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May 12, 2000



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MR 36090

Re: **3M Company -- Additional Information
On Perfluorinated Materials**

Contain NO CBI

Dear Charlie:

Pursuant to our earlier discussion, I am enclosing an analytical report from 3M's environmental monitoring program which has only just become available. 3M provided the study plans and other information regarding this program under cover of the May 4, 2000 letter to you from Bill Weppner.

Please do not hesitate to call with any questions.

Very truly yours,

Julia A. Hatcher

Julia A. Hatcher
of LATHAM & WATKINS



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**ENVIRONMENTAL MONITORING:
PART TWO -- BIOSPHERE SAMPLING AND ANALYSIS**

The attached report is dated May 10, 2000 and contains recent analytical results from 3M's ongoing Biosphere Sampling And Analysis program. We provided EPA with information previously on this program under cover of our May 4, 2000 letter.

The attached report covers sera, liver and other tissue measurements from various wildlife for perfluorooctane sulfonate and for several other sulfonated perfluoro materials as well as for perfluorooctanoate, a carboxylated perfluoro material. These measurements are part of an ongoing program, and no analysis has yet been prepared. Also, please note that the attached report does not provide information on the locations in which the samples were taken and/or the dates on which the samples were taken. This information will be covered in a separate report, which 3M will provide to EPA as soon as available.

000002

COMPOSITE ANALYTICAL LABORATORY REPORT

ON THE

Quantitative Analysis of Fluorochemicals in Environmental Samples

**REPORT NO. FACT GEN-021, GEN-024, GEN-030, GEN-033
LRN—W2491, W2845, W3197, E00-1386**

ANALYTICAL STUDY INITIATION

GEN021: 08/25/99

GEN024: 10/12/99

GEN030: 12/13/99

GEN033: 03/14/00

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ANALYTICAL STUDY PERSONNEL AND CONTRIBUTORS

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Dale Bacon, *Sponsor Representative*

INTRODUCTION

Purpose

The purpose of this composite report is to provide a summary of the analytical data collected for 3M studies Gen-021, Gen-024, Gen-030, and Gen-033. All of the samples included in these studies are tissue samples collected from fish, birds, mammals, and amphibians; Dr. John Geisy of Michigan State University has supplied all samples to 3M. These analyses have been conducted to support studies designed by Dr. Geisy.

The target analytes for these four studies were perfluorooctane sulfonate (PFOS; CAS# 2795-39-3), perfluorooctanesulfonylamide (PFOSA; CAS# 754-91-6), perfluorooctanoate (PFOA or POAA; CAS# 3825-26-1), and perfluorohexane sulfonate (PFHS; no CAS# available).

Due to the variety of matrices analyzed (with respect to both species and tissues), and due to evolving analytical methods, some analytical data quality objectives, such as the limit of quantitation (LOQ) were quite variable. A summary of the achieved LOQ (by specie, tissue and study number) is presented in Table 2 of this report. The stated data quality is based on results of data collection quality controls, sample prep quality controls, and recovery of target analytes from prepared matrix spike samples. More specific data quality objectives and parameters for these analytical studies are outlined later in this report.

Test and Control Article

The test articles for each study consisted of various tissues from various species and are listed below, in Table 1. For all studies, the control article consisted of rabbit sera and rabbit liver, as appropriate. Rabbit tissues were chosen as the control articles because previous studies have indicated very low levels of endogenous fluorochemicals in these matrices. Samples of the control articles were provided by the 3M Environmental Laboratory.

This report does not include details for the collection of the test articles; these details should be obtained from Dr. Geisy.

Table 1. Description of Samples, by Study

STUDY NUMBER	SERA/PLASMA/BLOOD	LIVER	OTHER
Gen-021	Cormorant Blood, Caspian Seal Blood, Sea Otter Blood	California Sea Lion, Elephant Seal, Harbor Seal, Gozzi, Mink, River Otter, Sea Otter, Turtle	Sea Otter Brain, Sea Otter Kidney
Gen-024	Albatross sera, Albatross plasma, Cormorant plasma, Herring Gull Plasma, Bald Eagle plasma, Cormorant blood, Herring Gull blood	Loon, Brown Pelican, Albatross	Albatross kidney, Cormorant yolk, Gull yolk
Gen-030	Northern Fur Seal blood (juvenile, sub-adult, adult), Polar Bear blood, Stellar Sea Lion blood	Northern Fur Seal, Polar Bear, Mink, Map Turtle, Terrapin, Tuna, Green Frog, Chinook Salmon, Lake Whitefish, Brown Trout	Carp body, Frog muscle, Frog body, Green Frog eggs, Lake Whitefish eggs, Brown Trout eggs, Carp muscle, Chinook Salmon muscle, Lake Whitefish muscle, Brown Trout muscle
Gen-033	None Submitted	Mink, Baikal Seal, Ganges Dolphin, Cormorant (adult and juvenile), Bottlenose Dolphin, Striped Dolphin, Weddell Seal, Swordfish, Tuna, Blacktailed Gull	None Submitted

Following analysis, extracts generated from these samples have been retained in cold storage.

Sample Collection and Analysis

Tissue samples were submitted to the Environmental Laboratory- Fluorine Analytical Chemistry Team by Kurunthachalan Kannan of Michigan State University. Details of the sample receipt are documented on the chain of custody forms located in appendices of this report.

SAMPLE RECEIPT AND MAINTENANCE

Samples were received in the Environmental Lab cold or frozen on the following dates: Gen-021 (8/24/99), Gen-024 (10/11/99), Gen-030 (12/13/99), and Gen-033 (3/13/00). Sample receipt, identification, and chain of custody information are located in the study folder for each report; the folders are located in the 3M archives.

The sample extracts will be maintained in cold storage at the 3M Environmental Laboratory until the quality of preparation no longer affords preservation.

CHEMICAL CHARACTERIZATION

The target analytes characterized in the samples include PFOS, PFOSA, PFOA, and PFHS. Procurement details of the reference standards used for analysis are summarized below.

Procurement

Table 2. Procurement Information for Reference Materials in the Analysis of Environmental Samples

REFERENCE MATERIAL	LOT NUMBER	SOURCE
PFOS (potassium salt)	171	3M ICP/PCP Division
PFOSA	Gen-021: L-2353; all others: L-15709	3M Specialty Chemicals (R. Buckanin)
PFHS (potassium salt)	NB116638-16	3M Specialty Chemicals (G. Moore)
PFOA (ammonium salt)	Gen-024: 245; all others: commercial	Gen-024: 3M Specialty Chemicals; all others: Aldrich

Full chemical characterization studies, including purity and stability determination, have not been completed at this time. Upon completion of these studies, a report will be archived in the 3M Environmental Lab.

METHOD SUMMARIES

Following is a brief description of the methods used during this analytical study by the 3M Environmental Laboratory. Copies of the actual methods used for these studies are located in attachment H.

PREPARATORY AND ANALYTICAL METHODS

- ETS-8-004.1, "Extraction of PFOS or Other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves. When sample size permitted, two matrix spikes were prepared in each tissue sample from each specie tested to provide some level of extraction efficiency determination.

For some samples, less than 1 mL of sample was available. For these samples, the available volume was extracted according to the method with the exception that the final volume of extraction solvent was adjusted to match the volume of the initial sample.

This method was used for the extraction of sera, plasma, and whole blood samples.

- ETS-8-005.1, "Analysis of PFOS or Other Fluorochemical Compounds in Serum Extracts Using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves; as a result, all sample concentrations were adjusted by a factor of 1.25 to adjust for the removal of 4/5 of the MTBE from the extract. The factor is unnecessary when an extracted curve is used for evaluation.

- ETS-8-006, "Analysis of PFOS or Other Fluorochemical Compounds in Liver Extracts using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves. When sample size permitted, two matrix spikes were prepared in each tissue sample from each specie tested to provide some level of extraction efficiency determination.

For some samples, less than 1 g of sample (as called for in the method) was available. For these samples, the available mass of tissue was extracted according to the method.

Samples of kidney, brain, egg, and muscle were extracted by this method.

- ETS-8-007, "Extraction of PFOS or Other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray Mass Spectrometry" with some modifications, described below.

Because the matrices were so variable and sample size extremely limited, it was not possible to prepare extracted standard curves. All extracts were evaluated versus unextracted standard curves; as a result, all sample concentrations were adjusted by a factor of 1.25 to adjust for the removal of 4/5 of the MTBE from the extract. The factor is unnecessary when an extracted curve is used for evaluation.

For Gen-030 and Gen-033 only: Due to the lack of excess test material for method development, all samples determined to contain greater than 0.015 µg/g of PFOS were subject to an additional PFOS verification process. Each sample was analyzed separately with respect to the 499 → 99 transition and the 499 → 80 transitions. The quantitative results

obtained from each transition analysis were compared. When these results agreed to with 30%, the identity of PFOS was confirmed (see Reference 1). Those samples where the identity of PFOS could not be confirmed are noted in the data table.

In Gen-021, no PFHS standard was available. In these samples, qualitative determination of PFHS was conducted based on reasonable retention time and a known PFHS transition (399 → 99).

Specific instrumental parameters are available in appendix I-L of this report, stored in the 3M Environmental Lab archives.

ANALYTICAL EQUIPMENT

For HPLC-Electrospray Tandem Mass Spectrometry:

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system

Analytical column:

1×30 mm C18 Betasil™

Column temperature: 30 degrees C

Cycle Time: 10 minutes

Mobile phase components:

Component A: 2mM ammonium acetate

Component B: Methyl alcohol

Flow rate: 300 µL/min

Injection volume: 10 µL

Solvent Gradient:

Time (min)	%B
0	10
1	10
5.5	95
7.5	95
8	10
10	10

For Detection:

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro Ultima Triple Quadrupole system or Micromass® API/Mass Spectrometer Quattro II Triple Quadrupole system

Acquisition Mode: MRM (refer to Table 3)

Software: Mass Lynx™ 3.3

Mode: Electrospray Negative

Source Block Temperature: 125-150°C

Source: Z-spray

Table 3. Ions Monitored in the Analyses of Extracts of Groundwater

TARGET ANALYTE	PRIMARY ION (amu)	PRODUCT ION (amu)
PFOS	499.0	80, 99*
PFOSA	498	78
PFOA	413	169
PFHS	399	99

* Indicates the ion used for quantitation

Refer to the analytical methods and equipment logs found in the raw data for details on the actual analytical equipment settings used in the present study. These settings may have varied somewhat during actual data collection. However, slight variations in the instrument settings will not adversely affect the quality of the data. Exact settings during all phases of data collection are recorded and presented in the appendix of this report.

DATA SUMMARY, ANALYSES, AND RESULTS

Summary of Quality Control Analyses Results

- **Standard Curves:** The coefficient of determination (r^2) for all 1/X weighted curves bracketing useable data was ≥ 0.982 . High or low curve points may have been excluded to provide a better fit over the linear range appropriate to the data. High or low curve points were deactivated if the calculated concentration varied from the theoretical concentration by more than 30%. Acceptable data was evaluated versus a standard curve containing at least 5 points. All actions are acceptable and are documented in specific data sets. All standard curves used to evaluate quantitative data were acceptable.
- **Continuing Calibration Verifications:** On average, one calibration check is analyzed for every five samples. Acceptable data is bracketed by calibration checks quantitated to be within 30% of the theoretical value, evaluated at least every ten samples. All quantitative data is bracketed by acceptable calibration checks, as required.
- **Blanks:** Extraction blanks were compliant if no target analyte was detected above the limit of quantitation (LOQ) for a specific analyte. In this study, extraction blanks were often higher than low curve points. Because analyte levels in the blank are used to determine the LOQ, by default, all blanks were determined to be below the limit of quantitation for the compounds of interest.
- **Internal Standards:** Internal standard response was monitored in Gen-030 and Gen-033 only. Internal standard response was required to be within $\pm 50\%$ of the theoretical value. If samples showed an internal standard response that deviated more than $\pm 50\%$, the samples were reanalyzed. If the deviant IS response was confirmed, the analyte data was reported, but noted in the data table.

Summary of Sample Results

- **GEN-021:**
 - PFOS was detected in at least one sample from the following matrices: California Sea Lion liver, Harbor Seal liver, Gozzi liver, Mink liver, River Otter liver, Turtle liver, Cormorant blood, Otter blood, and Caspian Seal blood.
 - PFOSA was tentatively identified in at least one sample from the following matrices: California Sea Lion liver, River Otter liver, Sea Otter liver, Sea Otter brain, and Otter blood.
 - PFOA was tentatively identified in at least one sample from the following matrices: California Sea Lion liver and Caspian Seal blood.
 - PFHS was tentatively identified in at least one sample from the following matrices: California Sea Lion liver, Gozzi liver, Mink liver, River Otter liver, Sea Otter liver, Turtle liver, Cormorant blood, Caspian Seal blood, and Otter blood.
- **GEN-024:**
 - PFOS was detected identified in at least one sample from the following matrices: Albatross plasma, Albatross sera, Cormorant plasma, Cormorant blood, Herring Gull plasma, Herring Gull blood, Bald Eagle plasma, Loon liver, Albatross liver, Brown Pelican liver, Albatross kidney, Cormorant yolk, and Gull yolk.
 - PFOSA was tentatively identified in at least one sample from the following matrices: Cormorant blood, Bald Eagle plasma, Loon liver, Brown Pelican liver, and Albatross liver.
 - PFOA was tentatively identified in at least one sample from the following matrices: Cormorant blood, Albatross liver, Cormorant yolk, and Gull yolk.
 - PFHS was tentatively identified in at least one sample from the following matrices: Herring Gull plasma and Bald Eagle plasma, Loon liver, Albatross liver, Brown Pelican liver, Albatross kidney, Cormorant yolk, and Gull yolk.
- **GEN-030:**
 - PFOS was detected in at least one sample from the following matrices: Polar Bear blood, Polar Bear liver, Mink liver, Northern Fur Seal liver, Map Turtle liver, Tuna liver, Green Frog liver, Chinook Salmon liver, Lake Whitefish liver, Brown Trout liver, Whole Carp, Frog muscle, Lake Whitefish eggs, Brown Trout eggs, Carp muscle, Chinook Salmon muscle, Lake Whitefish muscle, and Brown Trout muscle.
 - PFOSA was tentatively identified in at least one sample from the following matrices: Mink liver.
 - PFOA was not tentatively identified in any sample analyzed.
 - PFHS was not tentatively identified in any sample analyzed.
- **GEN-033:**
 - PFOS was detected in at least one sample from the following matrices: Mink liver, Baikal Seal liver, Cormorant liver, Bottle Nosed Dolphin liver, Ganges Dolphin liver, Striped Dolphin liver, Swordfish Liver, Tuna liver, and Black Tailed Gull liver.
 - PFOSA was tentatively identified in at least one sample from the following matrices: Mink liver, Cormorant liver, and Bottle Nosed Dolphin liver.

- PFOA was tentatively identified in at least one sample from the following matrices: Cormorant liver.
- PFHS was tentatively identified in at least one sample from the following matrices: Mink liver, Striped Dolphin liver, and Swordfish Liver.

Appendices contain data summary tables.

DATA QUALITY OBJECTIVES

No circumstances existed during the present study that would have affected the quality or integrity of the data. The data quality objectives (DQOs) followed during the present are indicated below.

- **Linearity:** The coefficient of determination (r^2) of the standard curve was equal to or greater than 0.985 with at least 5 active points using a linear regression curve with 1/x weighting.
- **Instrument Quantitation Limit (IQL):** The IQL is equal to the lowest acceptable standard in the calibration curve (acceptable standard is defined as a standard within 30% of the theoretical value). As this value is not useful in consideration of the sample data, the IQL was not specifically determined or stated for every study.
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within 30% of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks. The LOQ may vary due to the amount of sample available for analysis (particularly for samples extracted according to ETS-8-006) or to day-to-day variations in the analytical system. The ranges of LOQs for various tissues are listed in Table 4 (sera, plasma, and blood) and Table 5 (liver, kidney, muscle, egg, and brain).

Table 4. Range of LOQs for Sera, by Study

ANALYTE	GEN-021	GEN024	GEN-030	GEN-033
PFOS	0.0116 µg/mL	0.00116 µg/mL	0.0029-0.0579 µg/mL	NA
PFOSA	0.00625µg/mL	0.00626 µg/mL	0.000625 µg/mL	NA
PFHS	NA	0.00114 µg/mL	0.00114 µg/mL	NA
PFOA	0.00599 µg/mL	0.0299 µg/mL	0.00240-0.00958 µg/mL	NA

Table 5. Range of LOQs for Liver and Other Tissues, by Study

ANALYTE	GEN-021	GEN024	GEN-030	GEN-033
PFOS	0.0348 µg/g	0.0348 µg/g	0.00696-0.0696 µg/g	0.00696- 0.0694µg/g
PFOSA	0.0375 µg/g	0.00750 µg/g	0.0188 µg/g	0.0376 µg/g
PFHS	NA	0.00683 µg/g	0.00683-0.0342 µg/g	0.00683 µg/g
PFOA	0.0359 µg/g	0.180 µg/g	0.0180-0.0719 µg/g	0.00719-0.0718 µg/g

NA = not applicable

- **Duplicate/acceptable precision (extraction):** Spikes conducted on samples of control tissues were reproducible to within 15%
- **Quality Control Response:** A continuing calibration verification (CCV) was analyzed every 5–10 samples. Acceptable CCV response was within $\pm 30\%$ of the theoretical value. No more than 10 samples were analyzed between acceptable CCVs.
- **Spike/acceptable recoveries:** Due to the number of different matrices analyzed, there was great variability in spike recoveries. For any given matrix (specie and tissue), spike recoveries within 70–130% of the expected concentration indicate quantitative data (good to $\pm 30\%$); spike recoveries between 50–150% indicate semi-quantitative data for that matrix (good to $\pm 50\%$). Spike recoveries outside of this range indicate that sample data should be used for qualitative purposes only. Due to sample limitations, matrix spike studies were not conducted for all matrices. For PFOS analyses, sample data that is not supported by matrix spike studies should be considered for qualitative purposes only. Since no identity verification experiments were performed for PFOA, PFHS, and PFOSA, for these analytes, all analyses that are not supported by matrix spike studies should be considered to provide unconfirmed qualitative data only.
- **Use of Internal Standards:** Tetrahydro-perfluorooctane sulfonate (THPFOS) was spiked into the extracts post-extraction and used as an internal standard for samples in Gen-030 and Gen-033. For all samples in these studies, THPFOS levels were monitored to verify the analytical soundness of the data. THPFOS levels that were determined to be deviant from expected values by more than $\pm 50\%$ were reanalyzed. If the deviant THPFOS levels were confirmed, analyte levels were reported but are noted in the results table.
- **Use of confirmatory methods:** Given the selectivity of the analytical tool used (HPLC-ESMSMS) and lack of a viable alternative for analysis, no confirmatory methods were used.
- **Demonstration of specificity:** Specificity was demonstrated by chromatographic retention time (matched to standards to within 3%) and the response of at least one characteristic product ion arising from collisions of an analyte-specific parent ion.

Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, matrix spike studies have been used as an indicator of data quality (see above). The validity of this assumption has not been verified by other techniques.

STATEMENT OF CONCLUSION

Under the conditions of the present studies, the presence of fluorochemicals was observed in the quantitative analysis of a selection of environmental matrices.

REFERENCES

- 1) "Acceptance Criteria for Ultratrace HPLC-Tandem Mass Spectrometry: Quantitative and Qualitative Determination of Sulfonylurea Herbicides in Soil"; Li, L.Y.; Campbell, D.A.; Bennet, P.K.; Henion, J.; *Anal. Chem.*, **68** (19), 3397-3404, 1996

FACT-GEN-021

Study: GEN021 Various Matrices from MSU
 Product Number(Test Substance): None
 Matrix: Blood
 Method/Revision: ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: Masslynx 3.2
 Filename: See list to right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Extraction/Analyst: 08/25/99 MCH/KK/SAL/SEE
 Dates of Analysis/Analyst: 08/26/99, 08/27/99, 08/28/99, 12/09/99 MEE/IAS
 Date of Data Reduction/Analyst: 08/27/99, 08/30/99, 08/31/99, 12/10/99, 01/20/00 MEE/IAS

Sample Data

BLOOD

Group Dose	Sample #	Concentration of PFOS ug/mL	Mean PFOS ug/mL	RSD Std. Dev.	Concentration of PFOSA ug/mL	Mean PFOSA ug/mL	RSD Std. Dev.
Method Blk	H2O Blk-1 8/25/99	<LOQ (0.0116)			<LOQ (0.00625)		
	H2O Blk-2 8/25/99	<LOQ (0.0116)		NA	<LOQ (0.00625)		NA
	H2O Blk-3 8/25/99	<LOQ (0.0116)		NA	<LOQ (0.00625)		NA
	H2O Blk-4 8/25/99	<LOQ (0.0116)	<LOQ		<LOQ (0.00625)	<LOQ	
Caspian Seal Blood	W2491-40,J 53	0.0180			<LOQ (0.00625)		
	W2491-41,J 11	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-42,J 46	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-43,J 17	0.0166			<LOQ (0.00625)		
	W2491-44,J 13	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-45,J 8	0.0131			<LOQ (0.00625)		
	W2491-46,J 12	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-47,J 9	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-48,J 14	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-49,J 18	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-50,J 52	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-51,J 55	<LOQ (0.0116)			<LOQ (0.00625)		
	W2491-52,J 10	<LOQ (0.0116)			<LOQ (0.00625)		NA
	W2491-53,J 15	<LOQ (0.0116)	<LOQ - 3 Outliers		NA	<LOQ (0.00625)	<LOQ

No curve analyzed for PFHS, PFDS. PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Deviant Surrogate levels are not noted and were not confirmed.
 Date Entered/By: 08/27/99, 09/01/99, 12/30/99, 01/20/00, 02/14/00 LAC
 Date Verified/ By: 02/22/00 MEE

LOQ = Limit of Quantitation
 NA = Not Applicable
 RSD = Relative Standard Deviation
 ND = Not Detected
 D = Detected

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

FACT-GEN-021

Study: GEN021 Various Matrices from MSU
 Product Number(Test Substance): None
 Matrix: Blood
 Method/Revision: ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: Masslynx 3.2
 Filename: See list to right
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments
 Dates of Extraction/Analyst: 08/25/99 MCH/KK/SAL/SEE
 Dates of Analysis/Analyst: 08/26/99, 08/27/99, 08/28/99, 12/09/99 MEE/IAS
 Date of Data Reduction/Analyst: 08/27/99, 08/30/99, 08/31/99, 12/10/99, 01/20/00 MEE/IAS

Sample Data

BLOOD

Group Dose	Sample #	Concentration of POAA ug/mL	Mean POAA ug/mL	RSD Std. Dev.	Concentration of PFHS ug/mL	Mean PFHS ug/mL	RSD Std. Dev.	
Method Blk	H2O Blk-1 8/25/99	0.00629			ND			
	H2O Blk-2 8/25/99	<LOQ (0.00599)			ND			
	H2O Blk-3 8/25/99	<LOQ (0.00599)		NA	ND		NA	
	H2O Blk-4 8/25/99	<LOQ (0.00599)	<LOQ - 1 Outlier	NA	ND	ND	NA	
Caspian Seal Blood	W2491-40,J 53	<LOQ (0.00599)			D			
	W2491-41,J 11	0.00759			D			
	W2491-42,L 46	<LOQ (0.00599)			ND			
	W2491-43,J 17	<LOQ (0.00599)			D			
	W2491-44,J 13	<LOQ (0.00599)			ND			
	W2491-45,J 8	<LOQ (0.00599)			ND			
	W2491-46,J 12	<LOQ (0.00599)			ND			
	W2491-47,J 9	<LOQ (0.00599)			ND			
	W2491-48,J 14	0.00728			ND			
	W2491-49,J 18	<LOQ (0.00599)			ND			
	W2491-50,J 52	0.0108			ND			
	W2491-51,J 55	0.0234			ND			
	W2491-52,J 10	<LOQ (0.00599)			ND			
	W2491-53,J 15	<LOQ (0.00599)	<LOQ - 4 Outliers		NA NA	ND	ND - 3 Outliers	NA NA

No curve analyzed for PFHS, PFDS. PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Deviant Surrogate levels are not noted and were not confirmed.
 Date Entered/By: 08/27/99, 09/01/99, 12/30/99, 01/20/00, 02/14/00 LAC
 Date Verified/ By: 02/22/00 MEE

