

**STUDY TITLE**

Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard Liver  
for Analysis Using HPLC-Electrospray/Mass Spectrometry

**DATA REQUIREMENTS**

Analytical Method Requirements

**STUDY DIRECTOR**

Sean Gallagher

**STUDY COMPLETED ON**

September 06, 2001

**PERFORMING LABORATORY / TESTING FACILITY**

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**STUDY SPONSOR**

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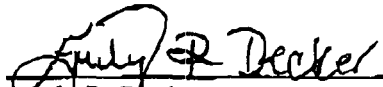
**PROJECT**

Centre Protocol Number: 00P-023-042  
Centre Study Number: 023-042

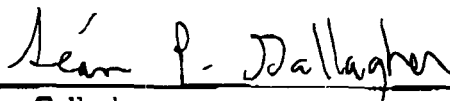
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**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

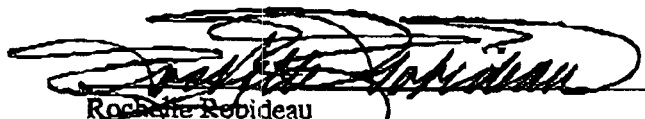
Centre Study Number 023-042, entitled "Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry," conducted for 3M Environmental Technology and Safety Services, was performed in compliance with US EPA TSCA Good Laboratory Practice Standards (40 CFR Part 792) by Centre Analytical Laboratories, Inc.

  
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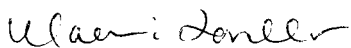
  
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## QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-042, entitled, "Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry". All reviewed phases were inspected for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

| <u>Phase</u>                        | <u>Date Inspected</u> | <u>Date Reported to Centre Management</u> | <u>Date Reported to Study Director and Sponsor Management</u> |
|-------------------------------------|-----------------------|-------------------------------------------|---------------------------------------------------------------|
| 1. Protocol Review                  | 11/20/00              | 11/30/00                                  | 12/28/00                                                      |
| 2. Extraction, Fortification        | 11/20/00              | 11/30/00                                  | 12/28/00                                                      |
| 3. Raw Data Review                  | 12/12/00              | 12/21/00                                  | 12/28/00                                                      |
| 4. Raw Data and Draft Report Review | 3/18-21/00            | 3/29/01                                   | 5/7/01                                                        |
| 5. Final Report Review              | 9/6/01                | 9/6/01                                    | 9/6/01                                                        |

  
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9/6/01  
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**CERTIFICATION OF AUTHENTICITY**

This report, for Centre Study Number 023-042, is a true and complete representation of the raw data for the study.

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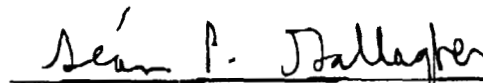
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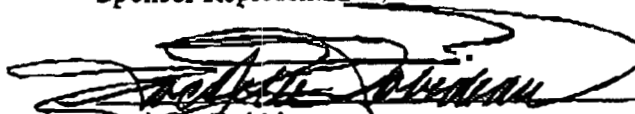
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## **STUDY IDENTIFICATION**

Extraction of Potassium Perfluorooctanesulfonate from Mallard Serum and Mallard Liver  
for Analysis Using HPLC-Electrospray/Mass Spectrometry

CENTRE PROTOCOL NUMBER: 00P-023-042

CENTRE STUDY NUMBER: 023-042

TYPE OF STUDY: Analytical

SAMPLE MATRIX: Mallard Serum and Mallard Liver

TEST SUBSTANCE: Perfluorooctanesulfonate (PFOS)

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TESTING FACILITY: Centre Analytical Laboratories, Inc.  
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State College, PA 16801

|                                |                                |          |
|--------------------------------|--------------------------------|----------|
| ANALYTICAL PHASE<br>TIMETABLE: | Study Initiation Date:         | 11/10/00 |
|                                | Experimental Start Date:       | 11/17/00 |
|                                | Experimental Termination Date: | 03/13/01 |
|                                | Study Completion Date:         | 09/06/01 |

**PROJECT PERSONNEL**

The Study Director for this project was Sean Gallagher at Wildlife International, Ltd. The following personnel from Centre Analytical Laboratories, Inc., were associated with various phases of the study:

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| Dave Bell               | Scientist         |
| Melissa Kennedy Whitsel | Group/Team Leader |
| Tiffany Proctor         | Technician        |
| Angela Morgan           | Technician        |
| Michelle Arjmand        | Technician        |
| Lawrence Ord            | Sample Custodian  |
| Rickey Keller           | Sample Custodian  |
| Karen Risha             | Scientist         |

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## **1.0 SUMMARY**

Centre Analytical Laboratories, Inc. (Centre) extracted mallard liver samples and mallard serum samples for the determination of perfluorooctanesulfonate (PFOS) in mallard serum and mallard liver according to protocol 00P-023-042 (Appendix A).

