE VII: Geographical distribution: a quantitative analysis of PFOS in sera collected around the United States
Number of samples with POAA > LOQ = 2 (1 at 22 ppb, 1 at 12 ppb)
Number of samples with POAA > LOD = 20
Total number of samples = 55
Comments: Data has not been examined carefully

PHASE VIII: Quantitative analysis of PFOS in historical samples from Sweden
Number of samples with POAA > LOQ = 0
Number of samples with POAA > LOD = 0
Total number of samples = 20
Comments: Data has not been precisely quantitated

PHASE IX, part 1: Quantitative analysis of PFOS in historical US samples, 1948-1951
Number of samples with POAA > LOQ = 0
Total number of samples = 20
Comments: Data has not been precisely quantitated

Experimental summary:

Sample preparation: Ion-pairing extraction

In a pH controlled environment, an ion-pairing reagent, tetrabutyl ammonium sulfate (TBA), is used to extract the analyte from the matrix. Anionic compounds, like PFOS and perfluorooctanoate (POAA), are selectively targeted by the cationic reagent. Subsequent to the formation of the TBA-anion pair, the analyte is transferred to a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC: Characteristic retention times for POAA

In HPLC, an aliquot of the extract is injected and passed through a chromatographic column. Based on the affinity of the analyte for the stationary-phase in the column relative to the liquid mobile-phase passing through the column, the analyte is retained for a characteristic amount of time. For example, in a standard solution, POAA may elute at 10.5 minutes. Retention times between a standard POAA solution and the analyte extracted from sera in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detection and monitoring of the molecular ion

Analysis of POAA standards indicates that the primary ion characteristic of POAA is m/z = 413 amu, corresponding to the mass of the anionic surfactant ($C_7F_{15}CO_2^-$). This ion was monitored selectively to maximize sensitivity. Fragmentation of the POAA anion can be induced; ion 369, characteristic of the

decarboxylation product, results. Both ions were monitored in most of the study phases listed above. A scan of m/z=100 to 1210 (negative only) was also collected.

ES/MSMS: Confirmation of analyte identification

Several samples in this set were analyzed by ESMSMS to verify the identity of the PFOS analyte ion. ES-MSMS is very similar to ESMS, except that it adds an additional dimension of certainty to compound identification. As in ESMS, a compound specific ion is selected. After selection, the selected ion is characterized further by smashing it apart with high energy gas. As a result of the smashing, ionic fragments, characteristic of the molecule, are created and detected.

For example, for PFOS analysis, ion 499 is selected as the compound specific primary ion. This ion is smashed into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). If PFOS is present in the samples, each of these secondary fragments is detected at the detector.

Quality control summary:

When possible, sera samples were extracted in duplicate and analyzed in duplicate. Often this was not possible, as samples size and/or time were limited. Methanol blanks were analyzed periodically to ensure complete isolation of the sample. Mid-level quality control samples were analyzed periodically through each analytical sequence, typically every five samples, to monitor instrument stability. Because human sera without PFOS is not available, samples of pooled sera purchased from Sigma were pre-extracted, spiked with a standard concentration of PFOS, and re-extracted. This pre-extracted sera is understood to be very similar to the sample matrix. In all cases PFOS was evaluated versus an extracted; for most phases of this study, POAA was also spiked and extracted to form a standard curve. In these cases, quantitation of the samples is based on the linear regression equation derived from the average of the two curves bracketing the samples.

Limit of Quantitation

The current analytical method has been used to evaluate a large range of PFOS levels extracted from sera; the method is not optimized for precise low-level (< 5 ppb) quantitation, but instead for samples with PFOS concentrations in the range of 20-60 ppb. A special, low-level curve has been prepared for precise quantitative evaluation of PFOS levels between 1-10 ppb. As the POAA levels observed in non-plant worker sera are very low, in future analysis, POAA will be evaluated against a special low-level curve, similar to that prepared for PFOS.

Samples designated <LOQ contain less than 10 ppb POAA. If further accuracy and precision in quantitation of the sera samples (samples less than 10 ppb) is required, samples will be re-evaluated versus a special low-level quantitative curve.

Limit of Detection

The limit of detection for this analytical method is based on analyst recognition of the distinct peak shape resulting from POAA analysis. POAA standard material and POAA extracted from sera analyzed by the conditions reported here, show near baseline resolution of 1 branched and 1 linear POAA isomer. The apex of each peak in the HPLC-trace occurs at a specific, reproducible retention time. Unlike, PFOS, and the ratio of the branched peak to the linear peak is not necessarily consistent, particularly at low levels. POAA, when present, seems to be present at such low levels that accurate characterization of the smaller peak (the branched isomer) is very difficult.

Qualifications:

This data will be considered preliminary until the analyte extracted from these samples can be verified as POAA using LC-MSMS techniques and until instrumental sensitivity can be optimized to more clearly identify the analyte response. The method used for the analysis of these samples has been validated by LC-MSMS previously, but confirmation of POAA for specific samples in this eight-phase study has not been performed.

Instrumental specifics:

HPLC system

Hewlett Packard Series 1100 Liquid Chromatograph

Column: Keystone Betasil C_{18}
2 x 100 mm
5 µm particle size

Flow rate: 300 µL/min.

Solvent A: 2.0 mM Ammonium Acetate

Solvent B: Methanol

Solvent Gradient:
45 to 90 %B in 9.50 mins.
Hold at 90 %B for 3.50 mins.
Return to 45 %B in 1.50 mins.
Hold at 45 %B for 3.5 mins.

Injection volume: 10 µL

Injections / sample: 2

Electrospray mass spectrometer

Micromass Platform II API Mass Spectrometer, "Chick"

MassLynx 2.4 Software
Cone voltages: -20v, -60v
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Ions: 369, 413; 499, 427
Electrode: cross-flow

Electrospray MSMS

Micromass Quattro II API Mass Spectrometer, "Madeline"

MassLynx 3.1 Software
Cone voltages: -20v
Collision gas energy: 45 V
Mode: electrospray negative
Source temperature: 115 °C.
Analyzer vacuum pressures: 0.000043 mBar, 0.000079 mBar
Primary Ion: 413
Daughter ions: 119, 169, 369
Electrode: Z-spray

004410