LOQ = Limit of Quantitation
 NA = Not Applicable
 RSD = Relative Standard Deviation
 ND = Not Detected
 D = Detected
 PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

000016

FACT-GEN-021

Study: GEN021 Various Matrices from MSU
 Product Number(Test Substance): None
 Matrix: Blood
 Method/Revision: ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: Masslynx 3.2, 3.3
 Filename: 08/28/99, 12/09/99 IAS
 R-Squared Value: 08/28/99, 12/09/99 IAS
 Slope: 08/30/99, 12/10/99 IAS
 Y-Intercept: See Attachments
 Dates of Extraction/Analyst: 08/25/99 MCH/KK/SAL/SEE
 Dates of Analysis/Analyst: 08/28/99, 12/09/99 IAS
 Date of Data Reduction/Analyst: 08/30/99, 12/10/99 IAS

Sample Data

Group Dose	Sample #	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL or % Rec	RSD Std. Dev.	Concentration of PFOSA ug/mL or % Rec	Mean PFOSA ug/mL or % Rec	RSD Std. Dev.	
Method Blk	H2O Blk-1 8/25/99	NA			NA			
	H2O Blk-2 8/25/99	NA			NA			
	H2O Blk-3 8/25/99	NA		NA	NA		NA	
	H2O Blk-4 8/25/99	NA	NA	NA	NA	NA	NA	
Casplan Seal Blood	W2491-40,J 53-MS	164%			106%			
	W2491-41,J 11-MS	115%			98%			
	W2491-42,J 46-MS	101%			73%			
	W2491-43,J 17-MS	2431%	*		1991%	*		
	W2491-44,J 13-MS	67%			50%			
	W2491-45,J 8-MS	103%			79%			
	W2491-46,J 12-MS	150%			116%			
	W2491-47,J 9-MS	103%			85%			
	W2491-48,J 14-MS	103%			90%			
	W2491-49,J 18-MS	73%			47%			
	W2491-50,J 52-MS	65%			49%			
	W2491-51,J 55-MS	17%			10%			
	W2491-52,J 10-MS	12%	* outlier excluded	58%	10%	* outlier excluded	58%	
	W2491-53,J 15-MS	17%		84%	48%		63%	37%

No curve analyzed for PFHS, PFDS. PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Deviant Surrogate levels are not noted and were not confirmed.
 Date Entered/By: 02/16/00, 02/17/00 LAC
 Date Verified/ By: 02/22/00 MEE

LOQ = Limit of Quantitation
 NA = Not Applicable
 RSD = Relative Standard Deviation
 ND = Not Detected
 D = Detected
 NS = Not Spiked

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

000017

FACT-GEN-021

Study: GEN021 Various Matrices from MSU
 Product Number(Test Substance): None
 Matrix: Blood
 Method/Revision: ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Analytical Equipment System Number: Amelia 062498
 Instrument Software/Version: Masslynx 3.2, 3.3
 Filename: 08/28/99, 12/09/99 IAS
 R-Squared Value: 08/28/99, 12/09/99 IAS
 Slope: 08/30/99, 12/10/99 IAS
 Y-Intercept: See Attachments
 Dates of Extraction/Analyst: 08/25/99 MCH/KK/SAL/SEE
 Dates of Analysis/Analyst: 08/28/99, 12/09/99 IAS
 Date of Data Reduction/Analyst: 08/30/99, 12/10/99 IAS

Sample Data

BLOOD QC

Group Dose	Sample #	Concentration of POAA ug/mL or % Rec	Mean POAA ug/mL or % Rec	RSD Std. Dev.	Concentration of PFHS ug/mL or % Rec	Mean PFHS ug/mL or % Rec	RSD Std. Dev.
Method Blk	H2O Blk-1 8/25/99	NA			NS		
	H2O Blk-2 8/25/99	NA			NS		
	H2O Blk-3 8/25/99	NA		NA	NS		NS
	H2O Blk-4 8/25/99	NA	NA	NA	NS	NS	NS
Caspian Seal Blood	W2491-40,J 53-MS	157%			NS		
	W2491-41,J 11-MS	115%			NS		
	W2491-42,J 46-MS	100%			NS		
	W2491-43,J 17-MS	2056%	*		NS		
	W2491-44,J 13-MS	89%			NS		
	W2491-45,J 8-MS	108%			NS		
	W2491-46,J 12-MS	174%			NS		
	W2491-47,J 9-MS	111%			NS		
	W2491-48,J 14-MS	112%			NS		
	W2491-49,J 18-MS	91%			NS		
	W2491-50,J 52-MS	78%			NS		
	W2491-51,J 55-MS	14%			NS		
	W2491-52,J 10-MS	19%	* outlier excluded	54%	NS		NS
	W2491-53,J 15-MS	19%	91%	50%	NS	NS	NS

No curve analyzed for PFHS, PFDS. PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Devient Surrogate levels are not noted and were not confirmed.
 Date Entered/By: 02/16/00, 02/17/00 LAC
 Date Verified/ By: 02/22/00 MEE

LOQ = Limit of Quantitation
 NA = Not Applicable
 RSD = Relative Standard Deviation
 ND = Not Detected
 D = Detected
 NS = Not Spiked

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

000018

FACT-GEN-021

Study:
 Product Number/Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
Sample Data

GEN021 Various Matrices from MSU
 None
 Various Matrices
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.2
 08/25/99 MCH/KK/SAL/SEE
 08/26/99, 08/27/99, 08/28/99, 12/09/99 MEE/IAS
 08/27/99, 08/30/99, 08/31/99, 12/10/99, 01/20/00 MEE/IAS

LIVER/WHOLE BLOOD

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev.	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev.	
Method Blk	H2O Blk-1 8/25/99	<LOQ (0.0348)			<LOQ (0.0375)			
	H2O Blk-2 8/25/99	<LOQ (0.0348)			<LOQ (0.0375)			
	H2O Blk-3 8/25/99	<LOQ (0.0348)		<LOQ	<LOQ (0.0375)		NA	
	H2O Blk-4 8/25/99	<LOQ (0.0348)	<LOQ	<LOQ	<LOQ (0.0375)	<LOQ	NA	
California Sea Lion Liver	W2491-3,CSL 3448	0.0384			0.0443			
	W2491-4,CSL 3395	0.0494			<LOQ (0.0375)			
	W2491-6,CSL 3020	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-7,CSL 2169	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-9,CSL 2839	<LOQ (0.0348)			<LOQ (0.0375)		NA	
	W2491-10,CSL 2367	<LOQ (0.0348)	<LOQ - 2 Outliers	NA	0.00773	<LOQ (0.0375)	<LOQ - 1 Outlier	NA
Elephant Seal Liver	W2491-2,ES 1500	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-5,ES 1552	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-11,ES 808	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-12,ES 772	<LOQ (0.0348)			<LOQ (0.0375)		NA	
	W2491-14,ES 782	<LOQ (0.0348)	<LOQ	NA	NA	<LOQ (0.0375)	<LOQ	NA
Harbor Seal Liver	W2491-8,HS	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-13,HS 1191	<LOQ (0.0348)			<LOQ (0.0375)		NA	
	W2491-15,HS 1199	0.0571	<LOQ - 1 Outlier	NA	<LOQ (0.0375)	<LOQ	NA	
Gozl Liver	W2491-1,NFS 100	0.133		0.133	NA	<LOQ (0.0375)	<LOQ	NA
Mink Liver	W2491-34,D1030 USFWS	4.85			<LOQ (0.0375)			
	W2491-35,D1146 USFWS	2.41		81.7	<LOQ (0.0375)		NA	
	W2491-36,D1158 USFWS	0.587	2.62	2.14	<LOQ (0.0375)	<LOQ	NA	
River Otter Liver	W2491-29,RAG 066	0.279			0.0371			
	W2491-30,RAG 028	0.994			0.0448			
	W2491-31,RAG 148	0.189			0.0716			
	W2491-32,RAG 230	0.0336			<LOQ (0.0375)		33.1	
	W2491-33,RAG 237	0.151	0.329	0.382	0.0393	0.0482 - 1 Outlier	0.0160	
Sea Otter Liver	W2491-16,SO 12593-001	<LOQ (0.0348)			0.0806			
	W2491-19,SO 11494-001	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-22,SO 11940-001	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-24,SO 11309-001	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-25,SO 12797-001	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-26,SO 13110-001	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-27,SO 12679-001	<LOQ (0.0348)			<LOQ (0.0375)		NA	
	W2491-28,SO 12707-001	<LOQ (0.0348)	<LOQ	NA	NA	<LOQ (0.0375)	<LOQ - 1 Outlier	NA
Turtle Liver	W2491-37,Male Turtle (-2,8),Turtle Liver	0.239			<LOQ (0.0375)			
	W2491-38,Male Turtle (2,12),Turtle Liver	0.358		56.0	<LOQ (0.0375)		NA	
	W2491-39,Female Turtle (-3,9),Turtle Liver	0.099	0.232	0.130	<LOQ (0.0375)	<LOQ	NA	
Sea Otter Brain	W2491-18,SO 12593-001,Sea Otter Brain	<LOQ (0.0348)			0.0664		NA	
	W2491-21,SO 11494-001,Sea Otter Brain	<LOQ (0.0348)	<LOQ	NA	<LOQ (0.0375)	NA	NA	
Sea Otter Kidney	W2491-17,SO 12593-001,Sea Otter Kidney	<LOQ (0.0348)			<LOQ (0.0375)			
	W2491-20,SO 11494-001,Sea Otter Kidney	<LOQ (0.0348)			<LOQ (0.0375)		NA	
	W2491-23,SO 11940-001,Sea Otter Kidney	<LOQ (0.0348)	<LOQ	NA	<LOQ (0.0375)	<LOQ	NA	
Whole Blood	W2491-54,Cormorant DCCO L Charity	0.190			<LOQ (0.0375)			
	W2491-55,Cormorant DCCO Hym Island, Lake Sup	0.0422		95.2	<LOQ (0.0375)		NA	
	W2491-56,Otter DCCO Great Lakes	0.0392	0.0904	0.0861	0.112	<LOQ - 1 Outlier	NA	

PFDS/PFHS = no curve analyzed PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Deviant Surrogate levels are not noted and were not confirmed.

LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 NA = Not Applicable
 ND = Not Detected
 D = Detected

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

Date Entered/Analyst: 08/27/99, 09/01/99, 12/30/99, 01/20/00, 02/14/00 LAC
 Date Verified/Analyst: 02/22/00 MEE

000019

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
Sample Data

GEN021 Various Matrices from MSU
 None
 Various Matrices
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.2
 08/25/99 MCH/KK/SAL/SEE
 08/26/99, 08/27/99, 08/28/99, 12/09/99 MEE/IAS
 08/27/99, 08/30/99, 08/31/99, 12/10/99, 01/20/00 MEE/IAS

LIVER/WHOLE BLOOD

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev.	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev.
Method Blk	H2O Blk-1 8/25/99	0.00602			ND		
	H2O Blk-2 8/25/99	<LOQ (0.0359)			ND		
	H2O Blk-3 8/25/99	<LOQ (0.0359)		NA	ND		NA
	H2O Blk-4 8/25/99	<LOQ (0.0359)	<LOQ - 1 Outlier	NA	ND	ND	NA
California Sea Lion Liver	W2491-3,CSL 3448	<LOQ (0.0359)			ND		
	W2491-4,CSL 3395	0.0409			ND		
	W2491-6,CSL 3020	<LOQ (0.0359)			ND		
	W2491-7,CSL 2169	<LOQ (0.0359)			ND		
	W2491-9,CSL 2839	<LOQ (0.0359)		NA	D		NA
	W2491-10,CSL 2367	<LOQ (0.0359)	<LOQ - 1 Outlier	NA	ND	ND - 1 Outlier	NA
Elephant Seal Liver	W2491-2,ES 1500	<LOQ (0.0359)			ND		
	W2491-5,ES 1532	<LOQ (0.0359)			ND		
	W2491-11,ES 808	<LOQ (0.0359)			ND		
	W2491-12,ES 772	<LOQ (0.0359)		NA	ND		NA
	W2491-14,ES 782	<LOQ (0.0359)	<LOQ	NA	ND	ND	NA
Harbor Seal Liver	W2491-8,HS	<LOQ (0.0359)			ND		
	W2491-13,HS 1191	<LOQ (0.0359)		NA	ND		NA
	W2491-15,HS 1199	<LOQ (0.0359)	<LOQ	NA	ND	ND	NA
Goazi Liver	W2491-1,NFS 100	<LOQ (0.0359)	<LOQ	NA	D	D	NA
Mink Liver	W2491-34,D1030 USFWS	<LOQ (0.0359)			D		
	W2491-35,D1146 USFWS	<LOQ (0.0359)		NA	ND		NA
	W2491-36,D1158 USFWS	<LOQ (0.0359)	<LOQ	NA	ND	ND - 1 Outlier	NA
River Otter Liver	W2491-29,RAG 066	<LOQ (0.0359)			D		
	W2491-30,RAG 028	<LOQ (0.0359)			D		
	W2491-31,RAG 148	<LOQ (0.0359)			D		
	W2491-32,RAG 230	<LOQ (0.0359)		NA	ND		NA
	W2491-33,RAG 237	<LOQ (0.0359)	<LOQ	NA	D	D - 1 Outlier	NA
Sea Otter Liver	W2491-16,SO 12593-001	<LOQ (0.0359)			ND		
	W2491-19,SO 11494-001	<LOQ (0.0359)			D		
	W2491-22,SO 11940-001	<LOQ (0.0359)			ND		
	W2491-24,SO 11309-001	<LOQ (0.0359)			ND		
	W2491-25,SO 12797-001	<LOQ (0.0359)			D		
	W2491-26,SO 13110-001	<LOQ (0.0359)			ND		
	W2491-27,SO 12679-001	<LOQ (0.0359)		NA	ND		NA
	W2491-28,SO 12707-001	<LOQ (0.0359)	<LOQ	NA	ND	ND - 2 Outliers	NA
Turtle Liver	W2491-37,Male Turtle (-2,8),Turtle Liver	<LOQ (0.0359)			ND		
	W2491-38,Male Turtle (2,12),Turtle Liver	<LOQ (0.0359)		NA	D		NA
	W2491-39,Female Turtle (-3,9),Turtle Liver	<LOQ (0.0359)	<LOQ	NA	D	D - 1 Outlier	NA
Sea Otter Brain	W2491-18,SO 12593-001,Sea Otter Brain	<LOQ (0.0359)			ND		
	W2491-21,SO 11494-001,Sea Otter Brain	<LOQ (0.0359)	<LOQ	NA	ND	ND	NA
Sea Otter Kidney	W2491-17,SO 12593-001,Sea Otter Kidney	<LOQ (0.0359)			ND		
	W2491-20,SO 11494-001,Sea Otter Kidney	<LOQ (0.0359)		NA	ND		NA
	W2491-23,SO 11940-001,Sea Otter Kidney	<LOQ (0.0359)	<LOQ	NA	ND	ND	NA
Whole Blood	W2491-54,Cormorant DCCO L Charity	<LOQ (0.0359)			ND		
	W2491-55,Cormorant DCCO Hymn Island, Lake Sup	<LOQ (0.0359)		NA	D		NA
	W2491-56,Otter DCCO Great Lakes	<LOQ (0.0359)	<LOQ	NA	D	D - 1 Outlier	NA

PFDS/PFHS = no curve analyzed PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 Deviant Surrogate levels are not noted and were not confirmed.

LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 NA = Not Applicable
 ND = Not Detected
 D = Detected

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

Date Entered/Analyst: 08/27/99, 09/01/99, 12/30/99, 01/20/00, 02/14/00 LAC
 Date Verified/Analyst: 02/22/00 MEE

000020

Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN021 Various Matrices from MSU
 None
 Various Matrices
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.2, 3.3
 08/25/99 MCH/KK/SAL/SEE
 08/28/99, 12/09/99 IAS
 08/30/99, 12/10/99 IAS

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Sample Data

LIVER/WHOLE BLOOD

QC

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec.	Mean PFOS Recovery	RSD Std. Dev.	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev.
Method Blk	H2O Blk-1 8/25/99	NA			NA		
	H2O Blk-2 8/25/99	NA			NA		
	H2O Blk-3 8/25/99	NA		NA	NA		NA
	H2O Blk-4 8/25/99	NA	NA	NA	NA	NA	NA
California Sea Lion Liver	W2491-3,CSL 3448-MS	91%			66%		
	W2491-4,CSL 3395-MS	78%			52%		
	W2491-6,CSL 3020-MS	69%			48%		
	W2491-7,CSL 2169-MS	45%			30%		
	W2491-9,CSL 2839-MS	31%		35%	15%		42%
	W2491-10,CSL 2367-MS	64%	63%	22%	43%	42%	18%
Elephant Seal Liver	W2491-2,ES 1500-MS	105%			78%		
	W2491-5,ES 1552-MS	77%			65%		
	W2491-11,ES 808-MS	51%			35%		
	W2491-12,ES 772-MS	44%		47%	18%		56%
	W2491-14,ES 782-MS	33%	62%	29%	29%	45%	25%
Harbor Seal Liver	W2491-8,HS-MS	35%			42%		
	W2491-13,HS 1191-MS	81%		42%	79%		39%
	W2491-15,HS 1199-MS	50%	55%	23%	42%	54%	21%
Gozzl Liver	W2491-1,NFS 100-MS	57%	NA	NA	63%	NA	NA
Mink Liver	W2491-34,D1030 USFWS-MS	NR			NR		
	W2491-35,D1146 USFWS-MS	NR		NA	NR		NA
	W2491-36,D1158 USFWS-MS	NR	NR	NA	NR	NR	NA
River Otter Liver	W2491-29,RAG 066-MS	NR			NR		
	W2491-30,RAG 028-MS	NR			NR		
	W2491-31,RAG 148-MS	48%			47%		
	W2491-32,RAG 230-MS	38%		15%	34%		26%
	W2491-33,RAG 237-MS	37%	41%	6%	29%	36%	9%
Sea Otter Liver	W2491-16,SO 12593-001-MS	42%			11%		
	W2491-19,SO 11494-001-MS	61%			50%		
	W2491-22,SO 11940-001-MS	44%			35%		
	W2491-24,SO 11309-001-MS	27%			16%		
	W2491-25,SO 12797-001-MS	34%			22%		
	W2491-26,SO 13110-001-MS	69%			44%		
	W2491-27,SO 12679-001-MS	36%		33%	26%		45%
	W2491-28,SO 12707-001-MS	65%	47%	16%	33%	30%	13%
	W2491-37, Male Turtle (-2,8)-MS	37%			42%		
Turtle Liver	W2491-38, Male Turtle (2,12)-MS	35%		20%	51%		15%
	W2491-39, Female Turtle (-3,9)-MS	51%	41%	8%	39%	44%	6%
Sea Otter Brain	W2491-18,SO 12593-001-MS	42%		7%	-3%		171%
	W2491-21,SO 11494-001-MS	46%	44%	3%	27%	12%	21%
Sea Otter Kidney	W2491-17,SO 12593-001-MS	61%			66%		
	W2491-20,SO 11494-001-MS	75%		49%	57%		61%
	W2491-23,SO 11940-001-MS	24%	53%	26%	14%	46%	28%
Whole Blood	W2491-54,Cormorant DCCO L Charity-MS	63%			65%		
	W2491-55,Cormorant DCCO Hym Island, Lake Sup-MS	69%		34%	64%		29%
	W2491-56,Otter DCCO Great Lakes-MS	115%	82%	28%	104%	77%	23%

PFDS/PFHS = no curve analyzed, PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 NR = Not reported, appears the spike wasn't detectable from endogenous levels.

LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 NA = Not Applicable
 NS = Not spiked

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

Date Entered/Analyst: 02/16/00, 02/17/00 LAC
 Date Verified/Analyst: 20/22/00 MEE

000021

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN021 Various Matrices from MSU
 None
 Various Matrices
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.2, 3.3
 08/25/99 MCH/KK/SAL/SEE
 08/28/99, 12/09/99 IAS
 08/30/99, 12/10/99 IAS

Sample Data

LIVER/WHOLE BLOOD

QC

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev.	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev.
Method Blk	H2O Blk-1 8/25/99	NA			NS		
	H2O Blk-2 8/25/99	NA			NS		
	H2O Blk-3 8/25/99	NA		NA	NS		NA
	H2O Blk-4 8/25/99	NA	NA	NA	NS	NS	NA
California Sea Lion Liver	W2491-3,CSL 3448-MS	109%			NS		
	W2491-4,CSL 3395-MS	85%			NS		
	W2491-6,CSL 3020-MS	68%			NS		
	W2491-7,CSL 2169-MS	45%			NS		
	W2491-9,CSL 2839-MS	20%		47%	NS		NA
	W2491-10,CSL 2367-MS	64%	65%	31%	NS	NS	NA
Elephant Seal Liver	W2491-2,ES 1500-MS	100%			NS		
	W2491-5,ES 1552-MS	80%			NS		
	W2491-11,ES 808-MS	44%			NS		
	W2491-12,ES 772-MS	31%		52%	NS		NA
	W2491-14,ES 782-MS	36%	58%	30%	NS	NS	NA
Harbor Seal Liver	W2491-8,HS-MS	48%			NS		
	W2491-13,HS 1191-MS	94%		37%	NS		NA
	W2491-15,HS 1199-MS	58%	67%	25%	NS	NS	NA
Gozzi Liver	W2491-1,NFS 100-MS	91%	NA	NA	NS	NS	NA
Mink Liver	W2491-34,D1030 USFWS-MS	NR			NS		
	W2491-35,D1146 USFWS-MS	NR		NA	NS		NA
	W2491-36,D1158 USFWS-MS	NR	NR	NA	NS	NS	NA
River Otter Liver	W2491-29,RAG 066-MS	21%			NS		
	W2491-30,RAG 028-MS	46%			NS		
	W2491-31,RAG 148-MS	69%			NS		
	W2491-32,RAG 230-MS	52%		39%	NS		NA
	W2491-33,RAG 237-MS	70%	52%	20%	NS	NS	NA
Sea Otter Liver	W2491-16,SO 12593-001-MS	47%			NS		
	W2491-19,SO 11494-001-MS	64%			NS		
	W2491-22,SO 11940-001-MS	47%			NS		
	W2491-24,SO 11309-001-MS	22%			NS		
	W2491-25,SO 12797-001-MS	30%			NS		
	W2491-26,SO 13110-001-MS	83%			NS		
	W2491-27,SO 12679-001-MS	44%		39%	NS		NA
	W2491-28,SO 12707-001-MS	64%	50%	20%	NS	NS	NA
Turtle Liver	W2491-37, Male Turtle (-2,8)-MS	48%			NS		
	W2491-38, Male Turtle (2,12)-MS	71%		29%	NS		NA
	W2491-39, Female Turtle (-3,9)-MS	42%	54%	15%	NS	NS	NA
Sea Otter Brain	W2491-18,SO 12593-001-MS	41%		13%	NS		NA
	W2491-21,SO 11494-001-MS	50%	46%	6%	NS	NS	NA
Sea Otter Kidney	W2491-17,SO 12593-001-MS	94%			NS		
	W2491-20,SO 11494-001-MS	95%		75%	NS		NA
	W2491-23,SO 11940-001-MS	9%	66%	49%	NS	NS	NA
Whole Blood	W2491-54,Cormorant DCCO L Charity-MS	118%			NS		
	W2491-55,Cormorant DCCO Hymn Island, Lake Sup-MS	122%		2%	NS		NA
	W2491-56,Otter DCCO Great Lakes-MS	123%	121%	3%	NS	NS	NA

PFDS/PFHS = no curve analyzed, PFHS based on PFOS response.
 No PFOS qualitative confirmation performed. Identifications are preliminary.
 NR = Not reported, appears the spike wasn't detectable from endogenous levels.

LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 NA = Not Applicable
 NS = Not spiked
 PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 POAA = Perfluorooctanoate
 PFHS = Perfluorohexanesulfonate

Date Entered/Analyst: 02/16/00, 02/17/00 LAC
 Date Verified/Analyst: 20/22/00 MEE

000022

Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y-Intercept:
 Dates of Extraction/Analyst:
 Dates of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN024 MSU Environmental Samples
 Various
 Various
 ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Amelia 062498
 Masslynx 3.3
 See Attachments
 See Attachments
 See Attachments
 See Attachments
 10/12/99 SAL/KK
 10/15/99, 10/19/99 IAS/MMH, 12/13/99 IAS
 10/18/99, 10/20/99 HOJ, 12/14/99 MMH

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SERA

Group Dose	Sample #	Concentration of POAA ug/mL or % Rec	Mean POAA ug/mL	RSD Std. Dev. RPD-MS/MSD	Concentration of PFOA ug/mL or % Rec	Mean PFOA ug/mL	RSD Std. Dev. RPD-MS/MSD
Method Blk	Bird10129-WBlk-5-1	<LOQ (0.0299 ug/mL)	<LOQ	<LOQ	<LOQ (0.00625 ug/mL)	<LOQ	<LOQ
Matrix Blk	NE	NE	NE	NE	NE	NE	NE
MS/MSD 250 ppb	Bird 040-Alb sera-MS-250 ppb-5-1	89%			70%		
	Bird 040-Alb sera-MSD-250 ppb-5-1	105%	97%	17%	80%	75%	14%
	Bird 060-E plasma-MS-250 ppb-5-2	77%			59%		
	Bird 060-E plasma-MSD-250 ppb-5-2	84%	80%	9%	65%	62%	9%
	Bird 054-C blood-MS-250 ppb-5	90%			77%		
	Bird 054-C blood-MSD-250 ppb-5	23%	56%	119%	14%	45%	140%
Albatross	Bird 034-Albatross Chick sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 035-Albatross plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 036-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 037-Albatross plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 038-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 039-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 040-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 041-Albatross sera	<LOQ (0.0299 ug/mL)	Plasma	NA	<LOQ (0.00625 ug/mL)	Plasma	NA
	Bird 042-Albatross sera	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ	NA
	Bird 043-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 044-Albatross sera	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 045-Albatross sera	<LOQ (0.0299 ug/mL)	Sera	NA	<LOQ (0.00625 ug/mL)	Sera	NA
	Bird 046-Albatross plasma	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ	NA
Comorant	Bird 047-Comorant plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 048-Comorant plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 049-Comorant plasma	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ	NA
	Bird 050-Comorant blood	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 051-Comorant blood	<LOQ (0.0299 ug/mL)			0.0145		
	Bird 052-Comorant blood	<LOQ (0.0299 ug/mL)			0.00871		
	Bird 053-Comorant blood	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 054-Comorant blood	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 055-Comorant blood	0.0489	<LOQ - 1 Outlier	NA	0.0426	0.0219 - 3 Outliers	82.7 0.0181
Herring	Bird 056-Herring Gull plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 057-Herring Gull plasma	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ	NA
	Bird 058-Herring Gull blood	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 059-Herring Gull blood	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ	NA
Bald Eagle	Bird 060-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 061-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 062-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 063-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 064-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 065-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 066-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 067-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 068-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			0.0751		
	Bird 069-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			0.0996		
	Bird 070-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 071-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 072-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 073-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 074-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 075-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 076-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 077-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 078-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 079-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 080-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 081-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 082-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 083-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 084-Bald Eagle plasma	<LOQ (0.0299 ug/mL)			<LOQ (0.00625 ug/mL)		
	Bird 085-Bald Eagle plasma	<LOQ (0.0299 ug/mL)	<LOQ	NA	<LOQ (0.00625 ug/mL)	<LOQ - 2 Outliers	NA

No PFOS qualitative confirmation performed. Identifications are preliminary.
 * Appears to not have been spiked. LAC 10/19/99

NE = Not extracted
 NA = Not Applicable
 NS = Not Spiked
 LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 RPD = Relative Percent Difference

PFOS = Perfluorooctanesulfonate
 PFHS = Perfluorohexanesulfonate
 POAA = Perfluorooctanoate
 PFOA = Perfluorooctanesulfonamide

Date Entered/By: 10/18/99, 10/20/99 LAC 12/30/99 MMH
 Date Verified/ By: 2/17/00 mmm

000024

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN024 MSU Environmental Samples
 Various
 Various
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.3
 10/12/99 SAL/KK
 10/14/99, 12/13/99 HOJ/IAS
 10/15/99, 11/15/99, 12/14/99 HOJ/MMH

Sample Data

LIVER, KIDNEY, YOLK

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD	
Method Blk	Bird10129-wblk-5-1 Bird10129-wblk-6-1	<LOQ (0.0348 ug/g) <LOQ (0.0348 ug/g)	<LOQ	NA	<LOQ (0.0683 ug/g) <LOQ (0.0683 ug/g)	<LOQ	NA	
QC 250 ng/g	Bird001-Loon Lvr-MS	28%			54%			
	Bird001-Loon Lvr-MSD	28%	28%	3%	65%	60%	18%	
	Bird023-Albatross Kdny-MS	71%			63%			
	Bird023-Albatross Kdny-MSD	87%	79%	20%	77%	70%	20%	
	Bird030-Cormorant Yolk-MS Bird030-Cormorant Yolk-MSD	121% 134%	128%	10%	71% 75%	73%	5%	
Liver	Bird001-Loon	0.345			<LOQ (0.0683 ug/g)			
	Bird002-Loon	0.689			<LOQ (0.0683 ug/g)			
	Bird003-Loon	0.185			<LOQ (0.0683 ug/g)			
	Bird004-Loon	0.199			<LOQ (0.0683 ug/g)			
	Bird005-Loon	0.202			<LOQ (0.0683 ug/g)			
	Bird006-Loon	0.105			<LOQ (0.0683 ug/g)			
	Bird007-Loon	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird008-Loon	<LOQ (0.0348 ug/g)	0.287 - 2 Outliers		73.6 0.212	<LOQ (0.0683 ug/g)	<LOQ	NA
Liver	Bird009-Brown Pelican	0.0460			103			
	Bird010-Brown Pelican	0.294	0.170	0.175	<LOQ (0.0683 ug/g)	<LOQ	NA	
Liver	Bird011-Albatross	0.617			<LOQ (0.0683 ug/g)			
	Bird012-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird013-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird014-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird015-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird016-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird020-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird021-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird022-Albatross	<LOQ (0.0348 ug/g)	<LOQ - 1 Outlier		NA	<LOQ (0.0683 ug/g)	<LOQ	NA
	Kidney	Bird017-Albatross	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)		
Bird018-Albatross		<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
Bird019-Albatross		<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
Bird023-Albatross		<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
Bird024-Albatross		<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
Bird025-Albatross		<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
Yolk	Bird026-Albatross	<LOQ (0.0348 ug/g)	<LOQ	NA	<LOQ (0.0683 ug/g)	<LOQ	NA	
	Bird027-Comorant	<LOQ (0.0348 ug/g)			<LOQ (0.0683 ug/g)			
	Bird028-Comorant	0.134			<LOQ (0.0683 ug/g)			
	Bird029-Comorant	0.317			<LOQ (0.0683 ug/g)			
Yolk	Bird030-Comorant	0.254	0.235 - 1 Outlier	0.0930	<LOQ (0.0683 ug/g)	<LOQ	NA	
	Bird031-Gull	0.146			<LOQ (0.0683 ug/g)			
	Bird032-Gull Bird033-Gull	<LOQ (0.0348 ug/g) 0.0541	0.0999 - 1 Outlier		64.8 0.0648	<LOQ (0.0683 ug/g)	<LOQ	NA

No PFOS qualitative confirmation performed. Identifications are preliminary.
 LOQ = Limit of Quantitation
 RSD = Relative Standard Deviation
 RPD = Relative Percent Difference
 Date Entered/Analyst: 10/23/99 LAC, 12/1/99 GML, 12/30/99 MMH
 Date Verified/Analyst: 2/17/00 MMH

NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 PFHS = Perfluorohexanesulfonate
 POAA = Perfluorooctanoate
 PFOA = Perfluorooctane sulfonamide

000025

Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN024 MSU Environmental Samples
 Various
 Various
 ETS-8-6.0 & ETS-8-7.0 using unextracted curves
 Amelia 062498
 Masslynx 3.3
 10/12/99 SAL/KK
 10/14/99, 12/13/99 HOJ/IAS
 10/15/99, 11/15/99, 12/14/99 HOJ/MMH

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Sample Data

LIVER, KIDNEY, YOLK

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	Bird10129-wblk-5-1 Bird10129-wblk-6-1	<LOQ (0.180 ug/g) <LOQ (0.180 ug/g)	<LOQ	NA	<LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	<LOQ	NA
QC 250 ng/g	Bird001-Loon Lvr-MS Bird001-Loon Lvr-MSD Bird023-Albatross Kdny-MS Bird023-Albatross Kdny-MSD Bird030-Cormorant Yolk-MS Bird030-Cormorant Yolk-MSD	101% 108% 113% 121% 105% 130%	104% 117% 117% 117%	6% 7% 22%	53% 51% 56% 58% 62% 72%	52% 67%	4% 5% 15%
Liver	Bird001-Loon Bird002-Loon Bird003-Loon Bird004-Loon Bird005-Loon Bird006-Loon Bird007-Loon Bird008-Loon	<LOQ (0.180 ug/g) <LOQ (0.180 ug/g)	<LOQ	NA NA	0.0153 <LOQ (0.00750 ug/g) 0.0147 0.0262 0.0213 0.0242 <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	0.0204 - 3 Outliers	25.5 0.00520
Liver	Bird009-Brown Pelican Bird010-Brown Pelican	<LOQ (0.180 ug/g) <LOQ (0.180 ug/g)	<LOQ	NA NA	<LOQ (0.00750 ug/g) 0.178	0.178 - 1 Outlier	NA
Liver	Bird011-Albatross Bird012-Albatross Bird013-Albatross Bird014-Albatross Bird015-Albatross Bird016-Albatross Bird020-Albatross Bird021-Albatross Bird022-Albatross	<LOQ (0.180 ug/g) <LOQ (0.180 ug/g) 0.182	<LOQ - 1 Outlier	NA NA	0.527 <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	<LOQ	NA
Kidney	Bird017-Albatross Bird018-Albatross Bird019-Albatross Bird023-Albatross Bird024-Albatross Bird025-Albatross Bird026-Albatross	<LOQ (0.180 ug/g) <LOQ (0.180 ug/g) <LOQ (0.180 ug/g) <LOQ (0.180 ug/g) <LOQ (0.180 ug/g) <LOQ (0.180 ug/g) <LOQ (0.180 ug/g)	<LOQ	NA NA	<LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	<LOQ	NA
Yolk	Bird027-Cormorant Bird028-Cormorant Bird029-Cormorant Bird030-Cormorant	<LOQ (0.180 ug/g) 0.245 <LOQ (0.180 ug/g) 0.192	0.218 - 2 outliers	17.2 0.0374	<LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	<LOQ	NA
Yolk	Bird031-Gull Bird032-Gull Bird033-Gull	0.197 <LOQ (0.180 ug/g) 0.196	0.196 - 1 Outlier	0.528 0.00104	<LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g) <LOQ (0.00750 ug/g)	<LOQ	NA

No PFOS qualitative confirmation performed. Identifications are preliminary.

LOQ = Limit of Quantitation

RSD = Relative Standard Deviation

RPD = Relative Percent Difference

Date Entered/Analyst: 10/23/99 LAC, 12/1/99 GML, 12/30/99 MMH

Date Verified/Analyst: 2/17/00 MMH

NA = Not Applicable

PFOS = Perfluorooctanesulfonate

PFHS = Perfluorohexanesulfonate

POAA = Perfluorooctanoate

PFOSA = Perfluorooctane sulfonamide

000026

FACT-GEN-030

Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y-Intercept:
 Dates of Extraction/Analyst:
 Dates of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
 Various
 Various Blood
 ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Soup020199
 Masslynx 3.3
 See Attachments
 See Attachments
 See Attachments
 See Attachments
 12/14/99 SAL/SRP/KK
 01/06/00, 01/07/00 MMH/IAS
 01/07/00, 01/10/00 IAS/MMH

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Sample Data
BLOOD

Group Dose	Sample #	Concentration of PFOS ug/mL or % Rec.	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/mL or % Rec.	Mean PFHS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	MSU12129-H2Obik unfiltered 5-3 MSU12129-H2Obik filtered 5-4	<LOQ (0.00290 ug/mL) <LOQ (0.00290 ug/mL)	<LOQ	NA NA	<LOQ (0.00114 ug/mL) <LOQ (0.00114 ug/mL)	<LOQ	NA NA
Matrix Blk	HMB12129-blood blk-5-1 HMB12129-blood blk-5-2 HMB12129-blood blk-5-3 HMB12129-blood blk-5-4 HMB12129-blood blk-5-5 HMB12129-blood blk-5-6* HMB12129-blood blk-5-7 HMB12129-blood blk-5-8 HMB12129-blood blk-5-9	0.0253 0.0248 0.0262 0.0243 <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL)	<LOQ - 4 outliers	NA 0.000790	<LOQ (0.00114 ug/mL) <LOQ (0.00114 ug/mL)	<LOQ	NA NA NA NA NA NA NA NA NA
QC	PBB-6255-250MS-5-1-2 PBB-6255-250MSD-5-1-2 FSB-S009-250ppb MS-5-1-2 FSB-S009-250ppb MSD-5-1-2 SSB-SSL49-250ppb MS-5-1-2 SSB-SSL49-250ppb MSD-5-1-2 HMB-FE52189-250 MS-5-1-2 HMB-FE52189-250 MSD-5-1-2	74% 91% -6% -5% 77% 65% -1% 77%	82% -6% -6% 71% 38%	21% 25% 17% 208%	76% 77% -1% -1% 83% 77% 1% 74%	76% 80%	2% 20% 9% 196%
Blood Northern Fur Seal Pups	P205 P206 P207 P208 P209 P210 P211 P212 P215 P217 P219 P220 P221 P222 P223 P224 P226 P229 P230	<LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL)	<LOQ	NA NA	<LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL)	<LOQ	NA NA
Blood Northern Fur Seal Adult Females	M104 M105 M106 M107 M112 M115 M116 M118 M119 M122	<LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL)	<LOQ	NA NA	<LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL)	<LOQ	NA NA
Blood Northern Fur Seal Subadult Males	S001 S002 S003 S006 S007 S008 S009	<LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL)	<LOQ	NA NA	<LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL)	<LOQ	NA NA
Blood Northern Fur Seal	P298B P236A 368C P406 P411 98 CUKB 3* 98 CUKB 7 98 CUKB 9	<LOQ (0.00579 ug/mL) <LOQ (0.00579 ug/mL)	<LOQ	NA NA	<LOQ (0.0114 ug/mL) <LOQ (0.0114 ug/mL)	<LOQ	NA NA

* Surrogate >50% deviation, not confirmed

I = May need to rerun all samples for PFOSA, interferent present in both analyses.

** PFOS NOT confirmed; MS transitions variation > 30%

Date Entered/By: 01/21/00 LAC

NE = Not Extracted
 E = Lost during extraction
 NA = Not Applicable
 LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
 PFOSA = Pefluorooctane sulfonamide
 PFHS = Perfluorohexanesulfonate
 POAA = Perfluorooctanoate

000027

FACT-GEN-030

Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y-Intercept:
 Dates of Extraction/Analyst:
 Dates of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
 Various
 Various Blood
 ETS-8-4.1 & ETS-8-5.1 using unextracted curves
 Soup020199
 Masslynx 3.3
 See Attachments
 See Attachments
 See Attachments
 See Attachments
 12/14/99 SAL/SRP/KK
 01/06/00, 01/07/00 MMH/LAS
 01/07/00, 01/10/00 IAS/MMH

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Sample Data
BLOOD

Group Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/mL or % Rec.	Mean PFOSA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	MSU12129-H2Oblk unfiltered 5-3 MSU12129-H2Oblk filtered 5-4	<LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL)	<LOQ	NA NA	<LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL)	<LOQ	NA NA
Matrix Blk	HMB12129-blood blk-5-1 HMB12129-blood blk-5-2 HMB12129-blood blk-5-3 HMB12129-blood blk-5-4 HMB12129-blood blk-5-5 HMB12129-blood blk-5-6* HMB12129-blood blk-5-7 HMB12129-blood blk-5-8 HMB12129-blood blk-5-9	<LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	0.00195 0.00190 0.00225 0.00189 I I I I I	I I I I I I I I I	NA NA NA NA NA NA NA NA NA
QC	PBB-6255-250MS-5-1-2 PBB-6255-250MSD-5-1-2 FSB-S009-250ppb MS-5-1-2 FSB-S009-250ppb MSD-5-1-2 SSB-SSL49-250ppb MS-5-1-2 SSB-SSL49-250ppb MSD-5-1-2 HMB-FE52189-250 MS-5-1-2 HMB-FE52189-250 MSD-5-1-2	76% 75% 0% 1% 76% 71% 1% 70%	76% 76% 0% 1% 74% 74% 35%	1% 1% 36% 7% 195%	50% 47% I I I I I I	49% 6% I I I I I I	6% NA NA NA NA NA NA NA
Blood Northern Fur Seal Pups	P205 P206 P207 P208 P209 P210 P211 P212 P215 P217 P219 P220 P221 P222 P223 P224 P226 P229 P230	<LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	I I I I I I I I I I I I I I I I I I I	I I I I I I I I I I I I I I I I I I I	NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA NA
Blood Northern Fur Seal Adult Females	M104 M105 M106 M107 M112 M115 M116 M118 M119 M122	<LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	I I I I I I I I I I	I I I I I I I I I I	NA NA NA NA NA NA NA NA NA NA
Blood Northern Fur Seal Subadult Males	S001 S002 S003 S006 S007 S008 S009	<LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	I I I I I I I	I I I I I I I	NA NA NA NA NA NA NA
Blood Northern Fur Seal NA2 EDTA	P298B P236A 368C P406 P411 98 CUKB 3* 98 CUKB 7 98 CUKB 9	<LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	I I I I I I I I	I I I I I I I I	NA NA NA NA NA NA NA NA

* Surrogate >50% deviation, not confirmed
 I = May need to rerun all samples for PFOSA, interferent present in both analyses.
 ** PFOS NOT confirmed; MS transitional variation > 30%
 Date Entered/By: 01/21/00 LAC

NE = Not Extracted
 E = Lost during extraction
 NA = Not Applicable
 LOQ = Limit of Quantitation
 PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctane sulfonamide
 PFHS = Perfluorohexanesulfonate
 POAA = Perfluorooctanoate

Study:
 Product Number (Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Filename:
 R-Squared Value:
 Slope:
 Y-Intercept:
 Dates of Extraction/Analyst:
 Dates of Analysis/Analyst:
 Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
 Various
 Various Blood
 ETS-8-4.1 & ETS-8-5.1 using unextracted-curves
 Soup020199
 Masslynx 3.3
 See Attachments
 See Attachments
 See Attachments
 See Attachments
 12/14/99 SAL/SRP/KKK
 01/06/00, 01/07/00 MMH/LAS
 01/07/00, 01/10/00 IAS/MMH

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Sample Data
BLOOD

Group Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/mL or % Rec.	Mean PFOSA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	MSU12129-H2Oblk unfiltered 5-3 MSU12129-H2Oblk filtered 5-4	<LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL)	<LOQ	NA NA	<LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL)	<LOQ	NA NA
Matrix Blk	HMB12129-blood blk-5-1 HMB12129-blood blk-5-2 HMB12129-blood blk-5-3 HMB12129-blood blk-5-4 HMB12129-blood blk-5-5 HMB12129-blood blk-5-6* HMB12129-blood blk-5-7 HMB12129-blood blk-5-8 HMB12129-blood blk-5-9	<LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)		NA NA	<LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL) I I I I I	I	NA NA
QC	PBB-6255-250MSD-5-1-2 PBB-6255-250MSD-5-1-2 FSB-S009-250ppb MS-5-1-2 FSB-S009-250ppb MSD-5-1-2 SSB-SSL49-250ppb MS-5-1-2 SSB-SSL49-250ppb MSD-5-1-2 HMB-FE52189-250 MS-5-1-2 HMB-FE52189-250 MSD-5-1-2	76% 75% 0% 1% 76% 71% 1% 70%	76%	1% 36% 7% 195%	50% 47% I I I I I I	49%	6% NA NA NA
Blood Polar Bear	6255 (Heparin) 20436 (NA2 EDTA) 20467 (NA2 EDTA) 20468 (EDTA) 20470 20472 20473* 20474 (NA2 EDTA) 20475 (NA2 EDTA) 20476 (NA2 EDTA) 20477 20485 (Heparin) 20486 (Heparin) 20487 (Heparin)	<LOQ (0.00240 ug/mL) <LOQ (0.00240 ug/mL)	<LOQ	NA NA	<LOQ (0.000625 ug/mL) <LOQ (0.000625 ug/mL)	<LOQ	NA NA
Blood Stellar Sealion	SSL49 (7.2 mg K2 EDTA) SSL50 (7.2 mg K2 EDTA) SSL51 (7.2 mg K2 EDTA) SSL52 (7.2 mg K2 EDTA) SSL53 (7.2 mg K2 EDTA) SSL54 (7.2 mg K2 EDTA) SSL55 (5.4 mg K2 EDTA) SSL56 (5.4 mg K2 EDTA)** SSL57 (5.4 mg K2 EDTA) SSL58 (5.4 mg K2 EDTA)* SSL59 (5.4 mg K2 EDTA) SSL60 (5.4 mg K2 EDTA)*	<LOQ (0.00958 ug/mL) <LOQ (0.00958 ug/mL)	<LOQ	NA NA	I I I I I I I I I I I I	I	NA NA

I = May need to rerun all samples for PFOSA, interferent present in both analyses
 ** Surrogate >50% deviation confirmed
 * Surrogate >50% deviation, not confirmed
 ** PFOS NOT confirmed; MS transitions variation > 30%
 Date Entered/By: 1/13/00, 01/21/00 MMH/LAC
 Date Verified/ By: 02/10/00 kjh

NE = Not Extracted
 E = Lost during extraction
 NA = Not Applicable
 NV = Not Verified
 LOQ = Limit of quantitation

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctane sulfonamide
 PFHS = Perfluorohexanesulfonate
 POAA = Perfluorooctanoate

000030

FACT-GEN-030
MSU Environmental Samples

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Study: GEN030 MSU Environmental Samples
 Product Number(Test Substance): Various
 Matrix: Various
 Method/Revision: ETS-8-6.0 & ETS-8-7.0
 Analytical Equipment System Number: Soup 020199, Amelia 062498
 Instrument Software/Version: Masslynx 3.3
 Date of Extraction/Analyst: 12/12/99 SAL/KK/SRP/CSH
 Date of Analysis/Analyst: 01/10/00 MMH
 Date of Data Reduction/Analyst: 01/12/00 IAS
Sample Data