The limit of quantitation for mallard liver was 10 ppb and 10 ng/mL for mallard serum. The LOQ for each matrix was determined in a method verification study performed at Centre (Centre Study: 023-044). Residues of PFOS in the mallard liver samples ranged from non-quantifiable levels (a peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL)) to 22.8 µg/g. These results are reported on a wet weight basis. PFOS in the mallard serum samples ranged from non-quantifiable levels to 218 µg/g.

The average percent recovery  $\pm$  standard deviation for PFOS in liver samples was 95%  $\pm$  12%. The average percent recovery  $\pm$  standard deviation for PFOS in serum samples was 93%  $\pm$  10%.

## **2.0 OBJECTIVE**

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of mallard liver and mallard serum using the analytical methods described in protocol 00P-023-042.

## **3.0 INTRODUCTION**

This report details the results of the analysis for the determination of PFOS in mallard liver and mallard serum, using the analytical methods entitled, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry".

The study was initiated on November 10, 2000, when the study director signed Centre protocol number 00P-023-042. The experimental start date was November 17, 2000, and the experimental termination date was March 13, 2001.

#### 4.0 TEST SYSTEM

The control mallard liver and serum used for the matrix blanks and matrix fortifications was received on May 18, 2000 from Wildlife International Ltd., Easton, MD. The control mallard liver was assigned the Centre ID of 0003804 and the control mallard serum was assigned the Centre ID of 0003803.

Sixty mallard liver samples and sixty mallard serum samples were received at Centre on August 10, 2000. The samples were stored frozen until they were logged in by Centre personnel on August 16, 2000 and stored frozen. An additional twenty mallard liver samples and forty mallard serum samples were received on February 24, 2001 and stored frozen until they were logged in by Centre personnel on March 1, 2001.

Sample log-in and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc. and a true copy of the storage records can be found in the raw data package associated with this study.

#### 5.0 REFERENCE MATERIAL

The analytical standard PFOS was received at Centre on June 3, 2000 from 3M Environmental Technology and Services.

The available information for the reference material is listed below. The reference material was stored frozen.

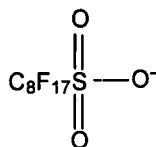
| <u>Compound</u> | <u>Centre Control No..</u> | <u>TCR No.</u> | <u>Purity (%)</u> | <u>Expiration Date</u> |
|-----------------|----------------------------|----------------|-------------------|------------------------|
| PFOS            | 00-023-042                 | TCR 00017-46   | 97.9              | 08/31/01               |

The molecular structure of PFOS is given below.

##### **PFOS**

Chemical Name = Perfluorooctanesulfonate

Molecular weight = 499 (C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup>)



**Note:** The neutral molecule and standard form from which PFOS (anion) is derived, is potassium perfluorooctanesulfonate [C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>K], molecular weight 538.

## 6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical methods entitled “Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry” and “Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry” were used for this study.

### 6.1 Extraction Procedure

A ~ 0.50 g sample of liver and a 100 µL aliquot of the serum was used for the extraction procedure. After fortification of appropriate samples, the samples were vortexed for ~ 15 seconds. One milliliter of 0.5 M TBA was added to the liver samples and 0.5 milliliter was added to the serum samples. Two milliliters of 0.25 M sodium carbonate/sodium bicarbonate was added to the liver samples and 1 milliliter was added to the serum samples. Five milliliters of MTBE was added to both the liver and serum samples. Each sample was placed on wrist-action shaker for ~ 20 min. and then centrifuged for ~ 15 min. Four milliliters of the organic layer was taken and dried on a nitrogen evaporator and then reconstituted with 1 milliliter of methanol. Each sample was analyzed by LC/MS/MS electrospray.

### 6.2 Preparation of Standards and Fortification Solutions

Standard solutions were prepared on September 18, 2000 as specified in Centre Analytical Laboratories’ protocol 00P-023-042. An individual stock standard solution of PFOS was prepared at a concentration of 100 µg/mL by dissolving 10 mg of the standard (corrected for purity and salt content) in methanol. From this solution, a 1.0 µg/mL fortification standard solution was prepared by taking 1 mL of the stock and bringing the volume up to 100 mL with methanol.