MUSCLE

Group Dose	Sample #	PFOS Verified	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12149-H2Ounfil 5-1*	NA	0.0163		NA	<LOQ (0.0342)		NA
	MSU12149-H2Ounfil 5-2*	NA	<LOQ (0.00696)	NA	NA	<LOQ (0.0342)	<LOQ	NA
Method Blk Filtered water	MSU12149-H2Ofil 5-1*	NA	<LOQ (0.00696)		NA	<LOQ (0.0342)		NA
	MSU12149-H2Ofil 5-2*	NA	0.0257	NA	NA	<LOQ (0.0342)	<LOQ	NA
Matrix Blk Fish Liver	MSU12149-Fishblk 5-1	NA	0.00948			<LOQ (0.0342)		NA
	MSU12149-Fishblk 5-2	NA	0.00985		37.2	<LOQ (0.0342)		NA
	MSU12149-Fishblk 5-3*	NA	0.00454	0.00796	0.00296	<LOQ (0.0342)	<LOQ	NA
QC 250 ng/g	CPM-B1N7-MS 5-1-2	NA	59%			60%		
	CPM-B2N6-MS 5-1-2	NA	31%	45%	63%	45%	53%	28%
	CSM-1999030-03-01-MS 5-1-2	NA	160%			148%		
	CSM-1999030-03-01-MSD 5-1-2	NA	127%	143%	23%	183%	166%	21%
	LWM-1999029-16-MS 5-1-2	NA	67%			129%		
	LWM-1999029-11-MS 5-1-3	NA	122%	95%	59%	130%	130%	0%
BTM-1999040-09-MS 5-1-2	BTM-1999040-09-MS 5-1-2	NA	148%			144%		
	BTM-1999040-10-MS 5-1-2	NA	166%	157%	11%	168%	156%	15%
Muscle Carp	B1N1	X	0.101			<LOQ (0.0342)		
	B1N2	X	0.0784			<LOQ (0.0342)		
	B1N7	X	0.0905			<LOQ (0.0342)		
	B1N10	X	0.0878			<LOQ (0.0342)		
	B2N2	X	0.105			<LOQ (0.0342)		
	B2N6	X	0.0894			<LOQ (0.0342)		
	B2N8	X	0.0596			<LOQ (0.0342)		
	B2N10	X	0.0838			<LOQ (0.0342)		
	Carp1*	X	0.297			<LOQ (0.0342)		NA
	Carp2*	X	0.243	0.124		64.2 0.0793	<LOQ (0.0342)	<LOQ
Muscle Chinook Salmon	1999030-01	NA	0.189			<LOQ (0.0342)		
	1999030-02	NA	0.126			<LOQ (0.0342)		
	1999030-02-01	X	<LOQ (0.00696)			<LOQ (0.0342)		
	1999030-02-04	X	0.113			<LOQ (0.0342)		
	1999030-03-01	X	0.0514		0.524	<LOQ (0.0342)		NA
	1999030-03-04	X	0.0573	0.107 - 1 outlier	0.0562	<LOQ (0.0342)	<LOQ	NA
Muscle Lake Whitefish	1999029-11*	X	0.168			<LOQ (0.0342)		
	1999029-12*	X	0.130			<LOQ (0.0342)		
	1999029-13	X	0.0967			<LOQ (0.0342)		
	1999029-14	X	0.0983		26.4	<LOQ (0.0342)		NA
	1999029-16	X	0.1659	0.132	0.0348	<LOQ (0.0342)	<LOQ	NA
Muscle Brown Trout	1999040-01	X	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-02 **	X **	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-03	NA	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-04	NA	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-05	X	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-06	X	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-07	NA	<LOQ (0.00696)			<LOQ (0.0342)		
	1999040-08	X	0.0460			<LOQ (0.0342)		
	1999040-09	NA	<LOQ (0.00696)		NA	<LOQ (0.0342)		NA
	1999040-10	NA	<LOQ (0.00696)	<LOQ - 1 outlier	NA	<LOQ (0.0342)	<LOQ	NA

* High (>50%) surrogate deviations

** PFOS NOT confirmed; MS transitions variation > 30%

Date Entered/Analyst: 01/24/00, 01/25/00, 01/28/00 LAC

Date Verified/Analyst: 02/10/00 kjh

NE = Not Extracted

E = Lost during extraction

NA = Not Applicable

LOQ = Limit of Quantitation

X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctane sulfonamide

PFHS = Perfluorohexanesulfonate

POAA = Perfluorooctanoate

000031

FACT-GEN-030
MSU Environmental Samples

Study: GEN030 MSU Environmental Samples
 Product Number(Test Substance): Various
 Matrix: Various
 Method/Revision: ETS-8-6.0 & ETS-8-7.0
 Analytical Equipment System Number: Soup 020199, Amelia 062498
 Instrument Software/Version: Masslynx 3.3
 Date of Extraction/Analyst: 12/12/99 SAL/KK/SRP/CSH
 Date of Analysis/Analyst: 01/10/00 MMH
 Date of Data Reduction/Analyst: 01/12/00 IAS

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Sample Data

MUSCLE

Group Desc	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12149-H2Ounfil 5-1*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	MSU12149-H2Ounfil 5-2*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Method Blk Filtered water	MSU12149-H2Ofil 5-1*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	MSU12149-H2Ofil 5-2*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Fish Liver	MSU12149-Fishblk 5-1	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	MSU12149-Fishblk 5-2	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	MSU12149-Fishblk 5-3*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
QC 250 ng/g	CPM-B1N7-MS 5-1-2	6%	23%	148%	11%	12%	19%
	CPM-B2N6-MS 5-1-2	39%			13%		
	CSM-1999030-03-01-MS 5-1-2	139%			77%		
	CSM-1999030-03-01-MSD 5-1-2	178%	158%	25%	69%	73%	11%
	LWM-1999029-16-MS 5-1-2	146%			61%		
	LWM-1999029-11-MS 5-1-3	105%	125%	33%	76%	68%	22%
BTM-1999040-09-MS 5-1-2		147%			80%		
	BTM-1999040-10-MS 5-1-2	152%	150%	3%	87%	83%	8%
Muscle Carp	B1N1	<LOQ (0.0359)			<LOQ (0.0188)		
	B1N2	<LOQ (0.0359)			<LOQ (0.0188)		
	B1N7	<LOQ (0.0359)			<LOQ (0.0188)		
	B1N10	<LOQ (0.0359)			<LOQ (0.0188)		
	B2N2	<LOQ (0.0359)			<LOQ (0.0188)		
	B2N6	<LOQ (0.0359)			<LOQ (0.0188)		
	B2N8	<LOQ (0.0359)			<LOQ (0.0188)		
	B2N10	<LOQ (0.0359)			<LOQ (0.0188)		
	Carp1*	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	Carp2*	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Muscle Chinook Salmon	1999030-01	<LOQ (0.0359)			<LOQ (0.0188)		
	1999030-02	<LOQ (0.0359)			<LOQ (0.0188)		
	1999030-02-01	<LOQ (0.0359)			<LOQ (0.0188)		
	1999030-02-04	<LOQ (0.0359)			<LOQ (0.0188)		
	1999030-03-01	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	1999030-03-04	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Muscle Lake Whitefish	1999029-11*	<LOQ (0.0359)			<LOQ (0.0188)		
	1999029-12*	<LOQ (0.0359)			<LOQ (0.0188)		
	1999029-13	<LOQ (0.0359)			<LOQ (0.0188)		
	1999029-14	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	1999029-16	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Muscle Brown Trout	1999040-01	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-02 **	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-03	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-04	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-05	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-06	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-07	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-08	<LOQ (0.0359)			<LOQ (0.0188)		
	1999040-09	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	1999040-10	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA

* High (>50%) surrogate deviations

** PFOS NOT confirmed; MS transitions variation > 30%

Date Entered/Analyst: 01/24/00, 01/25/00, 01/28/00 LAC

Date Verified/Analyst: 02/10/00 kjh

NE = Not Extracted

E = Lost during extraction

NA = Not Applicable

LOQ = Limit of Quantitation

X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctane sulfonamide

PFHS = Perfluorohexanesulfonate

POAA = Perfluorooctanoate

000032

FACT-GEN-030
MSU Environmental Samples

Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:
Sample Data

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRP/CSH
01/10/00 MMH
01/11/00 IAS

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MUSCLE and EGGS

Group Dose	Sample #	PFOS Verified	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Water	MSU12159-H2OBlk-unfiltered-5-9	NA	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
	MSU12159-H2OBlk-filtered-5-9	NA	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
Matrix Blk Egg	EggBlk-5-1	NA	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
	EggBlk-5-2	NA	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
	EggBlk-5-3	NA	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
QC 250 ng/g	FGW-ALSWCR-TD2 6/25/98-MS-5-1-2	X	44%			16%		
	FGW-ALSWCR-TD2 6/25/98-MSD-5-1-2	X	47%	46%	8%	16%	16%	3%
	FGE-AL118-HP26/25/98-MS-5-1-2	X	13%			10%		
	FGE-AL118-HP26/25/98-MSD-5-1-2	X	15%	14%	8%	12%	11%	13%
	LWE-1999029-13-MS-5-1-2	X	146%			30%		
	LWE-1999029-13-MSD-5-1-2	X	104%	125%	33%	35%	33%	16%
Carp	BTE-1999040-01-MS-5-1-2	X	121%			57%		
	BTE-1999040-01-MSD-5-1-2	X	167%	144%	32%	57%	57%	1%
Frog Muscle Wholebody	Diet 1 (Carp5) **	X **	<LOQ (0.0174)			<LOQ (0.0342)		
	Diet 2 (Carp6)	X	<LOQ (0.0174)			<LOQ (0.0342)		
	Diet 3 (Carp4)	X	0.0267		NA	<LOQ (0.0342)		NA
	Diet 4 (Carp3)	X	0.0278	0.0272 - 2 outliers	NA	<LOQ (0.0342)	<LOQ	NA
Green Frog Eggs	AL-118-YOY 08/26/98 **	X **	0.00243			<LOQ (0.0342)		
	ALSWCR-TD 06/03/98 **	X **	<LOQ (0.0174)			<LOQ (0.0342)		
	ALSWCR-TD2 06/25/99 **	X **	<LOQ (0.0174)			<LOQ (0.0342)		
	KZCKDM-JUV 08/26/98 **	X **	<LOQ (0.0174)			<LOQ (0.0342)		
	KZCKDM-TD 06/05/98 **	X **	0.0216			<LOQ (0.0342)		
	KZCKDM-TD-2 06/25/98 **	X **	<LOQ (0.0174)			<LOQ (0.0342)		
	SJ0002-TD-2 06/05/98 **	X **	<LOQ (0.0174)		NA	<LOQ (0.0342)		NA
SJ0002-TD-2 06/25/98 **	X **	<LOQ (0.0174)	<LOQ - 2 outliers	NA	<LOQ (0.0342)	<LOQ	NA	
Lake Whitefish Eggs	AL-118-HP89 06/25/98	X	<LOQ (0.0174)			<LOQ (0.0342)		
	AL-118-HP94 06/25/98	X	<LOQ (0.0174)			<LOQ (0.0342)		
	SJ0001 06/03/98 **	X **	<LOQ (0.0174)		NA	<LOQ (0.0342)		NA
	SJ0001 06/05/98	X	<LOQ (0.0174)	<LOQ	NA	<LOQ (0.0342)	<LOQ	NA
Brown Trout Eggs	1999029-13	X	0.145		63.5	<LOQ (0.0342)		NA
	1999029-14	X	0.381	0.263	0.167	<LOQ (0.0342)	<LOQ	NA
	1999040-01	X	0.0749			<LOQ (0.0342)		
	1999040-04	X	0.0675		21.1	<LOQ (0.0342)		NA
	1999040-06	X	0.0488	0.0637	0.0134	<LOQ (0.0342)	<LOQ	NA

* High (>50%) surrogate deviations
** PFOS NOT confirmed; MS transitions variation > 30%
Date Entered/Analyst: 01/21/00, 01/24/00 LAC
Date Verified/Analyst: 02/10/00 kjh

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration
PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

000033

FACT-GEN-030
MSU Environmental Samples

Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:
Sample Data

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRP/CSH
01/10/00 MMH
01/11/00 IAS

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MUSCLE and EGGS

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Water	MSU12159-H2OBik-unfiltered-5-9	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	MSU12159-H2OBik-filtered-5-9	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Egg	EggBlk-5-1	<LOQ (0.0359)			<LOQ (0.0188)		
	EggBlk-5-2	<LOQ (0.0359)		NA	<LOQ (0.0188)		NA
	EggBlk-5-3	<LOQ (0.0359)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
QC 250 ng/g	FGW-ALSWCR-TD2 6/25/98-MS-5-1-2	34%			49%		
	FGW-ALSWCR-TD2 6/25/98-MSD-5-1-2	33%	34%	4%	49%	49%	1%
	FGE-AL118-HP26/25/98-MS-5-1-2	22%			46%		
	FGE-AL118-HP26/25/98-MSD-5-1-2	26%	24%	15%	54%	50%	16%
	LWE-19999029-13-MS-5-1-2	90%			68%		
	LWE-19999029-13-MSD-5-1-2	83%	86%	9%	64%	66%	6%
Carp	BTE-19999040-01-MS-5-1-2	98%			104%		
	BTE-19999040-01-MSD-5-1-2	112%	105%	13%	108%	106%	4%
	Diet 1 (Carp5) ** Diet 2 (Carp6) Diet 3 (Carp4) Diet 4 (Carp3)	<LOQ (0.0359) <LOQ (0.0359) <LOQ (0.0359) <LOQ (0.0359)	<LOQ	NA NA NA	<LOQ (0.0188) <LOQ (0.0188) <LOQ (0.0188) <LOQ (0.0188)	<LOQ	NA NA NA
Frog Muscle Wholebody	AL-118-YOY 08/26/98 **	<LOQ (0.0180)			<LOQ (0.0188)		
	ALSWCR-TD 06/03/98 **	<LOQ (0.0180)			<LOQ (0.0188)		
	ALSWCR-TD2 06/25/99 **	<LOQ (0.0180)			<LOQ (0.0188)		
	KZCKDM-JUV 08/26/98 **	<LOQ (0.0180)			<LOQ (0.0188)		
	KZCKDM-TD 06/05/98 **	<LOQ (0.0180)			<LOQ (0.0188)		
	KZCKDM-TD-2 06/25/98 **	<LOQ (0.0180)			<LOQ (0.0188)		
	SJ0002-TD-2 06/05/98 **	<LOQ (0.0180)		NA	<LOQ (0.0188)		NA
	SJ0002-TD-2 06/25/98 **	<LOQ (0.0180)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Green Frog Eggs	AL-118-HP89 06/25/98	<LOQ (0.0180)			<LOQ (0.0188)		
	AL-118-HP94 06/25/98	<LOQ (0.0180)			<LOQ (0.0188)		
	SJ0001 06/03/98 **	<LOQ (0.0180)		NA	<LOQ (0.0188)		NA
	SJ0001 06/05/98	<LOQ (0.0180)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Lake Whitefish Eggs	1999029-13	<LOQ (0.0180)		NA	<LOQ (0.0188)		NA
	1999029-14	<LOQ (0.0180)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Brown Trout Eggs	1999040-01	<LOQ (0.0180)			<LOQ (0.0188)		
	1999040-04	<LOQ (0.0180)		NA	<LOQ (0.0188)		NA
	1999040-06	<LOQ (0.0180)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	1999040-06	<LOQ (0.0180)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA

* High (>50%) surrogate deviations

** PFOS NOT confirmed; MS transitions variation > 30%

Date Entered/Analyst: 01/21/00, 01/24/00 LAC

Date Verified/Analyst: 02/10/00 kjh

NE = Not Extracted

E = Lost during extraction

NA = Not Applicable

LOQ = Limit of Quantitation

X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctane sulfonamide

PFHS = Perfluorohexanesulfonate

POAA = Perfluorooctanoate

000034

FACT-GEN-030
MSU Environmental Samples

Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRP/CSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/06/00 IAS/MMH
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/07/00 MMH/IAS

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Sample Data

Miscellaneous Liver

Group Dose	Sample #	PFOS Verified	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12129-H2OBik-unfiltered-5-1	NA	NA			NA		
	MSU12129-H2OBik-unfiltered-5-2	NA	NA			NA		
	MSU12129-H2OBik-unfiltered-5-3	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	MSU12129-H2OBik-unfiltered-5-4	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	MSU12129-H2OBik-unfiltered-5-5	NA	E			E		
	MSU12129-H2OBik-unfiltered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	MSU12129-H2OBik-unfiltered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
	MSU12129-H2OBik-unfiltered-5-8	NA	<LOQ (0.0696)	<LOQ		<LOQ (0.0171)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBik-filtered-5-1	NA	NA			NA		
	MSU12129-H2OBik-filtered-5-2	NA	NA			NA		
	MSU12129-H2OBik-filtered-5-3	NA	E			E		
	MSU12129-H2OBik-filtered-5-4	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	MSU12129-H2OBik-filtered-5-5	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	MSU12129-H2OBik-filtered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	MSU12129-H2OBik-filtered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
	MSU12129-H2OBik-filtered-5-8	NA	<LOQ (0.0696)	<LOQ		<LOQ (0.0171)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvrBlk-5-1	NA	0.0305			<LOQ (0.0171)		
	FSH12129-LvrBlk-5-2	NA	0.0331		32.1	<LOQ (0.0171)		NA
	FSH12129-LvrBlk-5-3	NA	0.0170	0.0269	0.00862	<LOQ (0.0171)	<LOQ	NA
Matrix Blk Rabbit Liver	RBL12129-LvrBlk-5-1	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	RBL12129-LvrBlk-5-2	NA	<LOQ (0.0347)			<LOQ (0.00683)		
	RBL12129-LvrBlk-5-3	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	RBL12129-LvrBlk-5-4	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	RBL12129-LvrBlk-5-5*	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
QC 250 ng/g	Mink Liver, D530, MS-5-1-1	NA	145%			61%		
	Mink Liver, D530, MSD-5-1-2*	NA	538%	342%	115%	50%	55%	21%
	CSL-1999030-03-01-MS-5-1-2*	NA	196%			116%		
	CSL-1999030-03-01-MSD-5-1-2*	NA	140%	168%	33%	112%	114%	4%
	LWL-1999029-12-MS-5-1-2*	NA	321%			57%		
	LWL-1999029-12-MSD-5-1-2*	NA	287%	304%	11%	68%	63%	18%
	BTL-1999040-01-MS-5-1-2*	NA	138%			81%		
	BTL-1999040-01-MSD-5-1-2*	NA	132%	135%	5%	84%	83%	4%
	TNL-TU54-MS-5-1-2	NA	68%			55%		
	TNL-TU54-MSD-5-1-2	NA	E	68%		E	55%	NA
	FSL-P295-MS-5-1	NA	91%			97%		
	FSL-P295-MSD-5-1	NA	86%	88%	6%	84%	90%	15%
	PBL-980390LB-MS-5-1	NA	18%			70%		
	PBL-980390LB-MSD-5-2*	NA	179%	99%	163%	76%	73%	9%
	GFL-KZCKDM-D1-MS-5-1-2	NA	93%			82%		
	GFL-KZCKDM-D1-MSD-5-1-1	NA	105%	99%	12%	89%	86%	9%
TTL-LCPT99503C-MS-5-1-2*	NA	90%			66%			
TTL-LCPT99503C-MSD-5-1-2	NA	100%	95%	11%	72%	69%	8%	
MTL-10Vancleave98-MS-5-1-2	NA	79%			74%			
MTL-10Vancleave98-MSD-5-1-2	NA	89%	84%	11%	84%	79%	13%	
Liver Chinook Salmon	1999030-01	X	0.109			<LOQ (0.0171)		
	1999030-02	X	0.169			<LOQ (0.0171)		
	1999030-02-01	NA	0.0328			<LOQ (0.0171)		
	1999030-02-04	NA	0.126			<LOQ (0.0171)		
	1999030-03-01	NA	0.173		56.1	<LOQ (0.0171)		NA
	1999030-03-04	NA	0.0405	0.108	0.0608	<LOQ (0.0171)	<LOQ	NA
Liver Lake Whitefish	1999029-11	NA	0.0679			<LOQ (0.0171)		
	1999029-12	NA	0.0812			<LOQ (0.0171)		
	1999029-13	X	0.0738			<LOQ (0.0171)		
	1999029-14	X	0.0329		29.3	<LOQ (0.0171)		NA
	1999029-16	NA	0.0778	0.0667	0.0195	<LOQ (0.0171)	<LOQ	NA
Liver Brown Trout	1999040-01	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-02	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-03	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-04	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-05	NA	0.0255			<LOQ (0.0171)		
	1999040-06	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-07	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-08	NA	<LOQ (0.0174)			<LOQ (0.0171)		
	1999040-09	NA	<LOQ (0.0174)			<LOQ (0.0171)		NA
	1999040-10	NA	<LOQ (0.0174)	<LOQ - 1 outlier		NA	<LOQ (0.0171)	<LOQ

* High (>50%) surrogate deviations

Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99, 01/12/00, 01/17/00, 01/18/00 LAC
Date Verified/Analyst: 0

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

FACT-GEN-030
MSU Environmental Samples

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Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRP/CSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/06/00 IAS/MMH
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/07/00 MMH/IAS

Sample Data

Miscellaneous Liver

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12129-H2OBlk-unfiltered-5-1	NA			NA		
	MSU12129-H2OBlk-unfiltered-5-2	NA			NA		
	MSU12129-H2OBlk-unfiltered-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-5	E			E		
	MSU12129-H2OBlk-unfiltered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-7	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	MSU12129-H2OBlk-unfiltered-5-8	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBlk-filtered-5-1	NA			NA		
	MSU12129-H2OBlk-filtered-5-2	NA			NA		
	MSU12129-H2OBlk-filtered-5-3	E			E		
	MSU12129-H2OBlk-filtered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-5	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-7	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	MSU12129-H2OBlk-filtered-5-8	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	FSH12129-LvrBlk-5-2	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	FSH12129-LvrBlk-5-3	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Rabbit Liver	RBL12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-2	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-5*	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
QC 250 ng/g	Mink Liver, D530, MS-5-1-1	86%			43%		
	Mink Liver, D530, MSD-5-1-2*	84%	85%	3%	81%	62%	60%
	CSL-1999030-03-01-MS-5-1-2*	131%			64%		
	CSL-1999030-03-01-MSD-5-1-2*	111%	121%	17%	52%	58%	21%
	LWL-1999029-12-MS-5-1-2*	133%			77%		
	LWL-1999029-12-MSD-5-1-2*	145%	139%	9%	83%	80%	7%
	BTL-1999040-01-MS-5-1-2*	127%			94%		
	BTL-1999040-01-MSD-5-1-2*	120%	124%	6%	92%	93%	3%
	TNL-TU54-MS-5-1-2	84%			71%		
	TNL-TU54-MSD-5-1-2	E	84%	NA	E	71%	NA
	FSL-P295-MS-5-1	72%			87%		
	FSL-P295-MSD-5-1	80%	76%	10%	82%	85%	7%
	PBL-980390LB-MS-5-1	85%			71%		
	PBL-980390LB-MSD-5-2*	129%	107%	41%	65%	68%	9%
	GFL-KZCKDM-D1-MS-5-1-2	82%			82%		
	GFL-KZCKDM-D1-MSD-5-1-	103%	92%	23%	93%	88%	12%
TTL-LCPTR99503C-MS-5-1-2*	73%			78%			
TTL-LCPTR99503C-MSD-5-1-2	64%	69%	13%	71%	74%	10%	
MTL-10Vancleave98-MS-5-1-2	100%			74%			
MTL-10Vancleave98-MSD-5-1-2	75%	88%	29%	74%	74%	0%	
Liver Chinook Salmon	1999030-01	<LOQ (0.0719)			<LOQ (0.0188)		
	1999030-02	<LOQ (0.0719)			<LOQ (0.0188)		
	1999030-02-01	<LOQ (0.0719)			<LOQ (0.0188)		
	1999030-02-04	<LOQ (0.0719)			<LOQ (0.0188)		
	1999030-03-01	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	1999030-03-04	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Liver Lake Whitefish	1999029-11	<LOQ (0.0719)			<LOQ (0.0188)		
	1999029-12	<LOQ (0.0719)			<LOQ (0.0188)		
	1999029-13	<LOQ (0.0719)			<LOQ (0.0188)		
	1999029-14	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	1999029-16	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Liver Brown Trout	1999040-01	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-02	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-03	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-04	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-05	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-06	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-07	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-08	<LOQ (0.0719)			<LOQ (0.0188)		
	1999040-09	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	1999040-10	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA

* High (>50%) surrogate deviations

Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99,
01/12/00, 01/17/00, 01/18/00 LAC
Date Verified/Analyst: 0

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

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Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Maanlyx 3.3
12/12/99 SAL/KK/SRP/CSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/05/00, 01/06/00, 01/08/00 IAS/MMH
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/06/00, 01/07/00, 01/11/00 MMH/IAS

Miscellaneous Liver

Group	Sample #	PFOS Verified	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12129-H2OBik-unfiltered-5-1	NA	NA			NA		
	MSU12129-H2OBik-unfiltered-5-2	NA	NA			NA		
	MSU12129-H2OBik-unfiltered-5-3	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	MSU12129-H2OBik-unfiltered-5-4	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	MSU12129-H2OBik-unfiltered-5-5	NA	E			E		
	MSU12129-H2OBik-unfiltered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	MSU12129-H2OBik-unfiltered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
	MSU12129-H2OBik-unfiltered-5-8	NA	<LOQ (0.0696)	<LOQ		<LOQ (0.0171)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBik-filtered-5-1	NA	NA			NA		
	MSU12129-H2OBik-filtered-5-2	NA	NA			NA		
	MSU12129-H2OBik-filtered-5-3	NA	E			E		
	MSU12129-H2OBik-filtered-5-4	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	MSU12129-H2OBik-filtered-5-5	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	MSU12129-H2OBik-filtered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	MSU12129-H2OBik-filtered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
	MSU12129-H2OBik-filtered-5-8	NA	<LOQ (0.0696)	<LOQ		<LOQ (0.0171)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvBik-5-1	NA	0.0303			<LOQ (0.0171)		
	FSH12129-LvBik-5-2	NA	0.0331		32.1	<LOQ (0.0171)		NA
Matrix Blk Rabbit Liver	FSH12129-LvBik-5-3	NA	0.0170	0.0269	0.00862	<LOQ (0.0171)	<LOQ	NA
	RBL12129-LvBik-5-1	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	RBL12129-LvBik-5-2	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	RBL12129-LvBik-5-3	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	RBL12129-LvBik-5-4	NA	<LOQ (0.0696)			<LOQ (0.0171)		
	RBL12129-LvBik-5-5*	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA
QC 250 ng/g	RBL12129-LvBik-5-6*	NA	<LOQ (0.0696)	<LOQ	NA	<LOQ (0.0171)	<LOQ	NA
	Mink Liver, D530, MS-5-1-1	NA	145%			61%		
	Mink Liver, D530, MSD-5-1-2*	NA	538%	342%	115%	50%	55%	21%
	CSL-1999030-03-01-MS-5-1-2*	NA	258%			116%		
	CSL-1999030-03-01-MSD-5-1-2*	NA	202%	230%	24%	112%	114%	4%
	LWL-1999029-12-MS-5-1-2*	NA	351%			57%		
	LWL-1999029-12-MSD-5-1-2*	NA	317%	334%	10%	68%	63%	18%
	BTL-1999040-01-MS-5-1-2*	NA	138%			81%		
	BTL-1999040-01-MSD-5-1-2*	NA	132%	135%	5%	84%	83%	4%
	TNL-TU54-MS-5-1-2	NA	68%			55%		
	TNL-TU54-MSD-5-1-2	NA	E	68%	NA	E	55%	NA
	FSL-P295-MS-5-1	NA	111%			97%		
	FSL-P295-MSD-5-1	NA	106%	108%	5%	84%	90%	15%
	PBL-980390LB-MS-5-1	NA	77%			70%		
	PBL-980390LB-MSD-5-2*	NA	238%	158%	102%	76%	73%	9%
	GFL-KZCKDM-D1-MS-5-1-2	NA	101%			82%		
	GFL-KZCKDM-D1-MSD-5-1-2	NA	112%	107%	11%	89%	86%	9%
	TTL-LCPT99903C-MS-5-1-2*	NA	92%			66%		
	TTL-LCPT99903C-MSD-5-1-2	NA	102%	97%	10%	72%	69%	8%
	MTL-10Vanceave98-MS-5-1-2	NA	108%			74%		
MTL-10Vanceave98-MSD-5-1-2	NA	118%	113%	8%	84%	79%	13%	
Liver Northern Fur Seal	P283	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	P285**	X	0.123			<LOQ (0.0683)		
	P295	NA	0.0547			<LOQ (0.0683)		
	97 CU 02	NA	NE			NE		
	98 CU KB 02	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 03	NA	<LOQ (0.0347)			0.0587		
	98 CU KB 07	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 09	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 10	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 11	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 12	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 13	NA	<LOQ (0.0347)			<LOQ (0.0683)		
	98 CU KB 14	NA	<LOQ (0.0347)			<LOQ (0.0683)		NA
	98 CU KB 15	NA	<LOQ (0.0347)	<LOQ - 2 outliers		NA	<LOQ - 2 outliers	NA
	0.0858							
Liver Polar Bear	970012	X	0.456			<LOQ (0.0683)		
	970201	X	0.301			<LOQ (0.0683)		
	980341	X	0.678			<LOQ (0.0683)		
	692-PLBR-0033	X	0.471			<LOQ (0.0683)		
	980127LB	X	0.539			<LOQ (0.0683)		
	980387LB	X	0.221			<LOQ (0.0683)		
	980390LB**	X	0.209			<LOQ (0.0683)		
	980563LA	X	0.438			<LOQ (0.0683)		
	980565LB	X	0.328			<LOQ (0.0683)		
	990112LB	X	0.175			<LOQ (0.0683)		
	990592LA	X	0.313			<LOQ (0.0683)		
	990594LB	X	0.356			<LOQ (0.0683)		
	990598LB	X	0.436			<LOQ (0.0683)		
	990600LB	X	0.224			<LOQ (0.0683)		
	990610LB	X	0.295			<LOQ (0.0683)		
	990652LC	X	0.235			<LOQ (0.0683)		
	990658LB	X	0.282	0.350		<LOQ (0.0683)	<LOQ	NA
0.135								
Liver Mink	D0530	X	0.974			<LOQ (0.0171)		
	D0566*	X	2.68			<LOQ (0.0171)		
	D0590	X	2.38			<LOQ (0.0171)		
	D0618	X	0.974			<LOQ (0.0171)		
	D0630*	X	2.75			<LOQ (0.0171)		
	D0684*	X	3.50			<LOQ (0.0171)		
	D1000	X	3.42			<LOQ (0.0171)		
	D1024	X	2.13			<LOQ (0.0171)		
	D1030	X	3.28			<LOQ (0.0171)		
	D1092	X	3.22			<LOQ (0.0171)		
	D1110*	X	3.35			<LOQ (0.0171)		
	D1134	X	3.67			<LOQ (0.0171)		
	D1150X*	X	1.96			<LOQ (0.0171)		
	D1194	X	1.88			<LOQ (0.0171)		
	D1198	X	2.82			<LOQ (0.0171)		
	D1244	X	3.42			<LOQ (0.0171)		
	D1248	X	1.21			<LOQ (0.0171)		NA
	D1660*	X	3.68	2.63		35.0	<LOQ	NA
	0.919							

* High (>50%) surrogate deviations confirmed.
** positive analyte confirmation was not achieved, used 499 -> 99 transition.
Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99,
01/12/00, 01/17/00, 01/18/00, 01/19/00, 01/20/00 LAC
Date Verified/Analyst: 0

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

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Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:
Sample Data

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Maanlynx 3.3
12/17/99 SAL/KK/SRP/CSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/05/00, 01/06/00, 01/08/00 IAS/MMH
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/06/00, 01/07/00, 01/11/00 MMH/IAS

Miscellaneous Liver

Group Dose	Sample #	Concentration of FOAA ug/g or % Rec.	Mean FOAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12129-H2OBB-unfiltered-5-1	NA			NA		
	MSU12129-H2OBB-unfiltered-5-2	NA			NA		
	MSU12129-H2OBB-unfiltered-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-unfiltered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-unfiltered-5-5	E			E		
	MSU12129-H2OBB-unfiltered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-unfiltered-5-7	<LOQ (0.0719)			<LOQ (0.0188)		NA
	MSU12129-H2OBB-unfiltered-5-8	<LOQ (0.0719)	<LOQ		<LOQ (0.0188)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBB-filtered-5-1	NA			NA		
	MSU12129-H2OBB-filtered-5-2	NA			NA		
	MSU12129-H2OBB-filtered-5-3	E			E		
	MSU12129-H2OBB-filtered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-filtered-5-5	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-filtered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBB-filtered-5-7	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	MSU12129-H2OBB-filtered-5-8	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	FSH12129-LvrBlk-5-2	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	FSH12129-LvrBlk-5-3	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Rabbit Liver	RBL12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-2	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-5*	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	RBL12129-LvrBlk-5-6*	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
QC 250 ng/g	Mink Liver, D530, MS-5-1-1	86%			43%		
	Mink Liver, D530, MSD-5-1-2*	84%	85%	3%	81%	62%	60%
	CSL-1999030-03-01-MS-5-1-2*	131%			53%		
	CSL-1999030-03-01-MSD-5-1-2*	111%	121%	17%	41%	47%	26%
	LWL-1999029-12-MS-5-1-2*	133%			67%		
	LWL-1999029-12-MSD-5-1-2*	145%	139%	9%	73%	70%	8%
	BTL-1999040-01-MS-5-1-2*	127%			83%		
	BTL-1999040-01-MSD-5-1-2*	120%	124%	6%	81%	82%	3%
	TNL-TU54-MS-5-1-2	84%			59%		
	TNL-TU54-MSD-5-1-2	E	84%	NA	E	59%	NA
	FSL-P295-MS-5-1	72%			76%		
	FSL-P295-MSD-5-1	80%	76%	10%	71%	73%	8%
	PBL-980390LB-MS-5-1	85%			60%		
	PBL-980390LB-MSD-5-2*	129%	107%	41%	54%	57%	11%
	GFL-KZCKDM-D1-MS-5-1-2	82%			71%		
GFL-KZCKDM-D1-MSD-5-1-2	103%	92%	23%	82%	77%	14%	
TTL-LCPT99503C-MS-5-1-2*	73%			67%			
TTL-LCPT99503C-MSD-5-1-2	64%	69%	13%	59%	63%	12%	
MTL-10Vanclave98-MS-5-1-2	100%			63%			
MTL-10Vanclave98-MSD-5-1-2	75%	88%	29%	63%	63%	1%	
Liver Northern Fur Seal	P283	<LOQ (0.0719)			<LOQ (0.0188)		
	P285**	<LOQ (0.0719)			<LOQ (0.0188)		
	P295	<LOQ (0.0719)			<LOQ (0.0188)		
	97 CU 02	NE			NE		
	98 CU: KB 02	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 03	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 07	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 09	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 10	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 11	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 12	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 13	<LOQ (0.0719)			<LOQ (0.0188)		
	98 CU: KB 14	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	98 CU: KB 15	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
	Liver Polar Bear	970012	<LOQ (0.0719)			<LOQ (0.0188)	
970201		<LOQ (0.0719)			<LOQ (0.0188)		
980241		<LOQ (0.0719)			<LOQ (0.0188)		
692-FLBR-0033		<LOQ (0.0719)			<LOQ (0.0188)		
980127LB		<LOQ (0.0719)			<LOQ (0.0188)		
980387LB		<LOQ (0.0719)			<LOQ (0.0188)		
980390LB**		<LOQ (0.0719)			<LOQ (0.0188)		
980563LA		<LOQ (0.0719)			<LOQ (0.0188)		
980565LB		<LOQ (0.0719)			<LOQ (0.0188)		
980112LB		<LOQ (0.0719)			<LOQ (0.0188)		
980592LA		<LOQ (0.0719)			<LOQ (0.0188)		
980594LB		<LOQ (0.0719)			<LOQ (0.0188)		
980598LB		<LOQ (0.0719)			<LOQ (0.0188)		
980600LB		<LOQ (0.0719)			<LOQ (0.0188)		
980610LB		<LOQ (0.0719)			<LOQ (0.0188)		
980652LC	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA	
980658LB	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA	
Liver Mink	D0530	<LOQ (0.0719)			0.0334		
	D0566*	<LOQ (0.0719)			0.0266		
	D0590	<LOQ (0.0719)			0.0209		
	D0618	<LOQ (0.0719)			0.0581		
	D0630*	<LOQ (0.0719)			0.0703		
	D0684*	<LOQ (0.0719)			0.0612		
	D1000	<LOQ (0.0719)			0.0294		
	D1024	<LOQ (0.0719)			<LOQ (0.0188)		
	D1050	<LOQ (0.0719)			<LOQ (0.0188)		
	D1092	<LOQ (0.0719)			<LOQ (0.0188)		
	D1110*	<LOQ (0.0719)			0.0367		
	D1134	<LOQ (0.0719)			<LOQ (0.0188)		
	D1150X*	<LOQ (0.0719)			0.0340		
	D1194	<LOQ (0.0719)			0.0833		
	D1198	<LOQ (0.0719)			0.0242		
D1244	<LOQ (0.0719)		NA	<LOQ (0.0188)			
D1248	<LOQ (0.0719)		NA	0.0345			
D1660*	<LOQ (0.0719)	<LOQ	NA	0.0621	0.0442 - 5 outliers	0.5 0.0201	

* High (>50%) surrogate deviations confirmed.
** positive analyte confirmation was not achieved, used 499 --> 99 transition.
Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99,
01/12/00, 01/17/00, 01/18/00, 01/18/00, 01/19/00, 01/20/00 LAC
Date Verified/Analyst: 0

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

000038

FACT-GEN-030
MSU Environmental Samples

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Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:
Sample Data

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRPCSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/06/00, 02/01/00 IAS/MMH/MEE
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/07/00, 02/03/00 MMH/IAS/MEE

Miscellaneous Liver

Group Dose	Sample #	PFOS Verified	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec.	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD	
Method Blk Unfiltered water	MSU12129-H2OBlk-unfiltered-5-1	NA	NA			NA			
	MSU12129-H2OBlk-unfiltered-5-2	NA	NA			NA			
	MSU12129-H2OBlk-unfiltered-5-3	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	MSU12129-H2OBlk-unfiltered-5-4	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	MSU12129-H2OBlk-unfiltered-5-5	NA	E			E			
	MSU12129-H2OBlk-unfiltered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	MSU12129-H2OBlk-unfiltered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA	
	MSU12129-H2OBlk-unfiltered-5-8	NA	<LOQ (0.0696)	<LOQ		NA	<LOQ (0.0171)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBlk-filtered-5-1	NA	NA			NA			
	MSU12129-H2OBlk-filtered-5-2	NA	NA			NA			
	MSU12129-H2OBlk-filtered-5-3	NA	E			E			
	MSU12129-H2OBlk-filtered-5-4	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	MSU12129-H2OBlk-filtered-5-5	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	MSU12129-H2OBlk-filtered-5-6	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	MSU12129-H2OBlk-filtered-5-7	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA	
	MSU12129-H2OBlk-filtered-5-8	NA	<LOQ (0.0696)	<LOQ		NA	<LOQ (0.0171)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvrBlk-5-1	NA	0.0305			<LOQ (0.0171)			
	FSH12129-LvrBlk-5-2	NA	0.0331		32.1	<LOQ (0.0171)		NA	
	FSH12129-LvrBlk-5-3	NA	0.0170	0.0269	0.00862	<LOQ (0.0171)	<LOQ	NA	
Matrix Blk Rabbit Liver	RBL12129-LvrBlk-5-1	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	RBL12129-LvrBlk-5-2	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	RBL12129-LvrBlk-5-3	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	RBL12129-LvrBlk-5-4	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	RBL12129-LvrBlk-5-5*	NA	<LOQ (0.0696)			<LOQ (0.0171)		NA	
	RBL12129-LvrBlk-5-6*	NA	<LOQ (0.0696)	<LOQ		NA	<LOQ (0.0171)	<LOQ	NA
QC 250 ng/g	Mink Liver, D530, MS-5-1-1	NA	145%			61%			
	Mink Liver, D530, MSD-5-1-2*	NA	538%	342%		50%	55%	21%	
	CSL-1999030-03-01-MS-5-1-2*	NA	196%			116%			
	CSL-1999030-03-01-MSD-5-1-2*	NA	140%	168%		112%	114%	4%	
	LWL-1999029-12-MS-5-1-2*	NA	321%			57%			
	LWL-1999029-12-MSD-5-1-2*	NA	287%	304%		68%	63%	18%	
	BTL-1999040-01-MS-5-1-2*	NA	138%			81%			
	BTL-1999040-01-MSD-5-1-2*	NA	132%	135%		84%	83%	4%	
	TNL-TU54-MS-5-1-2	NA	68%			55%			
	TNL-TU54-MSD-5-1-2	NA	E	68%		E	55%	NA	
	FSL-P295-MS-5-1	NA	91%			97%			
	FSL-P295-MSD-5-1	NA	86%	88%		84%	90%	15%	
	PBL-980390LB-MS-5-1	NA	18%			70%			
	PBL-980390LB-MSD-5-2*	NA	179%	99%		163%	76%	73%	9%
	GFL-KZCKDM-D1-MS-5-1-2	NA	93%			82%			
	GFL-KZCKDM-D1-MSD-5-1	NA	105%	99%		12%	89%	86%	9%
TTL-LCPTR99503C-MS-5-1-2*	NA	90%			66%				
TTL-LCPTR99503C-MSD-5-1-2	NA	100%	95%		11%	72%	69%	8%	
MTL-10Vanceleave98-MS-5-1-2	NA	79%			74%				
MTL-10Vanceleave98-MSD-5-1-2	NA	89%	84%		11%	84%	79%	13%	
Liver Map Turtle	F, #10, Vanceleave 98	NA	0.0801			<LOQ (0.00683)			
	F, #09, Vanceleave 98	NA	0.0514			<LOQ (0.00683)			
	F, #02, Leeksville 98	NA	0.0739			<LOQ (0.00683)			
	F, #06, Leeksville 99	NA	0.0394			<LOQ (0.00683)			
	M, (-1)	NA	0.703			<LOQ (0.00683)			
Liver Terrapin	F, (89, 8912)	NA	0.179			<LOQ (0.00683)			
	LCPTR 9503C	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	LCPTR 9504C	NA	<LOQ (0.0347)		0.730	<LOQ (0.00683)		NA	
	LCPTR 9505C	NA	<LOQ (0.0347)	0.188 - 3 outliers		0.257	<LOQ (0.00683)	<LOQ	NA
Liver Tuna	TU25*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU34*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU41*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU48*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU49	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	TU54*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU58	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	TU63*	X	<LOQ (0.0696)			<LOQ (0.0171)			
	TU66	X	0.00698			<LOQ (0.0171)			
	TU84	NA	<LOQ (0.0696)			<LOQ (0.0171)			
	TU88	X	<LOQ (0.00696)			<LOQ (0.0171)		NA	
TU90	NA	<LOQ (0.0696)	<LOQ - 1 outlier		NA	<LOQ (0.0171)	<LOQ	NA	
Liver Green Frog	KZCKDM-D1	NA	<LOQ (0.0347)			<LOQ (0.00683)			
	KZCKDM-D2	X	0.285			<LOQ (0.00683)			
	Pool of 4	NA	<LOQ (0.0347)			<LOQ (0.00683)		NA	
	SJ0001	X	<LOQ (0.0347)	<LOQ - 1 outlier		NA	<LOQ (0.00683)	<LOQ	NA

* High (>50%) surrogate deviations

Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99,
01/12/00, 01/17/00, 01/18/00, 02/04/00 LAC
Date Verified/Analyst:

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

FACT-GEN-030
MSU Environmental Samples

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Study:
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Date of Extraction/Analyst:
Date of Analysis/Analyst:
Date of Data Reduction/Analyst:
Sample Data

GEN030 MSU Environmental Samples
Various
Various
ETS-8-6.0 & ETS-8-7.0
Soup 020199, Amelia 062498
Masslynx 3.3
12/12/99 SAL/KK/SRP/CSH
12/17/99, 12/20/99, 12/28/99, 12/29/99, 01/03/00, 01/06/00, 02/01/00 IAS/MMH/MEB
12/20/99, 12/21/99, 12/22/99, 12/30/99, 01/03/00, 01/05/00, 01/07/00, 02/03/00 MMH/IAS/MEE

Miscellaneous Liver

Group Dose	Sample #	Concentration of POAA ug/g or % Rec.	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk Unfiltered water	MSU12129-H2OBlk-unfiltered-5-1	NA			NA		
	MSU12129-H2OBlk-unfiltered-5-2	NA			NA		
	MSU12129-H2OBlk-unfiltered-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-5	E			E		
	MSU12129-H2OBlk-unfiltered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-unfiltered-5-7	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	MSU12129-H2OBlk-unfiltered-5-8	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Method Blk Filtered water	MSU12129-H2OBlk-filtered-5-1	NA			NA		
	MSU12129-H2OBlk-filtered-5-2	NA			NA		
	MSU12129-H2OBlk-filtered-5-3	E			E		
	MSU12129-H2OBlk-filtered-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-5	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-6	<LOQ (0.0719)			<LOQ (0.0188)		
	MSU12129-H2OBlk-filtered-5-7	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	MSU12129-H2OBlk-filtered-5-8	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Fish Liver	FSH12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	FSH12129-LvrBlk-5-2	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	FSH12129-LvrBlk-5-3	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Matrix Blk Rabbit Liver	RBL12129-LvrBlk-5-1	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-2	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-3	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-4	<LOQ (0.0719)			<LOQ (0.0188)		
	RBL12129-LvrBlk-5-5*	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	RBL12129-LvrBlk-5-6*	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
QC 250 ng/g	Mink Liver, D530, MS-5-1-1	86%			43%		
	Mink Liver, D530, MSD-5-1-2*	84%	85%	3%	81%	62%	60%
	CSL-1999030-03-01-MS-5-1-2*	131%			64%		
	CSL-1999030-03-01-MSD-5-1-2*	111%	121%	17%	52%	58%	21%
	LWL-1999029-12-MS-5-1-2*	133%			77%		
	LWL-1999029-12-MSD-5-1-2*	145%	139%	9%	83%	80%	7%
	BTL-1999040-01-MS-5-1-2*	127%			94%		
	BTL-1999040-01-MSD-5-1-2*	120%	124%	6%	92%	93%	3%
	TNL-TU54-MS-5-1-2	84%			71%		
	TNL-TU54-MSD-5-1-2	E	84%	NA	E	71%	NA
	FSL-P295-MS-5-1	72%			87%		
	FSL-P295-MSD-5-1	80%	76%	10%	82%	85%	7%
	PBL-980390LB-MS-5-1	85%			71%		
	PBL-980390LB-MSD-5-2*	129%	107%	41%	65%	68%	9%
	GFL-KZCKDM-D1-MS-5-1-2	82%			82%		
	GFL-KZCKDM-D1-MSD-5-1-1	103%	92%	23%	93%	88%	12%
	TTL-LCPTR99503C-MS-5-1-2*	73%			78%		
TTL-LCPTR99503C-MSD-5-1-2	64%	69%	13%	71%	74%	10%	
MTL-10Vancleave98-MS-5-1-2	100%			74%			
MTL-10Vancleave98-MSD-5-1-2	75%	88%	29%	74%	74%	0%	
Liver Map Turtle	F, #10, Vancleave 98	<LOQ (0.0719)			<LOQ (0.0188)		
	F, #09, Vancleave 98	<LOQ (0.0719)			<LOQ (0.0188)		
	F, #02, Leeksville 98	<LOQ (0.0719)			<LOQ (0.0188)		
	F, #06, Leeksville 99	<LOQ (0.0719)			<LOQ (0.0188)		
	M, (-1)	<LOQ (0.0719)			<LOQ (0.0188)		
Liver Terrapin	F, (89, 8912)	<LOQ (0.0719)			<LOQ (0.0188)		
	LCPTR 9503C	<LOQ (0.0719)			<LOQ (0.0188)		
	LCPTR 9504C	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
	LCPTR 9505C	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA
Liver Tuna	TU25*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU34*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU41*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU48*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU49	<LOQ (0.0719)			<LOQ (0.0188)		
	TU54*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU58	<LOQ (0.0719)			<LOQ (0.0188)		
	TU63*	<LOQ (0.0719)			<LOQ (0.0188)		
	TU66	<LOQ (0.0719)			<LOQ (0.0188)		
	TU84	<LOQ (0.0719)			<LOQ (0.0188)		
	TU88	<LOQ (0.0719)		NA	<LOQ (0.0188)		NA
TU90	<LOQ (0.0719)	<LOQ	NA	<LOQ (0.0188)	<LOQ	NA	
Liver Green Frog	KZCKDM-D1	<LOQ (0.0719)			<LOQ (0.0188)		
	KZCKDM-D2	<LOQ (0.0719)			<LOQ (0.0188)		
	Pool of 4 SJ0001	<LOQ (0.0719)	<LOQ		<LOQ (0.0188)		NA

* High (>50%) surrogate deviations

NE = Not Extracted
E = Lost during extraction
NA = Not Applicable
LOQ = Limit of Quantitation
X = Verified PFOS concentration

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctane sulfonamide
PFHS = Perfluorohexanesulfonate
POAA = Perfluorooctanoate

Date Entered/Analyst: 12/22/99, 12/28/99, 12/29/99, 12/30/99,
01/12/00, 01/17/00, 01/18/00, 02/04/00 LAC
Date Verified/Analyst:

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
 Sample Data

GEN033, MSU - Liver Samples
 NA
 Various livers - Unextracted Curves
 ETS-8-6.0 and ETS-8-7.0
 Davey 070799, Amelia 062498
 Masslynx 3.3
 03/14/00 SAL/CSH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of POAA ug/g or % Rec	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD
Mink Liver	C23 (45)	0.801			< LOQ (0.0359 ug/g)		
	C26 (44)	0.443			< LOQ (0.0359 ug/g)		
	C27 (49)	0.145			< LOQ (0.0359 ug/g)		
	C33 (46)	0.435			< LOQ (0.0359 ug/g)		
	C35 (47)	0.355			< LOQ (0.0359 ug/g)		
	C37 (48)	0.833			< LOQ (0.0359 ug/g)		
	C42 (43)	0.420			< LOQ (0.0359 ug/g)		
	C44 (54)	0.237			< LOQ (0.0359 ug/g)		
	D10 (50)	1.67			< LOQ (0.0359 ug/g)		
	F15 (55)	0.548			< LOQ (0.0359 ug/g)		
	F19 (56)	0.783			< LOQ (0.0359 ug/g)		
	F21 (57)	1.03			< LOQ (0.0359 ug/g)		
	F24 (58)	0.868			< LOQ (0.0359 ug/g)		
	P01(60)	2.16			< LOQ (0.0359 ug/g)		
	P03 (64)	4.80			< LOQ (0.0359 ug/g)		
	P09 (61)	0.841			< LOQ (0.0359 ug/g)		
	S11 (37)	0.902			< LOQ (0.0359 ug/g)		
	S15 (41)	1.27			< LOQ (0.0359 ug/g)		
	S18 (40)	1.99			< LOQ (0.0359 ug/g)		
	S19 (59)	2.68			< LOQ (0.0359 ug/g)		
	S25 (39)	0.509			< LOQ (0.0359 ug/g)		
	S30 (36)	0.186			< LOQ (0.0359 ug/g)		
	S35 (42)	0.0933			< LOQ (0.0359 ug/g)		
S39 (38)	0.317			< LOQ (0.0359 ug/g)			
T01 (51)	0.633			< LOQ (0.0359 ug/g)			
T04 (53)	1.35			< LOQ (0.0359 ug/g)			
T03 (52)	0.565		1.23	103	< LOQ (0.0359 ug/g)		
V12 (62)	4.87			1.27	< LOQ (0.0359 ug/g)		
V03 (65)	1.52				< LOQ (0.0359 ug/g)		NA
V08 (63)	3.65				< LOQ (0.0359 ug/g)	<LOQ	NA
Baikal Seal Liver	J08 (81)	0.0127			< LOQ (0.0359 ug/g)		
	J09 (87)	0.0228			< LOQ (0.0718 ug/g)		
	J10 (86)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	J12 (89)	0.0141			< LOQ (0.0359 ug/g)		
	J19 (84)	0.00931			< LOQ (0.0359 ug/g)		
	J20 (88)	0.0154			< LOQ (0.0718 ug/g)		
	J24 (82)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	J27 (83)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	J36 (85)	0.0146			< LOQ (0.0359 ug/g)		
	J37 (80)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	R04 (69)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	R13 (78)	0.0100			< LOQ (0.0359 ug/g)		
	R14 (74)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
	R16 (71)	0.00808			< LOQ (0.0359 ug/g)		
	R29 (72)	0.00795			< LOQ (0.0359 ug/g)		
	R42 (66)	0.0138			< LOQ (0.0359 ug/g)		
	R43 (73)	0.0156			< LOQ (0.0359 ug/g)		
	R45 (79)	0.00848			< LOQ (0.0359 ug/g)		
	R46 (77)	0.00778			< LOQ (0.0359 ug/g)		
	R47 (70)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)		
R54 (67)	<LOQ (0.0347 ug/g)			< LOQ (0.0718 ug/g)			
R55 (75)	0.0133			< LOQ (0.0718 ug/g)			
R57 (76)	0.0158			< LOQ (0.0359 ug/g)			
R64 (68)	0.00786		0.0123	33.7	< LOQ (0.0359 ug/g)	<LOQ	NA
Ganges Dolphin Liver	L04 (91)	<LOQ (0.0347 ug/g)		NA	<LOQ (0.0718 ug/g)		NA
	L05 (90)	0.0813	NA	NA	<LOQ (0.0718 ug/g)	<LOQ	NA

LOQ = Limit of Quantitation
 NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 PFHS = Perfluorohexane sulfonate
 POAA = Perfluorooctanoate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

000041

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
 Sample Data

GEN033, MSU - Liver Samples
 NA
 Various livers - Unextracted Curves
 ETS-8-6.0 and ETS-8-7.0
 Davey 070799, Amelia 062498
 Masslynx 3.3
 03/14/00 SAL/CSSH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Below
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD
Mink Liver	C23 (45)	0.0383			< LOQ (0.00683 ug/g)		
	C26 (44)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	C27 (49)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	C33 (46)	< LOQ (0.0376 ug/g)			0.00833		
	C35 (47)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	C37 (48)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	C42 (43)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	C44 (54)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	D10 (50)	0.0828			< LOQ (0.00683 ug/g)		
	F15 (55)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	F19 (56)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	F21 (57)	0.0579			< LOQ (0.00683 ug/g)		
	F24 (58)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	P01 (60)	0.551			0.0315		
	P03 (64)	0.590			0.0852		
	P09 (61)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S11 (37)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S15 (41)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S18 (40)	0.0414			< LOQ (0.00683 ug/g)		
	S19 (59)	0.132			0.0102		
	S25 (39)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S30 (36)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S35 (42)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S39 (38)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T01 (51)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T04 (53)	< LOQ (0.0376 ug/g)			0.0104		
	T03 (52)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	V12 (62)	0.345			< LOQ (0.00683 ug/g)		
V03 (65)	0.0594			< LOQ (0.00683 ug/g)			
V08 (63)	0.0586	<LOQ - 10 Outliers	NA	NA	< LOQ (0.00683 ug/g)	<LOQ - 5 Outliers	NA
Baikal Seal Liver	J08 (81)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J09 (87)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J10 (86)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J12 (89)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J19 (84)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J20 (88)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J24 (82)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J27 (83)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J36 (85)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	J37 (80)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R04 (69)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R13 (78)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R14 (74)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R16 (71)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R29 (72)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R42 (66)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R43 (73)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R45 (79)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R46 (77)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	R47 (70)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
R54 (67)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)			
R55 (75)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)			
R57 (76)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)			
R64 (68)	< LOQ (0.0376 ug/g)	<LOQ	NA	NA	< LOQ (0.00683 ug/g)	<LOQ	NA
Ganges Dolphin Liver	L04 (91)	< LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	L05 (90)	< LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ	NA

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 NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 PFOSA = Perfluorooctanesulfonamide
 PFHS = Perfluorohexane sulfonate
 POAA = Perfluorooctanoate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

000042

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 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
Sample Data

GEN033, MSU - Liver Samples
 NA
 Various livers - unextracted curves
 ETS-8-6.0 and ETS-8-7.0
 Davey 070799, Amelia 062498
 Masslynx 3.3
 03/14/00 SAL/CSH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of POAA ug/g or % Rec	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD
Comorant Liver Female, Adult	6F (1),F.A	0.0432			0.0414		
	29F (5),F.A**	0.0565			0.102		
	30F (6),F.A	0.0485			0.0506		
	32F (7),F.A	0.0977			0.0897		
	34F (9),F.A**	0.0335			0.0297		
	42F (11),F.A	0.150	0.0715	61.9 0.0442	0.143	0.0761	56.9 0.0432
Comorant Liver Female, Juvenile	7F (2),F.J	0.0913			0.0841		
	9F (3),F.J	0.468		113	0.444		115
	36F (10),F.J	0.0499	0.203	0.230	0.0467	0.192	0.219
Comorant Liver Male, Juvenile	22F (4),M.J	0.0433			0.0454		
	33F (8),M.J	0.0316		17.2	0.0303		25.7
	44F (12),M.J	0.0337	0.0362	0.00623	0.0293	0.0350	0.00899
Bottlenose Dolphin Liver	T15/91 (13)	0.181			<LOQ (0.0359 ug/g)		
	T18/91 (17)	0.296			<LOQ (0.0718 ug/g)		
	T161 (14)	0.169			<LOQ (0.0718 ug/g)		
	T1A11 (16)	0.425		44.5	<LOQ (0.0718 ug/g)		NA
	T1X12 (15)	<LOQ (0.00696 ug/g)	0.268 - One Outlier	0.119	<LOQ (0.0718 ug/g)	<LOQ	NA
Striped Dolphin Liver	SCF02 (18)	0.161			<LOQ (0.0718 ug/g)		
	SCV1 (19)	0.0891			<LOQ (0.0718 ug/g)		
	SCF03 (20)	0.0944		40.2	<LOQ (0.0718 ug/g)		NA
	SCF04 (21)	0.0647	0.102	0.0410	<LOQ (0.0718 ug/g)	<LOQ	NA
Weddell Seal Liver	WS1 (22)	< LOQ (0.0347 ug/g)	<LOQ	NA	<LOQ (0.0718 ug/g)	<LOQ	NA
Swordfish Liver	S23 (23)	< LOQ (0.00696 ug/g)			<LOQ (0.0359 ug/g)		
	S24 (24)	< LOQ (0.00696 ug/g)			<LOQ (0.0359 ug/g)		
	S25 (25)	0.00774			<LOQ (0.0359 ug/g)		
	S32 (27)	< LOQ (0.00696 ug/g)		NA	<LOQ (0.0359 ug/g)		NA
	S48 (26)	0.0133	<LOQ - Two Outliers	NA	<LOQ (0.0359 ug/g)	<LOQ	NA
Tunaflsh Liver	T1 (33)	< LOQ (0.0347 ug/g)			<LOQ (0.0359 ug/g)		
	T1LF156 (35)	0.0433			<LOQ (0.0718 ug/g)		
	T2 (29)	0.0874			<LOQ (0.0359 ug/g)		
	T15 (28)	0.0568			<LOQ (0.0718 ug/g)		
	T17 (31)	0.0491			<LOQ (0.0359 ug/g)		
	T20 (32)	0.0207			<LOQ (0.0359 ug/g)		
	T23 (30)	0.0560			<LOQ (0.0359 ug/g)		
	T25 (34)	0.0250	0.0483 - One Outlier	46.2 0.0223	<LOQ (0.0718 ug/g)	<LOQ	NA
Blacktailed Gull Liver	BHG01 (100)	0.292			<LOQ (0.0359 ug/g)		
	BHG02 (101)	0.260			<LOQ (0.0359 ug/g)		
	BHG03 (102)	0.148			<LOQ (0.0359 ug/g)		
	BHG04 (103)	0.503			<LOQ (0.0359 ug/g)		
	BHG05 (104)	0.271			<LOQ (0.0359 ug/g)		
	BTG9305 (92)	0.0881			<LOQ (0.0359 ug/g)		
	BTG9306 (99)	0.107			<LOQ (0.0359 ug/g)		
	BTG9310 (96)	0.215			<LOQ (0.0359 ug/g)		
	BTG9311 (98)	0.143			<LOQ (0.0359 ug/g)		
	BTG9312 (97)	0.0705			<LOQ (0.0359 ug/g)		
	BTG9401 (93)	0.126			<LOQ (0.0359 ug/g)		
	BTGhongdo1 (94)	0.0737			<LOQ (0.0359 ug/g)		
	BTGnando1 (95)	0.0707			<LOQ (0.0359 ug/g)		
	HRG04 (106)	0.116			<LOQ (0.0359 ug/g)		NA
	HRG09 (105)	0.0939	0.172	69.2 0.119	<LOQ (0.0359 ug/g)	<LOQ	NA

** NO PFOS confirmation performed.
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PFOS = Perfluorooctanesulfonate
 POAA = Perfluorooctanoate
 PFOSA = Perfluorooctanesulfonamide
 PFHS = Perfluorohexane sulfonate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

000043

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Study:
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 Instrument Software/Version:
 Date of Extraction/Analyst:
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 Date of Data Reduction/Analyst:
 Sample Data

GEN033, MSU - Liver Samples
 NA
 Various livers - unextracted curves
 ETS-4-6.0 and ETS-4-7.0
 Davey 070799, Amelia 062498
 Masslynx 3.3
 03/14/00 SALC/SH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Below
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOA ug/g or % Rec	Mean PFOA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Cormorant Liver Female, Adult	6F (1),F,A	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	29F (5),F,A**	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	30F (6),F,A	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	32F (7),F,A	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	34F (9),F,A**	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		NA
	42F (11),F,A	<LOQ (0.0376 ug/g)	<LOQ	NA NA	< LOQ (0.00683 ug/g)	<LOQ	NA NA
Cormorant Liver Female, Juvenile	7F (2),F,J	0.0883			< LOQ (0.00683 ug/g)		
	9F (3),F,J	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	36F (10),F,J	<LOQ (0.0376 ug/g)	<LOQ - One Outlier	NA	< LOQ (0.00683 ug/g)	<LOQ	NA
Cormorant Liver Male, Juvenile	22F (4),M,J	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	33F (8),M,J	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	44F (12),M,J	<LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ	NA
Bottlenose Dolphin Liver	T5/91 (13)	0.224			< LOQ (0.00683 ug/g)		
	T8/91 (17)	0.358			< LOQ (0.00683 ug/g)		
	T61 (14)	0.129			< LOQ (0.00683 ug/g)		
	TA11 (16)	0.129		54.1	< LOQ (0.00683 ug/g)		NA
	TX12 (15)	0.115	0.191	0.103	< LOQ (0.00683 ug/g)	<LOQ	NA
Striped Dolphin Liver	SCPO2 (18)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	SCV1 (19)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	SCPO3 (20)	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	SCPO4 (21)	<LOQ (0.0376 ug/g)	<LOQ - One Outlier	NA	0.0270	< LOQ (0.00683 ug/g)	<LOQ - One Outlier
Weddell Seal Liver	WS1 (22)	<LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ	NA
Swordfish Liver	S23 (23)	<LOQ (0.0376 ug/g)			0.00954		
	S24 (24)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S25 (25)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	S32 (27)	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	S48 (26)	<LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ - One Outlier	NA
Tuna/Liver	T1 (33)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T1LF156 (35)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T2 (29)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T15 (28)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T17 (31)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T20 (32)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	T23 (30)	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	T25 (34)	<LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ	NA
Blacktailed Gull Liver	BHG01 (100)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BHG02 (101)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BHG03 (102)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BHG04 (103)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BHG05 (104)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9305 (92)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9306 (99)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9310 (96)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9311 (98)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9312 (97)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTG9401 (93)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTGhongdol (94)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	BTGnando1 (95)	<LOQ (0.0376 ug/g)			< LOQ (0.00683 ug/g)		
	HRG04 (106)	<LOQ (0.0376 ug/g)		NA	< LOQ (0.00683 ug/g)		NA
	HRG09 (105)	<LOQ (0.0376 ug/g)	<LOQ	NA	< LOQ (0.00683 ug/g)	<LOQ	NA

** NO PFOS confirmation performed
 LOQ = Limit of Quantitation
 NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 POAA = Perfluorooctanoate
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 PFHS = Perfluorohexane sulfonate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

000044

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Study:
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Sample Data

GEN033, MSU - Liver Samples
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 Various livers - unextracted curves
 ETS-8-6.0 and ETS-8-7.0
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 Masslynx 3.3
 03/14/00 SAL/CSH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Attachments
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of POAA ug/g or % Rec	Mean POAA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL03140-H2O Blk-5-1	< LOQ (0.00696 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-H2O Blk-5-2	< LOQ (0.00696 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-H2O Blk-5-3	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-4	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-5	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-6	< LOQ (0.0347 ug/g)			<LOQ (0.00719 ug/g)		
	RBL03140-H2O Blk-5-7	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-8	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-9	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-H2O Blk-5-10	< LOQ (0.0347 ug/g)			<LOQ (0.00719 ug/g)		
	RBL03140-H2O Blk-5-11	< LOQ (0.0347 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-H2O Blk-5-12	< LOQ (0.0347 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-H2O Blk-5-13	< LOQ (0.0347 ug/g)		<LOQ	NA NA	<LOQ (0.0359 ug/g)	<LOQ
Matrix Blk	RBL03140-Liver Blk-5-1	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-2	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-3	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-4	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-5	0.00883			<LOQ (0.0359 ug/g)		
	RBL03140-Liver Blk-5-6	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-7	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-8	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-9	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-10	< LOQ (0.0694 ug/g)			<LOQ (0.0718 ug/g)		
	RBL03140-Liver Blk-5-11	< LOQ (0.0347 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-Liver Blk-5-12	< LOQ (0.0347 ug/g)			<LOQ (0.0359 ug/g)		
	RBL03140-Liver Blk-5-13	< LOQ (0.0347 ug/g)		<LOQ - One Outlier	NA NA	<LOQ (0.0359 ug/g)	<LOQ
QC 250 ppb	RBL03140-MS-1 ppb-5-1*	104%			112%		
	RBL03140-MSD-1 ppb-5-2*	114%	109%	9%	106%	109%	6%
	RBL03140-MS-250 ppb-5-1	120%			128%		
	RBL03140-MSD-250 ppb-5-2	133%	127%	10%	128%	128%	0%
	44F (12)-MS	98%			106%		
	44F (12)-MSD	107%	103%	9%	109%	107%	2%
	Tt8/91 (17)-MS	131%			59%		
	Tt8/91 (17)-MSD	140%	135%	6%	64%	61%	9%
	SCV1 (19)-MS	66%			56%		
	SCV1 (19)-MSD	93%	79%	35%	76%	66%	30%
	WS1 (22)-MS	81%			63%		
	WS1 (22)-MSD	73%	77%	10%	62%	62%	2%
	S24 (24)-MSD	92%		NA	101%	NA	NA
	T15 (28)-MS	84%			93%		
	T15 (28)-MSD	103%	94%	21%	105%	99%	12%
	TILF156 (35)-MS	93%			92%		
	TILF156 (35)-MSD	72%	82%	25%	80%	86%	15%
	S19 (59)-MS	326%			126%		
	S19 (59)-MSD	234%	280%	33%	154%	140%	20%
	P9 (61)-MS	231%			102%		
P9 (61)-MSD	142%	187%	48%	112%	107%	9%	
R47 (70)-MS	68%			70%			
R47 (70)-MSD	69%	69%	2%	68%	69%	3%	
L4 (91)-MS	69%			73%			
L4 (91)-MSD	187%	128%	92%	165%	119%	78%	
BTG9305 (92)-MS	143%		NA	153%	NA	NA	
BTGhongd01 (94)-MS	122%			137%			
BTGhongd01 (94)-MSD	120%	121%	2%	156%	146%	13%	

LOQ = Limit of Quantitation
 NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 POAA = Perfluorooctanoate
 PFOA = Perfluorooctanesulfonamide
 PFHS = Perfluorohexane sulfonate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

000045

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Study:
 Product Number(Test Substance):
 Matrix:
 Method/Revision:
 Analytical Equipment System Number:
 Instrument Software/Version:
 Date of Extraction/Analyst:
 Date of Analysis/Analyst:
 Date of Data Reduction/Analyst:
 Sample Data

GEN033, MSU - Liver Samples
 NA
 Various livers - unextracted curves
 ETS-8-6.0 and ETS-8-7.0
 Davey 070799, Amelia 062498
 Masslynx 3.3
 03/14/00 SAL/CSH/KKK
 03/16/00, 03/17/00, 3/19/00, 03/20/00, 03/21/00, 03/29/00, 04/07/00 IAS/MMH
 03/20/00, 03/22/00, 03/23/00, 03/24/00, 04/04/00, 04/11/00 IAS/MMH

Filename: See Below
 R-Squared Value: See Attachments
 Slope: See Attachments
 Y-Intercept: See Attachments

Various Livers

Group Dose	Sample #	Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	Concentration of PFHS ug/g or % Rec	Mean PFHS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL03140-H2O Blk-5-1	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-2	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-3	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-4	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-5	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-6	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-7	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-8	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-9	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-10	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-11	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-H2O Blk-5-12	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		NA
	RBL03140-H2O Blk-5-13	< LOQ (0.0376 ug/g)	<LOQ		NA	<LOQ (0.00683 ug/g)	<LOQ
Matrix Blk	RBL03140-Liver Blk-5-1	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-2	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-3	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-4	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-5	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-6	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-7	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-8	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-9	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-10	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-11	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		
	RBL03140-Liver Blk-5-12	< LOQ (0.0376 ug/g)			<LOQ (0.00683 ug/g)		NA
	RBL03140-Liver Blk-5-13	< LOQ (0.0376 ug/g)	<LOQ		NA	<LOQ (0.00683 ug/g)	<LOQ
QC 250 ppb	RBL03140-MS-1 ppb-5-1*	66%			87%		
	RBL03140-MSD-1 ppb-5-2*	67%	67%	2%	57%	72%	42%
	RBL03140-MS-250 ppb-5-1	114%			61%		
	RBL03140-MSD-250 ppb-5-2	84%	99%	30%	48%	54%	25%
	44F (12)-MS	94%			84%		
	44F (12)-MSD	89%	92%	5%	87%	86%	3%
	T18/91 (17)-MS	146%			65%		
	T18/91 (17)-MSD	191%	168%	26%	65%	65%	1%
	SCV1 (19)-MS	53%			54%		
	SCV1 (19)-MSD	87%	70%	48%	64%	59%	17%
	WS1 (22)-MS	69%			59%		
	WS1 (22)-MSD	69%	69%	0%	60%	60%	1%
	S24 (24)-MSD	98%	NA	NA	76%	NA	NA
	T15 (28)-MS	66%			67%		
	T15 (28)-MSD	95%	81%	36%	102%	84%	40%
	T11F156 (35)-MS	86%			88%		
	T11F156 (35)-MSD	66%	76%	26%	73%	80%	19%
	S19 (59)-MS	140%			97%		
	S19 (59)-MSD	2%	71%	195%	-1%	48%	203%
	P9 (61)-MS	73%			52%		
P9 (61)-MSD	68%	70%	7%	48%	50%	6%	
R47 (70)-MS	68%			55%			
R47 (70)-MSD	78%	73%	14%	58%	57%	5%	
L4 (91)-MS	50%			58%			
L4 (91)-MSD	119%	85%	82%	145%	102%	86%	
BTG9303 (92)-MS	70%	NA	NA	31%	NA	NA	
BTGhongdol (94)-MS	62%			18%			
BTGhongdol (94)-MSD	63%	63%	2%	22%	20%	20%	

LOQ = Limit of Quantitation
 NA = Not Applicable

PFOS = Perfluorooctanesulfonate
 POAA = Perfluorooctanoate
 PFOSA = Perfluorooctanesulfonamide
 PFHS = Perfluorohexane sulfonate

Date Entered/Analyst: 03/28/00, 04/05/00, 04/07/00, 05/07/00 MMH/LAC
 Date Verified/Analyst:

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3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCETANESULFONATE OR OTHER FLUORO-CHEMICAL 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.

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

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 μ L to 50 μ L
- 7.10 Graduated pipettes
- 7.11 Syringes, disposable plastic, 3 cc
- 7.12 Syringe filters, nylon, 0.2 μ m, 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 (Na₂CO₃), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO₃), 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

- 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 ($\mu g/mL$).
- 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, C₈F₁₃SO₃H 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.

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:

$$\frac{\text{mL of standard} \times \text{concentration of standard } (\mu\text{g}/\text{mL})}{\text{mL of standard} + \text{mL of surrogate standard} + \text{initial matrix volume (mL)}} =$$

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.

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

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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

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

4.2 Cautions:

- 4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). 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 shall 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 air 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 (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent
 - 8.1.3.1 When preparing different amounts than those listed, adjust accordingly.
 - 8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to ETS-8-6.0.

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 may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 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 Checks

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 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 sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted $1/x$, not forced through the origin, 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 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). 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. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

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

12.1.4 Samples are analyzed with a continuing calibration verification injected standard 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 μ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 mi.	40%	60%

12.2.2.5 Press the “Start” button.

12.3 Instrument Set-up

12.3.1 Refer to **ETS-9-24.0**, “Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source,” for more details.

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

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 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to “On”

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. 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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 – 500 µL/min

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

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 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). 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:

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

13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentrations in matrix ($\mu\text{g/g}$):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\frac{(\text{Initial Weight of Liver (g)})}{\text{Final Volume (mL)}}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to **ETS-8-6.0, 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 must be below the lowest standard in the calibration curve.