A 0.1 µg/mL fortification standard was prepared by taking 10 mL of the 1.0 µg/mL fortification standard and bringing the volume up to 100 mL with methanol. A 0.01 µg/mL standard was prepared by taking 10 mL of the 0.1 µg/mL standard and bringing to 100 mL with methanol.

A set of standards containing PFOS was prepared by serial dilution of the 0.1 µg/mL and 0.01 µg/mL solutions in the following manner:

| Initial Conc. (µg/mL) <sup>1</sup> | Volume (mL) | Diluted to (mL) | Final Conc. (µg/mL) |
|------------------------------------|-------------|-----------------|---------------------|
| 0.1                                | 5           | 100             | 0.005               |
| 0.1                                | 2           | 100             | 0.002               |
| 0.1                                | 1           | 100             | 0.001               |
| 0.01                               | 5           | 100             | 0.0005              |
| 0.01                               | 2           | 100             | 0.0002              |
| 0.01                               | 1           | 100             | 0.0001              |

<sup>1</sup> of PFOS

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator ( $4^{\circ} \pm 2^{\circ}\text{C}$ ) when not in use. Documentation of standard preparation can be found in the raw data associated with this report.

### 6.3 Chromatography

Quantification of PFOS was accomplished by LC/MS/MS electrospray. The retention time of PFOS was  $\sim 4.4$  min. Peaks were detected in the control matrices corresponding to the analyte retention time, but the amounts detected were only significant enough to alter several fortification recoveries and the rest were less than the lowest calibration standard ( $0.0001 \mu\text{g/mL}$ ).

### 6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of  $0.0001 \mu\text{g/mL}$  of PFOS.

### 6.5 Description of Instrument and Operating Conditions

Instrument: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX Turbolon Spray Liquid Introduction Interface

Computer: Dell UltraScan P1110

Software: PE SciexAnalyst version 1.1  
Windows NT, version 4

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

HPLC Column: Genesis C<sub>8</sub> (Jones Chromatography), 2.1 mm x 50 mm, 4 $\mu$

Column Temp.: 35 $^{\circ}$  C

Injection Vol.: 10  $\mu\text{L}$

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

Mobile Phase (B): Methanol

Flow Rate: 0.3 mL/min.

| <u>Time</u> | <u>% A</u> | <u>% B</u> |
|-------------|------------|------------|
| 0           | 60         | 40         |
| 1.0         | 0          | 100        |
| 7.0         | 0          | 100        |
| 7.5         | 40         | 60         |
| 11.0        | 40         | 60         |

Ions monitored:

| <u>Analyte</u> | <u>Mode</u> | <u>Transition Monitored</u> | <u>Approximate Retention Time (min)</u> |
|----------------|-------------|-----------------------------|-----------------------------------------|
| PFOS           | negative    | 499 → 99                    | 4.20                                    |

### Tune File Parameters

| <u>Controls</u>                   | <u>Set</u> |
|-----------------------------------|------------|
| IS-Iospray                        | -4200.0    |
| DP-Declustering Potential         | -51.0      |
| FP-Focusing Potential             | -230.0     |
| EP-Entrance Potential             | 10.0       |
| CE-Collision Energy               | -70        |
| CXP-Collision Cell Exit Potential | -5         |
| DF-Deflector                      | 300.0      |
| CEM-Channel Electron Multiplier   | 2800.0     |
| <u>Gas Flows</u>                  | <u>Set</u> |
| Nebulizer Gas                     | 12         |
| Curtain Gas                       | 13         |
| Collision Gas                     | 4          |
| TIS Temperature                   | 350°C      |

## 6.6 Quantitation and Example Calculation

Ten microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x fit weighted linear regression) by Analyst software using six concentrations of standards. The concentration for mallard liver ( $\mu\text{g/g}$ ) and mallard serum ( $\mu\text{g/mL}$ ) was determined from the equations below.

Equation 1 calculated the amount of analyte found (in  $\text{ng/mL}$ , based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program. Then Equation 2 calculated the amount of analyte found in  $\mu\text{g/mL}$  (the equations for serum are shown).

Equation 1:

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

Equation 2:

$$\text{Analyte found (}\mu\text{g/mL)} = \frac{((\text{anal. found (ng/mL)} \times \text{FV (mL)} \times \text{DF} \times \text{EV (mL)}) \times 1 \mu\text{g}}{(\text{AV (mL)} \times \text{sample vol. (mL)})} \quad 1000 \text{ ng}$$

Where:

FV = Final Volume

DF = Dilution Factor

EV = Extraction Volume

AV = Aliquot Volume

For samples fortified with known amounts of PFOS prior to extraction, Equation 3 calculated the percent recovery.

Equation 3:

Recovery (%) =

$$\frac{((\text{anal. found (}\mu\text{g/mL)} - \text{avg. anal. in ctrl (}\mu\text{g/mL)}) \times (1000 \text{ ng/1 } \mu\text{g})) \times 100}{\text{amount added (ng/mL)}}$$

An example of a calculation using an actual sample follows:

Mallard serum sample Centre ID 0003803 Spk A (Set: 112100B), fortified at 10 ng/mL with PFOS, where:

|                        |   |                      |
|------------------------|---|----------------------|
| peak area              | = | 6558                 |
| intercept              | = | 515                  |
| slope                  | = | 7740                 |
| dilution factor        | = | 1                    |
| ng added (fort level)  | = | 1 ng                 |
| avg. amt in controls   | = | 0 (Not quantifiable) |
| final volume           | = | 1 mL                 |
| extraction volume      | = | 5 mL                 |
| aliquot volume         | = | 4 mL                 |
| sample weight (volume) | = | 0.1 mL               |

**From equation 1:**

$$\text{Analyte found (ng/mL)} = \frac{[6558 - 515]}{7740}$$

$$= 0.8 \text{ ng/mL}$$

**From equation 2:**

$$\text{Analyte found (}\mu\text{g/mL)} = \frac{(0.8 \text{ ng/mL} \times 1 \text{ mL} \times 1 \times 5 \text{ mL}) \times 1 \mu\text{g}}{(4 \text{ mL} \times 0.1 \text{ mL})} \quad 1000 \text{ ng}$$

$$= 0.00976 \mu\text{g/mL}$$

From equation 3:

$$\begin{aligned}\% \text{ Recovery} &= \\ &= \frac{((0.00976 \mu\text{g/mL} \times -0 \text{ ng/mL}) \times 1000) \times 100}{10 \text{ ng/mL}} \\ &= 98\%\end{aligned}$$

## **7.0 EXPERIMENTAL DESIGN**

Each set of samples (liver or serum) consisted of one reagent blank, one matrix blank, two matrix blanks fortified at known concentrations, and ~ 20 samples. Each sample was extracted using the appropriate method and then analyzed in duplicate.

## **8.0 RESULTS**

The PFOS found in the reagent blanks are listed in **Table I**. The PFOS in the mallard liver blanks and mallard serum blanks are given in **Tables II & III**.

Individual recoveries for PFOS in the liver samples are detailed in **Table IV**. The average percent recovery  $\pm$  standard deviation for PFOS in liver samples was  $95\% \pm 12\%$ . Individual recoveries for PFOS in the serum samples are detailed in **Table V**. The average percent recovery  $\pm$  relative standard deviation for PFOS in serum samples was  $93\% \pm 10\%$ .

PFOS in the mallard liver samples ranged from non-quantifiable levels (a peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards ( $0.0001 \mu\text{g/mL}$ )) to  $22.8 \mu\text{g/g}$ . Individual results are listed in **Tables VI-XIII**. The results are reported on a wet weight basis.

PFOS in the mallard serum samples ranged from non-quantifiable levels (a peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards ( $0.0001 \mu\text{g/mL}$ )) to  $218 \mu\text{g/g}$ . Individual results are listed in **Tables XIV-XXIII**.

The percent moistures for the mallard liver samples are given in **Table XXIV**.

## **9.0 CONCLUSIONS**

The mallard liver and serum samples were successfully extracted and analyzed according to protocol 00P-023-042.

## **10.0 RETENTION OF DATA AND SAMPLES**

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 40 CFR Part 792. Retained samples of reference substances are archived by the sponsor.