14.3 Calibration Curves

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

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.6 If criteria listed in the method performance section are not 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, refer to **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-8-7.0 Data summary spreadsheet

18.0 REFERENCES

- 18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver 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-8-6.0 & 7.0-V-1**

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision
Number

Reason For Revision

Revision
Date

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 ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

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

3M ENVIRONMENTAL LABORATORY

METHOD

EXTRACTION OF POTASSIUM PERFLUOROOCETANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-6.0

Adoption Date:

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 is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 μ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

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 Ultra-Turrax T25 Grinder for grinding liver samples
- 6.1.2 Vortex mixer, VWR, Vortex Genie 2
- 6.1.3 Centrifuge, Mistral 1000 or IEC
- 6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

- 7.1 Gloves
- 7.2 Dissecting scalpels
- 7.3 Eppendorf or disposable pipettes
- 7.4 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.5 Volumetric flasks, glass, type A
- 7.6 I-CHEM vials, 40 mL glass
- 7.7 Plastic sample vials, Wheaton, 6 mL (or appropriate size)
- 7.8 Centrifuge tubes, polypropylene, 15 mL
- 7.9 Labels
- 7.10 Oxford Dispensor – 3.0 to 10.0 ml
- 7.11 Syringes, capable of measuring 5 µL to 50 µL
- 7.12 Graduated pipettes
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2 µm, 25 mm
- 7.15 Timer
- 7.16 Crimp cap autovials and caps
- 7.17 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 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 (Na₂CO₃), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO₃), J.T. Baker or equivalent
- 8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Liver, frozen from supplier
- 8.9 Dry ice from supplier
- 8.10 **Fluorochemical standards**
 - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

- 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
- 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H, 1-H, 2-H, 2-H C₈F₁₃SO₃H) molecular weight = 428
- 8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

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

- 8.11.1 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.
- 8.11.2 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.
- 8.11.3 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.
 - 8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na₂CO₃/NaHCO₃): Weigh approximately 26.5 g of sodium carbonate (Na₂CO₃) and 21.0 g of sodium bicarbonate (NaHCO₃) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

- 8.12.1 Prepare PFOS standards for the standard curve.
- 8.12.2 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.)
- 8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).
- 8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, C₈F₁₃SO₃H into a 50 ml volumetric flask and record the actual weight.

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

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 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.

10.0 QUALITY CONTROL

10.1 Matrix blanks and method 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 liver homogenate following this procedure and use as matrix blanks. Refer to 11.1.6.

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 a control liver 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 verifications

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

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

- 10.3.3** Prepare each continuing calibration verification 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** Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.
- 11.1.2** If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.
- 11.1.3** Refer to **13.0** to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.
- 11.1.5** Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.
- 11.1.6** Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.
- 11.1.7** 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 samples, two matrix blanks, and two method blanks.
- 11.1.8** Refer to validation reports **ETS-8-6.0** and **ETS-8-7.0-V-1** or **Attachment B**, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.9** Use **Attachment C** as an aid in calculating the concentrations of the working standards. Refer to **13.0** to calculate actual concentrations of PFOS in calibration standards.
- 11.2** To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

11.3 Extract spiked liver homogenates following 12.14-12.25 of this method. Use these standards to establish each initial curve on the mass spectrometer.

Working Standard (Approx. Conc.)	μ l	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

12.3 Weigh the sample directly into a tared plastic sample vial.

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sample vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sample vial with the study number, weight, liver ID, date and analyst initials.

- 12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sample vial. Refer to attached worksheet for documenting the remaining steps.
- 12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.
- 12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section 11.2.
- 12.14 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.
- 12.21 Label a fresh 15 mL centrifuge tube with the same information as in 12.10.
- 12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.
- 12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.
- 12.25 Vortex mix for 30 seconds.
- 12.26 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.
- 12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{1.0 \text{ mL aliquot}}$$

13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver} \times \text{Average density* of homogenate (mg/mL)}}{\text{(g of Liver + g of Water)}}$$

* refer to 13.1.1 for details.

13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration } (\mu\text{g / mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration } (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

*refer to 13.1.2 for details.

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 (refer to **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 verification samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of ETS-8-7.0 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.

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 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

- 17.1 Attachment A, Extraction worksheet
17.2 Attachment B, MDL/LOQ values and summary
17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

- 18.1 The validation report associated with this method is ETS-8-6.0 & 7.0-V-1.
18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"
18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-7.0, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	8.45	26.9	30 ppb – 1200 ppb
PFOSA	3.50	11.1	12 ppb – 1200 ppb
PFOSAA	24.6	78.3	30 ppb – 1200 ppb
EtFOSE-OH	108	345	60 ppb – 900 ppb*
M556	82.3	262	60 ppb – 1200 ppb
PFOSEA	33.9	108	30 ppb- 1200 ppb

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information

* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	6 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.19 - 1237	12 - 1200	12 - 1200	12 - 300	12 - 300	60 - 1200	60 - 1200

Compound: PFOSAA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.16 - 1232	12 - 1200	30 - 1200	30 - 900	60 - 900	N/A	N/A

Compound: EtFOSE-OH

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 900	31 - 900	N/A	N/A	N/A	N/A

Compound: PFOSEA

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	31 - 1200	N/A	N/A	N/A	N/A

Compound: M556

Liver matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	6.17 - 1235	31 - 1200	60 - 1200	N/A	N/A	N/A	N/A

Ion Pair Standard Curves – Tissue

Prep date(s):
 Analyte(s):
 Sample matrix:

Standard number:
 Equipment number:
 Final solvent and TN:
 Blank liver/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

Actual concentrations of standards in the FC mix

PFOS Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density g
0.500	0.500	0.500	0.500	0.500	0.500		0.002	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.004	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.010	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.020	0.167
0.500	0.500	0.500	0.500	0.500	0.500		0.040	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.010	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.020	0.167
5.00	5.00	5.00	5.00	5.00	5.00		0.030	0.167
50.0	50.0	50.0	50.0	50.0	50.0		0.004	0.167

Calculated concentrations of standards in the sample matrix

PFOS Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked mL
5.99	5.99	5.99	5.99	5.99	5.99		100	0.005
12.0	12.0	12.0	12.0	12.0	12.0		Surrogate Final conc ng/mL 0.500	
29.9	29.9	29.9	29.9	29.9	29.9			
59.9	59.9	59.9	59.9	59.9	59.9			
120	120	120	120	120	120			
299	299	299	299	299	299			
599	599	599	599	599	599			
898	898	898	898	898	898			
1198	1198	1198	1198	1198	1198			

Validated ranges – approximate concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb	5-1000 ppb
Bovine	Estimates only, use rabbit values.					
Rat	Estimates only, use rabbit values.					
Monkey	Estimates only, use rabbit values.					

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCCTANESULFONATE OR OTHER FLUORO-CHEMICALS 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.

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 μ L 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.

- 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:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

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 $\pm 30\%$ of the spiked concentration.
- 14.5 Continuing Calibration Verifications**
- 14.5.1** Continuing calibration verification percent recoveries must be $\pm 30\%$ 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

<u>Revision Number</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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

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

3M ENVIRONMENTAL LABORATORY

METHOD

ANALYSIS OF POTASSIUM PERFLUOROOCCTANESULFONATE OR OTHER FLUORO-CHEMICALS IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-7.0

Adoption Date:

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 analysis of liver 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 liver, or other tissues as designated in the validation report.

2.0 SUMMARY OF METHOD

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from liver 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 selected 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 mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (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 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 refer to 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 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). 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 shall 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 air 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 (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to ETS-8-6.0.

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 may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

10.0 QUALITY CONTROL

10.1 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 Checks

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 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 sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted $1/x$, not forced through the origin, 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 Set up the sample list.

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). 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. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

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

12.1.4 Samples are analyzed with a continuing calibration verification injected standard 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 μ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
1.0 min.	40%	60%
4.5 min.	95%	5%
6.5 min.	95%	5%
7.0 min.	40%	60%
9.0 mi.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to ETS-9-24.0, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

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

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 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. 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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 - 500 µL/min

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

- 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 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). 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:

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

13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentrations in matrix ($\mu\text{g/g}$):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\left(\frac{\text{Initial Weight of Liver (g)}}{\text{Final Volume (mL)}} \right)} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

14.0 METHOD PERFORMANCE

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to ETS-8-6.0, 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 must be below the lowest standard in the calibration curve.

14.3 Calibration Curves

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

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within $\pm 30\%$ of the spiked concentration.

14.6 If criteria listed in the method performance section are not 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, refer to **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-8-7.0 Data summary spreadsheet

18.0 REFERENCES

- 18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver 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-8-6.0 & 7.0-V-1**

19.0 AFFECTED DOCUMENTS

- 19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision
Number

Reason For Revision

Revision
Date

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 ng/g	Initial Wt. g	Dilution Factor	Final Conc. ug/g

Slope: Taken from linear regression equation.

Group/Dose: Taken from the study folder.

Sample#: Taken from the study folder.

Concentration (ng/g): Taken from the MassLynx integration summary.

Initial Wt. (g): Taken from the study folder.

Dilution Factor: Taken from the study folder.

Final Conc. (ug/g): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-154.0

Adoption Date:

Revision Date:

Author: Kristen J. Hansen/Harold O. Johnson

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

William K. Reagen, Laboratory Management

Date

Kristen J. Hansen, Ph.D., Group Leader

Date

Kent R. Lindstrom, Technical Reviewer

Date

1.0 SCOPE AND APPLICATION

- 1.1 This method provides collection, extraction, and analytical procedures for the determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane Sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in groundwater, surface water, and drinking water samples.
- 1.2 This method was prepared according to the EPA document, "Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141" (see Reference 18.1), and is based in part on the report "Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water" (see Reference 18.2).

2.0 SUMMARY OF METHOD

- 2.1 Water samples are collected from a site of interest and shipped cold to an analytical facility. PFOS, PFOSA, and POAA are extracted from 40mL water samples using C₁₈ solid phase extraction (SPE) cartridges. The compounds are eluted from the C₁₈ cartridge, using methanol. Separation, identification, and measurement are accomplished by high-performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) analysis using multiple response monitoring (MRM).

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

3.0 DEFINITIONS

- 3.1 **Analytical Sample**—A portion of an extracted Laboratory sample prepared for analysis.
- 3.2 **Calibration Standard**—A solution prepared from the Working Standard (WS) and extracted according to this method. The calibration standard solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 **Duplicate Sample (DS)**—A separate aliquot of a sample, taken in the analytical laboratory and analyzed separately with identical procedures. Analysis of DSs compared to that of the first aliquot give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.4 **Field Blank Control Sample (FB)**—Type I water placed in a sample container in the laboratory and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FB is to determine if test substances or other interferences are present in the field environment.

- 3.5 Field Duplicate (FD)**—A sample collected in duplicate at the same time as the sample and placed under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analysis of FD compared to that of the first sample gives a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.
- 3.6 Field Matrix Spike (FMS)**—A sample collected in duplicate to which known quantities of the target analytes are added in the field at the time of sample collection. The FMS should be spiked at approximately 50–150% of the expected analyte concentration in the sample. The FMS is analyzed to ascertain if any matrix effects, interferences, or stability issues may complicate the interpretation of the sample analysis.
- 3.7 Field Spike Control Sample (FSCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the field at the time of sample collection (at an appropriate concentration to be determined by the project lead). The FSCS is extracted and analyzed exactly like a sample to determine whether a loss of analyte could be attributed to sample storage and/or shipment.
- 3.8 Laboratory Control Sample (LCS)**—An aliquot of type I water to which known quantities of the target analytes are added in the laboratory. Two levels are included, one at the LOQ (approx. 25Pg/mL), the other at a concentration of approx. 100–250Pg/mL or another concentration to be determined by the project lead. The LCS is extracted and analyzed exactly like a laboratory sample to determine whether the methodology is in control, and whether the laboratory is capable of making accurate measurements at the required method detection limit and higher.
- 3.9 Laboratory Sample**—A portion of a sample received from the field for testing.
- 3.10 Limit of Detection (LOD)**—The lowest concentration of an analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. The LOD can be determined in several ways, including signal-to-noise ratio and statistical calculations.
- 3.11 Limit of Quantitation (LOQ)**—The lowest concentration (LLOQ) or highest concentration (ULOQ) that can be reliably achieved within the specified limits of precision and accuracy during routine operating conditions.
- Note:** The LLOQ is generally 5–10 times the LOD. For many analytes, the LLOQ analyte concentration is selected as the lowest non-zero standard in the calibration curve. However, it may be nominally chosen within these stated guidelines to simplify data reporting. Sample LLOQs are matrix-dependent.
- 3.12 Matrix Spike (MS)**—An aliquot of a sample, to which known quantities of target analytes are added in the laboratory. The MS is extracted and analyzed exactly like a laboratory sample to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS corrected for background concentrations.
- 3.13 Method Blank**—An aliquot of type I water that is treated exactly like a laboratory sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other laboratory samples. The method blank

is used to determine if test substances or other interferences are present in the laboratory environment, the reagents, or the apparatus.

- 3.14 Method Detection Limit (MDL) Determination**—One of several processes that may be used to establish a LOD value. The statistically calculated minimum amount of an analyte that can be measured with 99% confidence that the reported value is greater than zero. This term is usually associated with the EPA definition in 40 CFR Part 136 Appendix B.
- 3.15 Sample**—A sample is a small portion collected from a larger quantity of material intended to represent the original source material.
- 3.16 Spiking Stock Standard (SSS)**—A solution prepared from stock standards used to prepare the working standard.
- 3.17 Stock Standard (SS)**—A concentrated solution of a single analyte prepared in the laboratory with an assayed reference compound.
- 3.18 Working Standard (WS)**—A solution of several analytes prepared in the laboratory from SSs and diluted as needed to prepare calibration standards and other required analyte solutions.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings

- 4.1.1** The acute and chronic toxicity of the standards for this method have not been precisely determined; however, each should be treated as a potential health hazard.
- 4.1.2** Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.
- 4.1.3** The laboratory is responsible for maintaining a safe work environment and a current awareness of local regulations regarding the handling of the chemicals used in this method. A reference file of material safety data sheets (MSDS) should be available to all personnel involved in these analyses.

5.0 INTERFERENCES

- 5.1** During extraction and analysis, major potential contaminant sources are reagents and liquid-solid extraction devices.
- 5.2** All materials used in the analyses shall be demonstrated to be free from interferences under conditions of analysis by running method blanks.
- 5.3** Teflon[®] containing materials (e.g. caps, wash bottles) contain fluorocompounds which may cause interferences and should not be used during collection, storage, extraction, or analysis of the samples.

6.0 EQUIPMENT, SUPPLIES, AND MATERIALS

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

6.1 Sampling Equipment

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

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

6.1.2 Coolers for sample shipment.

6.1.3 Ice for sample shipment.

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

6.2 Laboratory Equipment (Extraction and Analytical)

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

6.2.2 Vacuum pump, Bchi.

6.2.3 Visiprep vacuum manifold, Supelco.

6.2.4 Sep Pak Vac 6cc (1g) tC₁₈ cartridges (part # WAT 036795), Waters.

6.2.5 50mL disposable polypropylene centrifuge tubes, VWR.

6.2.6 15mL disposable polypropylene centrifuge tubes, VWR.

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

6.2.8 Class A pipettes and volumetric flasks, various.

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

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

6.2.11 125mL LDPE narrow-mouth bottles, Nalgene.

6.2.12 HPLC pump (LC10AD), Shimadzu.

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

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

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

6.3 Equipment Notes

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

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

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

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

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

7.0 REAGENTS AND STANDARDS

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

7.1 Chemicals

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

7.2 Standards

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

7.3 Reagent Preparation

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

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

7.4 Spiking Stock Standard (SSS) Preparation

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

7.5 Calibration Standards

- 7.5.1 100µg/mL each PFOS, PFOSA, and POAA stock standard solutions**—Weigh out 10mg of analytical standard (corrected for percent salt and purity) and dilute to 100mL with methanol in a 100mL volumetric flask. Transfer to a 125mL LDPE bottle. Prepare a **separate** solution for each analyte. Store solutions in a refrigerator at 4°±2°C for a maximum period of 6 months from the date of preparation.
- 7.5.2 1µg/mL Working Standard**—Add 1.0mL each of the 100µg/mL SS solutions (from 7.5.1) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.3 0.1µg/mL Working Standard**—Add 10.0mL of the 1.0µg/mL mixed solution (from 7.5.2) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.4 0.01µg/mL Working Standard**—Add 10.0mL of the 0.1µg/mL mixed solution (from 7.5.3) to a 100mL volumetric flask and bring up to volume with methanol.
- 7.5.5 Storage Conditions**—Store all WSs in a refrigerator (in 125mL LDPE bottles) at 4°±2°C for a maximum period of 3 months from the date of preparation.
- 7.5.6 Calibration Standard**—Prepare a minimum of five calibration solutions in filtered type I water according to the following table:

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

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

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

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

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

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

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

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

- 8.1 Tap Water**—Open the tap and allow the system to flush until the water temperature ($15^{\circ}\pm 10^{\circ}\text{C}$) has stabilized (usually about two minutes). Adjust the flow to about 500mL/min and collect samples from the flowing stream.
- 8.2 Ground Water**—Purge the well of standing water using a pump or a bailer. Collect the sample directly from the pump or from the bailer.
- 8.3 Surface Water**—When sampling from an open body of water, fill the sample container with water from a representative area.
- 8.4 Sample Dechlorination**—All samples should be iced or refrigerated at $4^{\circ}\pm 2^{\circ}\text{C}$ and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced by adding 200 μL of a 250mg/mL sodium thiosulfate solution to each water sample, FB, and FSCS (which may be placed in each bottle before leaving for the sampling site.).
- 8.5 Holding Time (HT)**— Results of the time/storage study of all target analytes showed that the three compounds are stable for 14 days in water samples when the samples are dechlorinated and stored as described in section 8.4 (see also reference 18.3). Therefore, laboratory samples must be extracted within 14 days and the extracts analyzed within 30 days of sample collection. If the HT exceeds 14 days, great care is used when evaluating field spikes to avoid misrepresentation of the sample concentration.
- 8.6 Field Blanks**
- 8.6.1** Process a Field Blank Control Sample (FB) along with each sample set (samples collected from the same general sample site at approximately the same time). At the laboratory, prior to sample collection, fill a sample container with filtered type I water, seal, and ship the FB to the sampling site along with the empty sample containers. Return the FB to the laboratory with the filled sample bottles.
- 8.6.2** When sodium thiosulfate is added to samples, use the same procedure to preserve the FB.
- 8.7 Field Duplicates**
- 8.7.1** Collect a Field Duplicate (FD) for every ten (10) samples collected or per each sampling set, if less than 10 samples are collected.
- 8.7.2** Separate FDs must be collected for each type of water sample (ground, tap, etc.) collected.
- 8.7.3** Collect the FD immediately after the sample.
- 8.7.4** Preserve, store and ship FD using the same procedures as used for the samples.

8.8 Field Spike Control Sample (FSCS)

- 8.8.1** A Field Spike Control Sample (FSCS) must be prepared for each sample shipment. If multiple coolers are used to ship a set of samples, each cooler must contain a FSCS.
- 8.8.2** At the laboratory, fill a sample container with 100mL of type I water. Seal and ship to the sampling site along with the empty sample containers and FBs.
- 8.8.3** When sodium thiosulfate is added to samples, use the same procedure to add the same amounts to the FSCS.
- 8.8.4** Seal and gently invert the FSCS to mix. Store and ship the FSCS using the same procedures as used for the samples.

9.0 EXTRACTION PROCEDURE

9.1 Extraction Scheme

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

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

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

9.1.3 Condition the C₁₈ SPE cartridges (1g, 6mL) by passing 10mL methanol followed by 5mL filtered type I water (~2drop/sec). Do not let column run dry.

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

9.1.4 Load the analytical sample onto the C₁₈ SPE cartridge. Discard eluate.

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

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

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

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

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

9.1.9 Standardization of C₁₈ SPE columns—If poor recoveries are observed, it may be necessary to standardize the C₁₈ SPE columns in the following manner before analyzing samples.

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

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

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

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

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

10.0 CALIBRATION AND STANDARDIZATION (ANALYTICAL SETUP)

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

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

Mass Spec: Micromass Quattro Ultima (Micromass)

Interface: Electrospray (Micromass)

Mode: Electrospray Negative, Multiple Response Monitoring (MRM)

Harvard infusion pump (Harvard Instruments), for tuning

Computer: COMPAQ Professional Workstation AP200

Software: Windows NT, MassLynx 3.3

HPLC: Hewlett Packard (HP) Series 1100

HP Quat Pump

HP Vacuum Degasser

HP Autosampler

HP Column Oven

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

HPLC Column: Genesis C₈ (Jones Chromatography), 2.1mm x 50mm, 4µm

Column Temperature: 35°C

Injection Volume: 15µL

Mobile Phase (A): 2mM Ammonium Acetate in filtered type I water (See 7.3.1)

Mobile Phase (B): Methanol

HPLC Gradient Program:

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

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

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1mm x 30mm) and columns from different manufacturers (Keystone Betasil C₁₈ etc.) may be used.

Ions Monitored:

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

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

10.2 Tune File Parameters

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

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

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

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

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

Pressures	Set
Gas Cell	3.0e–3mbar

11.0 ANALYTICAL QUALITY CONTROL

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

12.0 ANALYTICAL PROCEDURE

12.1 Sample Analysis

12.1.1 Set up analysis sample queue.

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

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

12.2 Calibration Curve

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

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

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

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

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

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

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

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

14.0 METHOD PERFORMANCE PARAMETERS

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

- 14.1 Linearity**—Linear standard curves for quantitation generated for each analyte by linear regression with 1/x weighting of peak area versus calibration standard concentration. The correlation coefficient (r) for the calibration curves must be ≥ 0.990 ($r^2 \geq 0.980$).
- 14.2 Calibration Curve Standards**—The measured value for each curve point must be within $\pm 30\%$ of theoretical values when curve is evaluated over a range appropriate to the data. High or low points may be deactivated to achieve these criteria, but an acceptable curve must contain at least five active curve points.
- 14.3 CCV Performance**—Mid and low level calibration checks to be analyzed every 5–10 injections. The analyte level measured in the CCVs should be within $\pm 30\%$ of theoretical values. If CCVs fall outside of this range, data collected subsequent to the last passing CCV should not be used. Only data collected between acceptable CCVs can be used.
- 14.4 Limit of Detection (LOD)**—The lowest calibration standard with a peak area at least 2X the peak area of the extraction blank that can be measured at a concentration greater than zero.
- 14.5 Limits of Quantitation (LOQ)**—The lower LOQ (LLOQ) is the lowest non-zero active standard in the calibration curve; the peak area of the LLOQ must be at least 2X that of the extraction blank. By definition, the measured value of the LLOQ must be within 30% of the theoretical value.
- 14.6 Matrix Spikes**—Matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.
- 14.7 Solvent Blanks, Method Blanks, and Matrix Blanks**—Values must be below the lowest non-zero active standard in the calibration curve. Matrix blanks are considered compliant if no test substance is detected above the LOD for that analyte.
- 14.8 Reproducibility**—Reproducibility of the method is defined by the results of the matrix spikes and matrix spike duplicates. The MS/MSD should be reproducible to within 20%.
- 14.9 Use of Confirmatory Methods**—None
- 14.10 Demonstration of Specificity**—Specificity is demonstrated by chromatographic retention time (within 3% of standard) and the mass spectral response of unique product ions generated from a characteristic primary ion.
- 14.11 Documentation**
- 14.11.1** If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed, or other actions taken as determined by the analyst. Document all actions in the appropriate logbook.
- 14.11.2** If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

- 16.1** Each page generated for a study must have the following information included, either in the header or hand-written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument run log.
- 16.3** Plot the calibration curves as described in this method, then print these graphs and store in the study folder.
- 16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.
- 16.5** Summarize data using suitable software (MS Excel 97) and store in the study folder.
- 16.6** Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

17.0 ATTACHMENTS

- 17.1 Attachment A: Figures—Fluorochemical Compounds**

18.0 REFERENCES

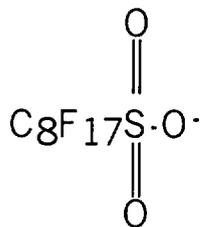
- 18.1** “Guidelines and Format for Methods to be Proposed at 40 CFR Part 136 or Part 141”, U.S. Environmental Protection Agency, Office of Science and Technology Office of Water, Washington, D.C. Draft 1996.
- 18.2** “Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water”, E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, January 2000.
- 18.3** Validation report for the “Method of Analysis for the Determination of Perfluorooctane sulfonate (PFOS), Perfluorooctane sulfonylamide (PFOSA), and Perfluorooctanoate (POAA) in Water”, E. Wickremesinhe and J. Flaherty, Study Number 023–002, Centre Analytical Laboratories, Inc., State College, Pennsylvania, (Approval pending)

19.0 REVISIONS

<u>Revision Number.</u>	<u>Reason For Revision</u>	<u>Revision Date</u>
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Figure 1: PFOS

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

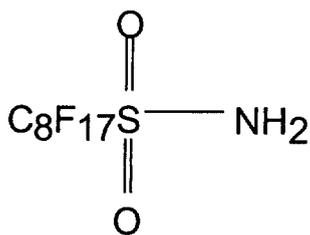


PFOS

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

Figure 2: PFOSA

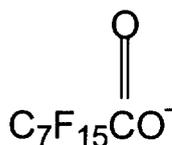
Chemical Name = Perfluorooctane sulfonylamide
Molecular ion = 498 ($C_8F_{17}SO_2NH_2$)



PFOSA

Figure 3: POAA

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



POAA

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

Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices

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Abstract

Since the early 1980s, there has been a steady increase in the use of nonvolatile fluorinated organic compounds for a variety of industrial applications. The industrial use of these relatively stable compounds has initiated debate over the fate of fluorochemicals in the environment and, ultimately, the bioavailability of these compounds (1,2). Until recently, levels of organic fluorochemicals in biological matrices have been determined by non-chemical specific analytical methods such as total fluoride analysis (2-6). In this manuscript, we present a compound-specific method for the extraction of extremely low levels of several commercial organic fluorochemicals from sera and liver with quantitative detection by negative ion electrospray tandem mass spectrometry. This technique represents a robust, previously undescribed approach to quantifying specific organic fluorochemicals in biological matrices. This method should prove useful in future studies designed to determine the levels of organic fluorochemicals in humans and the environment. Results from a study of 65 human sera samples purchased from biological supply companies and the details of the analytical method for the quantitative analysis of specific organic fluorine containing compounds are described.