# TABLES

**Table I. Summary of PFOS in Reagent Blanks**

| <b>Sponsor<br/>ID</b>      | <b>Centre<br/>ID</b> | <b>Set<br/>Number</b> | <b>Extraction<br/>Date</b> | <b>Analysis<br/>Date</b> | <b>Analyte<br/>Found (µg/g)</b> |
|----------------------------|----------------------|-----------------------|----------------------------|--------------------------|---------------------------------|
| na                         | Reagent Blank A      | 111700A               | 11/17/00                   | 11/18-19/00              | NQ                              |
| na                         | Reagent Blank A*     | 111700A               | 11/17/00                   | 11/18-19/00              | NQ                              |
| na                         | Reagent Blank A      | 112000A               | 11/20/00                   | 11/20-21/00              | NQ                              |
| na                         | Reagent Blank A*     | 112000A               | 11/20/00                   | 11/20-21/00              | NQ                              |
| na                         | Reagent Blank A      | 112100A               | 11/21/00                   | 11/22-23/00              | NQ                              |
| na                         | Reagent Blank A*     | 112100A               | 11/21/00                   | 11/22-23/00              | NQ                              |
| na                         | Reagent Blank A      | 112100B               | 11/21/00                   | 11/23/00                 | NQ                              |
| na                         | Reagent Blank A*     | 112100B               | 11/21/00                   | 11/23/00                 | NQ                              |
| na                         | Reagent Blank A      | 112700A               | 11/27/00                   | 11/28-29/00              | NQ                              |
| na                         | Reagent Blank A*     | 112700A               | 11/27/00                   | 11/28-29/00              | NQ                              |
| na                         | Reagent Blank A      | 112700B               | 11/27/00                   | 11/29/00                 | NQ                              |
| na                         | Reagent Blank A*     | 112700B               | 11/27/00                   | 11/29/00                 | NQ                              |
| na                         | Reagent Blank A      | 030701A               | 3/7/01                     | 3/9/01                   | NQ                              |
| na                         | Reagent Blank A*     | 030701A               | 3/7/01                     | 3/9/01                   | NQ                              |
| na                         | Reagent Blank A      | 030701B               | 3/7/01                     | 3/9-10/01                | NQ                              |
| na                         | Reagent Blank A*     | 030701B               | 3/7/01                     | 3/9-10/01                | NQ                              |
| na                         | Reagent Blank A      | 030801A               | 3/8/01                     | 3/11/01                  | NQ                              |
| na                         | Reagent Blank A*     | 030801A               | 3/8/01                     | 3/11/01                  | NQ                              |
| <b>AVERAGE:</b>            |                      |                       |                            |                          | <b>NQ</b>                       |
| <b>STANDARD DEVIATION:</b> |                      |                       |                            |                          | <b>NQ</b>                       |

\* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/g (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table II. Summary of PFOS in Mallard Liver Blanks**

| Sponsor ID                 | Centre ID        | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|------------------|------------|-----------------|---------------|----------------------|
| na                         | 0003804 Blank A  | 111700A    | 11/17/00        | 11/18-19/00   | 0.000294             |
| na                         | 0003804 Blank A* | 111700A    | 11/17/00        | 11/18-19/00   | 0.000300             |
| na                         | 0003804 Blank A  | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| na                         | 0003804 Blank A* | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| na                         | 0003804 Blank A  | 112100A    | 11/21/00        | 11/22-23/00   | NQ                   |
| na                         | 0003804 Blank A* | 112100A    | 11/21/00        | 11/22-23/00   | NQ                   |
| na                         | 0003804 Blank A  | 030801A    | 3/8/01          | 3/11/01       | NQ                   |
| na                         | 0003804 Blank A* | 030801A    | 3/8/01          | 3/11/01       | NQ                   |
| <b>AVERAGE:</b>            |                  |            |                 |               | <b>NQ</b>            |
| <b>STANDARD DEVIATION:</b> |                  |            |                 |               | <b>NQ</b>            |

**Table III. Summary of PFOS in Mallard Serum Blanks**

| Sponsor ID                 | Centre ID        | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|----------------------------|------------------|------------|-----------------|---------------|-----------------------|
| na                         | 0003803 Blank A  | 112100B    | 11/21/00        | 11/23/00      | NQ                    |
| na                         | 0003803 Blank A* | 112100B    | 11/21/00        | 11/23/00      | NQ                    |
| na                         | 0003803 Blank A  | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| na                         | 0003803 Blank A* | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| na                         | 0003803 Blank A  | 112700B    | 11/27/00        | 11/29/00      | NQ                    |
| na                         | 0003803 Blank A* | 112700B    | 11/27/00        | 11/29/00      | 0.00159               |
| na                         | 0003803 Blank A  | 030701A    | 3/7/01          | 3/9/01        | 0.00826               |
| na                         | 0003803 Blank A* | 030701A    | 3/7/01          | 3/9/01        | 0.00825               |
| na                         | 0003803 Blank A  | 030701B    | 3/7/01          | 3/9-10/01     | 0.00159               |
| na                         | 0003803 Blank A* | 030701B    | 3/7/01          | 3/9-10/01     | 0.00397               |
| <b>AVERAGE:</b>            |                  |            |                 |               | <b>0.00239</b>        |
| <b>STANDARD DEVIATION:</b> |                  |            |                 |               | <b>0.00333</b>        |

\* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

**Table IV. Summary of PFOS Recoveries in Mallard Liver**

| <b>Sponsor ID</b>                   | <b>Centre ID</b> | <b>Set Number</b> | <b>Extraction Date</b> | <b>Analysis Date</b> | <b>Amt Added (ng/g)</b> | <b>% Recovery</b> |
|-------------------------------------|------------------|-------------------|------------------------|----------------------|-------------------------|-------------------|
| na                                  | 0003804 Spk A    | 111700AD          | 11/17/00               | 11/19/00             | 10                      | 79                |
| na                                  | 0003804 Spk A*   | 111700AD          | 11/17/00               | 11/19/00             | 10                      | 77                |
| na                                  | 0003804 Spk B    | 111700A           | 11/17/00               | 11/18-19/00          | 50                      | 85                |
| na                                  | 0003804 Spk B*   | 111700A           | 11/17/00               | 11/18-19/00          | 50                      | 84                |
| na                                  | 0003804 Spk A    | 112000AR          | 11/20/00               | 11/22/00             | 100                     | 114               |
| na                                  | 0003804 Spk A*   | 112000AR          | 11/20/00               | 11/22/00             | 100                     | 115               |
| na                                  | 0003804 Spk B    | 112000A           | 11/20/00               | 11/20-21/00          | 4000                    | 106               |
| na                                  | 0003804 Spk B*   | 112000A           | 11/20/00               | 11/20-21/00          | 4000                    | 107               |
| na                                  | 0003804 Spk A    | 112100A           | 11/21/00               | 11/22-23/00          | 1000                    | 89                |
| na                                  | 0003804 Spk A*   | 112100A           | 11/21/00               | 11/22-23/00          | 1000                    | 88                |
| na                                  | 0003804 Spk B    | 112100AD          | 11/21/00               | 11/27/00             | 20000                   | 90                |
| na                                  | 0003804 Spk B*   | 112100AD          | 11/21/00               | 11/27/00             | 20000                   | 89                |
| na                                  | 0003804 Spk A    | 030801A           | 3/8/01                 | 3/11/01              | 20                      | 92                |
| na                                  | 0003804 Spk A*   | 030801A           | 3/8/01                 | 3/11/01              | 20                      | 92                |
| na                                  | 0003804 Spk B    | 030801AD          | 3/8/01                 | 3/11-12/01           | 200                     | 107               |
| na                                  | 0003804 Spk B*   | 030801AD          | 3/8/01                 | 3/11-12/01           | 200                     | 101               |
| <b>AVERAGE:</b>                     |                  |                   |                        |                      |                         | <b>95</b>         |
| <b>STANDARD DEVIATION:</b>          |                  |                   |                        |                      |                         | <b>11</b>         |
| <b>RELATIVE STANDARD DEVIATION:</b> |                  |                   |                        |                      |                         | <b>12</b>         |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table V. Summary of PFOS Recoveries in Mallard Serum**

| <b>Sponsor ID</b>                   | <b>Centre ID</b> | <b>Set Number</b> | <b>Extraction Date</b> | <b>Analysis Date</b> | <b>Amt Added (ng/mL)</b> | <b>% Recovery</b> |
|-------------------------------------|------------------|-------------------|------------------------|----------------------|--------------------------|-------------------|
| na                                  | 0003803 Spk A    | 112100B           | 11/21/00               | 11/23/00             | 10                       | 98                |
| na                                  | 0003803 Spk A*   | 112100B           | 11/21/00               | 11/23/00             | 10                       | 98                |
| na                                  | 0003803 Spk B    | 112100B           | 11/21/00               | 11/23/00             | 500                      | 84                |
| na                                  | 0003803 Spk B*   | 112100B           | 11/21/00               | 11/23/00             | 500                      | 86                |
| na                                  | 0003803 Spk A    | 112700A           | 11/27/00               | 11/28-29/00          | 10                       | 81                |
| na                                  | 0003803 Spk A*   | 112700A           | 11/27/00               | 11/28-29/00          | 10                       | 81                |
| na                                  | 0003803 Spk B    | 112700A           | 11/27/00               | 11/28-29/00          | 10000                    | 91                |
| na                                  | 0003803 Spk B*   | 112700A           | 11/27/00               | 11/28-29/00          | 10000                    | 91                |
| na                                  | 0003803 Spk A    | 112700B           | 11/27/00               | 11/29/00             | 500                      | 97                |
| na                                  | 0003803 Spk A*   | 112700B           | 11/27/00               | 11/29/00             | 500                      | 97                |
| na                                  | 0003803 Spk B    | 112700B           | 11/27/00               | 11/29/00             | 100000                   | 89                |
| na                                  | 0003803 Spk B*   | 112700B           | 11/27/00               | 11/29/00             | 100000                   | 89                |
| na                                  | 0003803 Spk A    | 030701A           | 3/7/01                 | 3/9/01               | 100                      | 79                |
| na                                  | 0003803 Spk A*   | 030701A           | 3/7/01                 | 3/9/01               | 100                      | 77                |
| na                                  | 0003803 Spk B    | 030701A           | 3/7/01                 | 3/9/01               | 1000                     | 89                |
| na                                  | 0003803 Spk B*   | 030701A           | 3/7/01                 | 3/9/01               | 1000                     | 93                |
| na                                  | 0003803 Spk A2   | 030701B           | 3/7/01                 | 3/9-10/01            | 100                      | 90                |
| na                                  | 0003803 Spk A2*  | 030701B           | 3/7/01                 | 3/9-10/01            | 100                      | 90                |
| na                                  | 0003803 Spk B2   | 030701B           | 3/7/01                 | 3/9-10/01            | 1000                     | 118               |
| na                                  | 0003803 Spk B2*  | 030701B           | 3/7/01                 | 3/9-10/01            | 1000                     | 119               |
| <b>AVERAGE:</b>                     |                  |                   |                        |                      |                          | <b>92</b>         |
| <b>STANDARD DEVIATION:</b>          |                  |                   |                        |                      |                          | <b>11</b>         |
| <b>RELATIVE STANDARD DEVIATION:</b> |                  |                   |                        |                      |                          | <b>12</b>         |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table VI. Summary of PFOS in Adult Liver Samples at 0 ppm a.i.  
(Test Termination 19 Weeks)**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| 1961 <sup>M</sup>          | 0008006   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000813             |
| 1961*                      | 0008006   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000763             |
| 1962 <sup>F</sup>          | 0008007   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000509             |
| 1962*                      | 0008007   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000631             |
| 1963 <sup>M</sup>          | 0008008   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000937             |
| 1963*                      | 0008008   | 111700A    | 11/17/00        | 11/18-19/00   | 0.000887             |
| 1964 <sup>F</sup>          | 0008009   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00155              |
| 1964*                      | 0008009   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00157              |
| 1965 <sup>M</sup>          | 0008010   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00110              |
| 1965*                      | 0008010   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00111              |
| 1966 <sup>F</sup>          | 0008011   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00446              |
| 1966*                      | 0008011   | 111700A    | 11/17/00        | 11/18-19/00   | 0.00432              |
| 1967 <sup>M</sup>          | 0008012   | 111700ADD  | 11/17/00        | 11/20/00      | 0.662                |
| 1967*                      | 0008012   | 111700ADD  | 11/17/00        | 11/20/00      | 0.684                |
| 1968 <sup>F</sup>          | 0008013   | 111700AD   | 11/17/00        | 11/19/00      | 0.238                |
| 1968*                      | 0008013   | 111700AD   | 11/17/00        | 11/19/00      | 0.246                |
| 1969 <sup>M</sup>          | 0008014   | 111700AD   | 11/17/00        | 11/19/00      | 0.115                |
| 1969*                      | 0008014   | 111700AD   | 11/17/00        | 11/19/00      | 0.106                |
| 1970 <sup>F</sup>          | 0008015   | 111700AD   | 11/17/00        | 11/19/00      | 0.355                |
| 1970*                      | 0008015   | 111700AD   | 11/17/00        | 11/19/00      | 0.343                |
| <b>AVERAGE:</b>            |           |            |                 |               | <b>0.138</b>         |
| <b>STANDARD DEVIATION:</b> |           |            |                 |               | <b>0.219</b>         |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table VII. Summary of PFOS in Adult Liver Samples at 1.8 ppm a.i.  
(Test Termination 6 Weeks)**