Introduction

The unique chemical/physical properties of fluorine make fluorinated organic compounds useful for many commercial applications, and industrial production of these compounds has increased significantly since the early 1980s. Fluorinated organics are used as refrigerants, surfactants, and polymers and as components of pharmaceuticals, fire retardants, lubricants, and insecticides (1). Fluorochemical compounds that are not perfluorinated may be susceptible to partial chemical breakdown at functional group bonds (7). However, given the energy of the carbon-fluorine bond, it is expected that many organic fluorochemical compounds will be resistant to hydrolysis, photolysis, biodegradation, or metabolism (8). For example, even in the high-energy environment of the stratosphere, the carbon-fluorine bonds in chlorofluorocarbons are exceptionally stable (9).

In 1974, Guy et al. reported results for the determination of organic fluorine levels in plasma from 106 individuals from five cities in the United States (2). These researchers demonstrated that although levels of inorganic fluorine in human plasma could be correlated to fluoride levels in drinking water, organic fluorine levels showed no such correlation. Guy et al. showed that the organic fluorine levels measured from samples collected within a particular city were, basically, log normally distributed with few outliers. The average organic fluorine level in human plasma samples included in the study was reported to be 1.35 ± 0.85 micromolar R-F (approximately 26 ppb organic fluorine). By concentrating the organic fluorine from 20 liters of plasma and performing nuclear magnetic resonance (NMR) analysis, Guy et al. postulated that the

perfluorooctanoate anion (PFOA) or a structurally related compound may be the source of the organic fluorine. Further, they suggested that there may be three or more different components of organic fluorine in plasma samples collected from the general population. Although the source of the organic fluorine in general population blood has been debated and never definitively determined, some have postulated that contamination of the environment with industrial fluorochemicals is the source of the organic fluorine compounds (4). Others suggest that the organic fluorine is likely to have a natural source (10). Despite the route of exposure, researchers agree that such low levels of organic fluorochemicals are unlikely to cause toxic effects (2,11,12).

A large number of studies in both humans and animals have been conducted to study the toxicity associated with PFOA. In these studies, when determinations of the PFOA levels in tissues were necessary, a total organic fluorine method was employed or a study using radiolabeled material was designed, because easy, sensitive, compound-specific methods have not been available (11-17). Historically, low-level detection of fluorochemicals such as PFOA and perfluorooctanesulfonate (PFOS) has been limited to relatively insensitive or non-mass-specific detection methods, such as gas chromatography-flame ionization detection, gas chromatography-electron capture detection and high performance liquid chromatography (HPLC)-ultraviolet detection (18-20).

In the work presented here, a new method for the analysis of several low-level fluorinated organic compounds in sera and liver tissue is described. After initial extraction of the tissue with an ion-pairing reagent, extracts are analyzed with HPLC-negative ion electrospray tandem mass spectrometry (HPLC-ESMSMS). The ability to select a unique

product ion upon fragmentation of the molecular ion provides a very selective analysis that is not as likely to be affected by biological interferences.

Detection limits and the linear range of the method were determined for four fluorinated organic compounds [PFOA, PFOS, perfluorooctanesulfonylamide (PFOSA), and perfluorhexanesulfonate (PFHS)] in both liver and sera by spiking each matrix with standard material and quantitatively recovering the compounds. Although detection limits can be improved by concentrating sample extracts, extraction of non-concentrated sera produced detection limits for all target analytes of 1-3 ppb.

The method presented here has been used to quantitatively analyze four organic fluorochemicals in 65 human sera samples collected from several biological supply companies in the United States. High-resolution time-of-flight mass spectrometry was used to confirm the identity of PFOS, PFOA, PFHS, and PFOSA extracted from a single representative sera sample.

Experimental Materials and Methods

Rabbit and rat sera were purchased from Sigma (St. Louis, MO). HPLC-grade methyl-tert-butyl-ether (MTBE) and methanol were purchased from E.M. Science (Gibbstown, NJ); the tetra-butyl ammonium (TBA) hydrogen sulfate was purchased from Kodak (Rochester, NY); the pH of the TBA solution was adjusted with sodium hydroxide (J.T. Baker; Phillipsburg, NJ). Before use, water was purified with a Milli-Q[®] system (Millipore; Bedford, MA). Human sera samples were purchased from the following biological supply companies: Sigma (St. Louis, MO); Golden West Biologicals

(Temecila, CA); Biological Specialty Corporation (Colmar, PA); and Lampire Biological Laboratories (Pipersville, PA).

New Zealand White [Hra:(NZW)SPF] rabbit liver was obtained from Covance Laboratories, Inc., in Madison, WI.; Sprague Dawley rats were purchased from Harlan (Indianapolis, IN), and rat liver samples were harvested by 3M Toxicology personnel (St. Paul, MN).

The PFOS and PFOA used as standards and as matrix spikes were purchased from Fluka (Milwaukee, WI); standards of PFHS and PFOSA were made available from 3M Company (St. Paul, MN). The internal standard, *1H,1H,2H,2H* perfluorooctane sulfonate (THPFOS), was purchased from ICN (Costa Mesa, CA).

Extraction Procedure: One half mL of sera, 5 μ L of internal standard, 1 mL of 0.5 M TBA solution (adjusted to pH 10), and 2 mL of 0.25 M sodium carbonate buffer were added to a 15-mL polypropylene tube for extraction. After thorough mixing, 5 mL of MTBE was added to the solution, and the mixture was shaken for 20 minutes. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4.0 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice more; all rinses were combined in a second polypropylene tube. The solvent was allowed to evaporate under nitrogen before being reconstituted in 0.5 mL of methanol. The sample was vortex mixed for 30 seconds and passed through a 0.2 μ m nylon mesh filter into an autovial. Depending upon the species of test animal, the serum extract was typically either colorless or light yellow.

For the extraction of liver samples, a liver homogenate of 1 gram of liver to 5 mL of Milli-Q water was prepared. One mL of the homogenate was added to a polypropylene tube, and the sample was extracted according to the procedure for sera (described above).

Teflon[®] or glass containers were avoided in this procedure; the former may cause analytical interferences, and the latter may bind the surfactants in an aqueous solution. Disposable polypropylene or plastic lab wear was used to minimize the possibility of sample contamination that can occur when glassware is reused. Any glassware used in the preparation of the reagents was thoroughly rinsed with methanol prior to use.

To ensure that target analytes were not introduced to the matrix prior to extraction, blood collection supplies were extracted and analyzed. Blood bags were purchased from Baxter (Deerfield, IL), and five different types of Vacutainers[®] (two labeled “gel and clot activator,” two labeled “K₃EDTA,” and one labeled “no activator”) were purchased from Becton Dickinson (Franklin Lakes, NJ). Additional blood collection materials tested consisted of 3-cc and 10-cc syringes, 19G1 1/2 Precision Guide[®] sterile needles, multiple sample Vacutainer sterile needles, and Terumo[®] winged infusion sets, all of which were obtained from Becton Dickinson.

The inside surfaces of all blood collection supplies were exposed to methanol (from 0.5 mL to 80 mL, depending on the particular supply) for 1 hour. The extraction solvent was dried and reconstituted to exactly 1 mL of methanol. A second set of samples was

spiked with analyte and extracted in exactly the same way as the first set to ensure that analyte could be recovered.

Extraction blanks were prepared using Milli-Q water, and matrix blanks were prepared from rabbit or rat tissue spiked with THPFOS.

Analyte separation was performed using a Hewlett-Packard HP1100 liquid chromatograph modified with low dead-volume internal tubing. Prior to the autosampler, a 1 cm Hypercarb cartridge from Keystone (Bellefonte, PA) was added. Ten μL s of extract were injected onto a 50 x 2mm (5 μm) Keystone Betasil[®] C₁₈ column with a 2 mM ammonium acetate/methanol mobile phase starting at 45% methanol. At a flow rate of 300 $\mu\text{L}/\text{minute}$, the gradient increased to 90% methanol before reverting to original conditions at 9 minutes. Column temperature was maintained at 25° C.

For quantitative determination, the HPLC system was interfaced to a Micromass[®] (Beverly, MA) Quattro II atmospheric pressure ionization tandem mass spectrometer operated in the electrospray negative mode. Instrumental parameters were optimized to transmit the [M-H] ion for all analytes. When possible, multiple daughter ions were monitored, but quantitation was based on a single product ion. Refer to Table 2 for a summary of transitions monitored.

In all cases, the capillary was held between 1.6- 3.2 kV. For PFOA determination, the quantitation ion ($m/z=169$) corresponds to C₅F₉⁻; the product ion $m/z=99$ corresponds to

FSO_3^- for quantitative determination of PFOS. Quantitation of PFOSA occurs at $m/z=78$, corresponding to SO_2N^- ; quantitation of PFHS occurs at $m/z=80$ (SO_3^-).

In the ESMSMS system, the 499 Da. \rightarrow 80 Da. transition can provide a stronger signal than the 499 Da. \rightarrow 99 Da. transition of the PFOS analysis. However, in the analysis of tissue samples collected from some species of animals, an unidentified interferent was present in the 499 Da. \rightarrow 80 Da. transition. Although this interferent was rarely observed, to ensure complete selectivity, quantitation was based on the 499 Da. \rightarrow 99 Da. transition.

Exact mass determination was achieved by interfacing the chromatographic system to either a Micromass[®] LCT; product ion spectra were collected with a Micromass[®] Q-TOF. Both the LCT and Q-TOF are high-resolution time-of-flight mass spectrometers. An 800 ng/mL solution of raffinose in 50/50 ACN/water was infused into the source at 20 $\mu\text{L/hr}$ as a lock mass (503.1612 Da). PFOS, PFOSA, and PFHS were measured at a cone voltage of 70 V; PFOA was measured at a 10-V cone voltage. For analysis of all analytes, the capillary was maintained at 3200 V.

Results and Discussion

Characterization of the Method

A series of experiments, described in more detail below, was designed to characterize the analytical method. In general, all curves, extracted or unextracted, were plotted using linear regression, weighted $1/X$. Tables 3 and 4 show the extraction efficiency, limit of detection, and linear range for the target analytes.

With the exception of PFOA, the extraction efficiency was determined by extracting and analyzing six replicate rat or rabbit sera samples spiked at approximately the following levels: 10 ng/mL, 50 ng/mL, 100 ng/mL, and 500 ng/mL. For PFOA, only the three higher levels were used for extraction efficiency calculations. Extraction efficiency in liver was determined by extracting samples spiked at 50 ng/g, 100 ng/g, and 500 ng/g. For both sera and liver, the extracted samples were evaluated versus the average curve produced by two unextracted solvent curves analyzed before and after the extracts. The extraction efficiency for PFHS and PFOSA from liver was determined to be significantly lower than those determined for the PFOS and POAA. However, because sample analysis is conducted using extracted curves, the relatively low recoveries should not affect the results. Table 3 shows the compiled average for all spike levels along with the standard deviation.

For both sera and liver analyses, the internal standard was used for quantitative determination of PFOS and PFOA, only.

The limit of detection was determined as per EPA Regulation 40 CFR part 136, Appendix B. For each analyte, seven low-level spikes were prepared and analyzed. Based on the standard deviation associated with the replicate analysis, a limit of detection was calculated. This calculated limit of detection was verified by analyzing a sample that was spiked at that level and extracted.

The linear range was determined by analyzing duplicate curves extracted from each matrix over a wide range. Starting with the highest standard, points were removed from the curve until the correlation coefficient for the 1/x weighted fit was greater than 0.99. For the sera curves, all points except for the lowest standard level were evaluated to be within 20% of the expected value. For the standard curves extracted from liver, all points except the lowest point were within ± 30 .

Characterization of Blanks

Method blanks were prepared from Milli-Q water. Because analyte-free (less than 1 ng/mL) human sera matrix could not be located, surrogate matrix blanks were prepared from rabbit sera. None of the analytes were detected in either set of blanks. Instrument blanks, consisting of HPLC-grade methanol, were analyzed after high-level-standard-curve points and after periodic calibration checks, to monitor potential carry-over. No carry-over was observed.

Methanol extracts of blood collection supplies were analyzed; none of the target analytes were detected in these extracts. In addition, the Teflon cap liners of glass jars used for reagent storage were extracted with methanol. Low-levels of PFOS and PFOA were detected in some of the extracts of the Teflon liners. These materials were removed from the extraction procedure.

Figure 1 compares the results of the multiple response monitoring (MRM) analysis for PFOS in an extraction blank, in unspiked rabbit sera, and in unspiked, commercially available human sera.

Identification of Target Analytes

The retention times of the analytes extracted from human sera were matched to within 2% of the retention time of standard material spiked into and extracted from rabbit sera. MRM analysis was used for verification of analyte identity. For each analyte except PFOSA, at least two characteristic product ions were monitored, although quantitation was based on the response of a single product ion. PFOSA was detected at such low levels, only a single product ion could be monitored, even for qualitative purposes. In the human sera samples, for all analytes except PFOSA, the relative abundances of two or more product ions collected by MRM were confirmed to within 20% of standards as criteria for analyte verification (21).

To further verify the identity of the detected analytes, a 30-fold concentrated extraction of one sera sample was prepared. This concentrated extract was used for exact mass determination of all four analytes using high-resolution time-of-flight mass spectrometry. The concentration of the detected analytes were confirmed to within 5 ppm for all target analytes. Figure 2 shows the results of the PFOS and PFOSA high-resolution analysis. Additionally, using high-resolution time-of-flight mass spectrometry, full product ion spectra were collected for each analyte in the concentrated extract. The product ion spectra for the PFOS identified in human sera is shown in Figure 3.

Quantitation of Target Analytes in Human Sera

Quantitation of the analytes was based on comparison of a single product ion peak area to the response of two standard curves, weighted 1/X, bracketing each sample set. Mid-level calibration checks were analyzed every five to ten samples. Based on the precision

determined from repeat injections of the standard curves, results were considered quantitative to $\pm 30\%$. Quantitative results, presented as compound-specific average concentrations in sera are presented in Table 5. In addition to the average analyte concentration, the concentration of organic fluorine represented by each compound is presented. For example, by weight, PFOS is 65% fluorine; for samples reported here, the average PFOS concentration was determined to be 33 ng/mL of PFOS. This corresponds to about 22 ng/mL of organic fluorine.

Added together, the four specific fluorochemicals measured in this small set of samples account for approximately 31 ng of organic fluorine per milliliter of sera. Within experimental error associated with each technique, this value compares closely to the value obtained by Guy et al. (approx. 26 ng/mL) more than 20 years ago (2).

Also in accordance with Guy et al., PFOA has specifically been identified in the sera samples, although not necessarily as the major component. For the 65 samples reported here, PFOS was present at the highest concentration. Each analyte measured was detected in every sample, with the following significant exceptions: PFOSA was not measured above the detection limit in 60 of the 65 samples; PFHS was not detected in one sample.

A combination of extraction and analytical methods that do not require chemical derivitization, use small volumes of samples, and are highly sensitive and mass specific were developed for the low-level analysis of several fluorinated organic compounds in sera and liver. Using these methods, samples of human sera collected from biological supply companies were analyzed for four separate fluorochemicals, PFOA, PFOS, PFHS,

and PFOSA. Taken together, these fluorochemicals account for 31 ng/mL of organic fluorine in an examination of 65 human sera samples from biological supply companies, consistent with historical reports of total organic fluorine studies.

Although this study comprises a relatively small sample set, it does suggest the possibility of a more complete characterization of the organic fluorine compounds present in human sera. Additionally, these compound-specific analyses should be paired with a total organic fluorine analysis to determine what fraction of the total organic fluorine present is due to the four fluorochemicals quantified in this study.

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References

- 1 Key, B.D., Howell, R.D., and Criddle, C.S. Fluorinated Organics in the Biosphere. *Environ. Sci. Technol.* **1997**, *31*, 2445.
- 2 Guy, W.S., Taves, D.R., and Brey, W.S., Jr. Organic Fluorocompounds in Human Plasma: Prevalence and Characterization. *Biochemistry Involving Carbon-Fluorine Bonds*, **Edition number; Publisher; Place of Publication; Year; Volume number**; pp. 117-XXX.
- 3 Taves, D. Evidence that there are Two Forms of Fluoride in Human Serum. *Nature* **1968**, *217*, 1051.
- 4 Taves, D.R. Comparison of 'Organic' Fluoride in Human and Nonhuman Serum. *Nature* **1971**, *50*, 783.
- 5 Belisle, J., and Hagen, D.F. Method for the Determination of the Total Fluorine Content of Whole Blood, Serum/Plasma, and Other Biological Samples *Anal. Biochem.* **1978**, *87*, 545.
- 6 Yamamoto, G., Yoshitake, K., Sato, T., Kimura, T., and Ando, T. Distribution and Forms of Fluorine in Whole Blood of Human Male *Anal. Biochem.* **1989**, *182*, 371.
- 7 Hagen, D.F.; Belisle, J.; Johnson, J.D.; Venkateswarlu, V. Characterization of Fluorinated Metabolites by a Gas Chromatographic Helium Microwave Plasma Detector – The Biotransformation of 1H,1H,2H,2H-Perfluorodecanol to Perfluorooctanoate. *Anal. Biochem.* **1981**, *118*, 336.
- 8 *Organofluorine Chemistry Principles and Commercial Applications*; Banks, R.E.; Smart, B.E.; Tatlow, J.C., Eds.; Plenum Press: New York, 1994.
- 9 *Atmospheric Chemistry*; Finlayson-Pitts, B.J., Pitts, J.N. Jr., Eds; John Wiley & Sons: New York, 1986.
- 10 Belisle, J. Organic Fluorine in Human Serum: Natural Versus Industrial Sources. *Science* **1981**, *212*, 1509.
- 11 Gilliland, F.D., and Mandel, J.S. Serum Perfluorooctanoic Acid and Hepatic Enzymes, Lipoproteins, and Cholesterol: A Study of Occupationally Exposed Men *Am. J. Ind. Med.* **1996**, *29*, 560.
- 12 Griffith, F.D., and Long, J. E. Animal Toxicity Studies with Ammonium Perfluorooctanoate. *Am. Ind. Hyg. Assoc. J.* **1980**, *41*, 576.

- 13 Kennedy, J.R., and Gerald, L.; Dermal Toxicity of Ammonium Perfluorooctanoate. *Toxicol. Appl. Pharmacol.* **1985**, *81*, 348.
- 14 Kennedy, G.L., Jr., Hall, G.T., Brittelli, M.R., and Chen, H.C. Inhalation Toxicity of Ammonium Perfluorooctanoate. *Food Chem. Toxicol.* **1986**, *24*, 1325.
- 15 Hanhijarvi, H., Ophaug, R.H., and Singer, L. The Sex-related Difference in Perfluorooctanoate Excretion in the Rat. *Proceedings of the Society for Experimental Biology and Medicine* **1982**, *171*, 50.
- 16 Venden Heuvel, J.P., Kuslikis, B.I., Van Rafelghem, M.J., and Peterson, R.E. Tissue Distribution, Metabolism, and Elimination of Perfluorooctanoic Acid in Male and Female Rats. *J. Biochem. Toxicol.* **1991**, *6*, 83.
- 17 Venden Heuvel, J.P., Davis, J.W., II, Sommers, R., and Peterson, R.E.; Renal Excretion of Perfluorooctanoic Acid in Male Rats: Inhibitory Effect of Testosterone. *J. Biochem. Toxicol.* **1992**, *7*, 31.
- 18 Ohya, T., Kudo, N., Suzuki, E., and Kawashima, Y., Determination of Perfluorinated Carboxylic Acids in Biological Samples by High-performance Liquid Chromatography. *J. Chromatogr.* **1998**, *720*, 1.
- 19 Belisle, J., and Hagen, D.F. A Method for the Determination of Perfluorooctanoic Acid in Blood and Other Biological Samples. *Anal. Biochem.* **1980**, *101*, 369.
- 20 Ylinen, M., Hanhijarvi, H., Peura, P., Ramo, O. Quantitative Gas Chromatographic Determination of PFOA as the Benzyl Ester in Plasma and Urine. *Arch. Environ. Contam. Toxicol.* **1985**, *14*, 713.
- 21 Lily Y.T., Campbell, D.A., Bennett, P.K., and Henion, J. Acceptance Criteria for Ultratrace HPLC-Tandem Mass Spectrometry: Quantitative and Qualitative Determination of Sulfonylurea Herbicides in Soil, *Anal. Chem.* **1996**, *68*, 3397.

COMPOUND	PRIMARY ION (DA)	PRODUCT IONS (DA)	OPTIMAL CONE VOLTAGE (V)	OPTIMAL COLLISION ENERGY (eV)
PFOA	413	119, 169*, 219	25	20
PFOS	499	80, 99*, 130	60	45
PFHS	399	80, 99*, 130	60	45
PFOSA	498	78*	60	45
THPFOS	427	80*	60	40

*Product ions were used for quantitation.

Table 2. Summary of Primary Ions, Product Ions, and ESMSMS Conditions

ANALYTE	EXTRACTION EFFICIENCY ± STANDARD DEVIATION	LIMIT OF DETECTION	LINEAR RANGE, EXTRACTED	CORRELATION COEFFICIENT
PFOA	101 ± 9 %	1.0 ppb	5-1000 ppb	0.998
PFOS	93 ± 9 %	1.7 ppb	5-1000 ppb	0.995
PFOSA	95 ± 6 %	1.5 ppb	5-1000 ppb	0.998
PFHS	85 ± 7 %	2.0 ppb	5-1000 ppb	0.998

Table 3. Method Characteristics for the Analysis of Specific Organic Fluorochemicals in Sera. (All concentrations are expressed as ng/g.)

ANALYTE	EXTRACTION EFFICIENCY	LIMIT OF DETECTION	LINEAR RANGE, EXTRACTED	CORRELATION COEFFICIENT
PFOA	87 ± 12 %	5.0 ppb	10-1000	0.989
PFOS	100 ± 13 %	8.5 ppb	5-1000	0.991
PFOSA	56 ± 11 %	3.5 ppb	5-1000 ppb	0.995
PFHS	71 ± 23 %	2.0 ppb	5-1000 ppb	0.994

Table 4. Method Characteristics for the Analysis of Specific Organic Fluorochemicals in Liver. (All concentrations are expressed as ng/g.)

ANALYTE	AVERAGE CONCENTRATION IN SERA	RANGE OF CONCENTRATION IN SERA	CONCENTRATION OF ORGANIC FLUORINE REPRESENTED
PFOS	33 ± 15	5-85	22
PFOA	6.6 ± 3*	1-13	4.6
PFHS	6.4 ± 5*	1-13	3.7
PFOSA	1.8 ± 0.3*	<1-2	1.1

* Several samples were determined to contain the target analyte below the limit of quantitation, therefore, average concentration is estimated.

Table 5. Concentrations (ng/mL) of Various Organic Fluorochemicals in Human Sera

Figure 2. High Resolution Analysis of PFOS and PFOSA

Figure 3. Product Ion Spectra for PFOS Endogenous in Human Sera