| Sponsor<br>ID               | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/g) |
|-----------------------------|--------------|---------------|--------------------|------------------|-------------------------|
| E01-0263-21065 <sup>M</sup> | 0103870      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.359                   |
| E01-0263-21065*             | 0103870      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.350                   |
| E01-0263-21066 <sup>M</sup> | 0103871      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.647                   |
| E01-0263-21066*             | 0103871      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.631                   |
| E01-0263-21067 <sup>M</sup> | 0103872      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.886                   |
| E01-0263-21067*             | 0103872      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.880                   |
| E01-0263-21068 <sup>M</sup> | 0103873      | 030801AD      | 3/8/01             | 3/11-12/01       | 2.11                    |
| E01-0263-21068*             | 0103873      | 030801AD      | 3/8/01             | 3/11-12/01       | 2.04                    |
| E01-0263-21069 <sup>M</sup> | 0103874      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.222                   |
| E01-0263-21069*             | 0103874      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.232                   |
| E01-0263-21075 <sup>M</sup> | 0103880      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.317                   |
| E01-0263-21075*             | 0103880      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.326                   |
| E01-0263-21076 <sup>M</sup> | 0103881      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.219                   |
| E01-0263-21076*             | 0103881      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.230                   |
| E01-0263-21077 <sup>M</sup> | 0103882      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.194                   |
| E01-0263-21077*             | 0103882      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.197                   |
| E01-0263-21078 <sup>M</sup> | 0103883      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.346                   |
| E01-0263-21078*             | 0103883      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.342                   |
| E01-0263-21079 <sup>M</sup> | 0103884      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0893                  |
| E01-0263-21079*             | 0103884      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0888                  |
| <b>AVERAGE:</b>             |              |               |                    |                  | <b>0.535</b>            |
| <b>STANDARD DEVIATION:</b>  |              |               |                    |                  | <b>0.574</b>            |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table VIII. Summary of PFOS in Adult Liver Samples at 6.2 ppm a.i.  
(Test Termination 6 Weeks)**

| Sponsor<br>ID              | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/g) |
|----------------------------|--------------|---------------|--------------------|------------------|-------------------------|
| E01-0263-21070             | 0103875      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.04                    |
| E01-0263-21070*            | 0103875      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.01                    |
| E01-0263-21071             | 0103876      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.963                   |
| E01-0263-21071*            | 0103876      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.907                   |
| E01-0263-21072             | 0103877      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.92                    |
| E01-0263-21072*            | 0103877      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.81                    |
| E01-0263-21073             | 0103878      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.791                   |
| E01-0263-21073*            | 0103878      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.827                   |
| E01-0263-21074             | 0103879      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.28                    |
| E01-0263-21074*            | 0103879      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.34                    |
| E01-0263-21080             | 0103885      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.01                    |
| E01-0263-21080*            | 0103885      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.03                    |
| E01-0263-21081             | 0103886      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0614                  |
| E01-0263-21081*            | 0103886      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0578                  |
| E01-0263-21082             | 0103887      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.473                   |
| E01-0263-21082*            | 0103887      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.517                   |
| E01-0263-21083             | 0103888      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.66                    |
| E01-0263-21083*            | 0103888      | 030801AD      | 3/8/01             | 3/11-12/01       | 1.52                    |
| E01-0263-21084             | 0103889      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0862                  |
| E01-0263-21084*            | 0103889      | 030801AD      | 3/8/01             | 3/11-12/01       | 0.0878                  |
| <b>AVERAGE:</b>            |              |               |                    |                  | <b>0.920</b>            |
| <b>STANDARD DEVIATION:</b> |              |               |                    |                  | <b>0.576</b>            |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table IX. Summary of PFOS in Adult Liver Samples at 17.6 ppm a.i.  
(Test Termination 19 Weeks)**

| <b>Sponsor<br/>ID</b>      | <b>Centre<br/>ID</b> | <b>Set<br/>Number</b> | <b>Extraction<br/>Date</b> | <b>Analysis<br/>Date</b> | <b>Analyte<br/>Found (µg/g)</b> |
|----------------------------|----------------------|-----------------------|----------------------------|--------------------------|---------------------------------|
| 1991 <i>AI</i>             | 0008016              | 111700ADD             | 11/17/00                   | 11/20/00                 | 15.9                            |
| 1991*                      | 0008016              | 111700ADD             | 11/17/00                   | 11/20/00                 | 16.4                            |
| 1992 <i>F</i>              | 0008017              | 111700ADD             | 11/17/00                   | 11/20/00                 | 22.8                            |
| 1992*                      | 0008017              | 111700ADD             | 11/17/00                   | 11/20/00                 | 21.9                            |
| 1993 <i>AI</i>             | 0008018              | 111700ADD             | 11/17/00                   | 11/20/00                 | 7.02                            |
| 1993*                      | 0008018              | 111700ADD             | 11/17/00                   | 11/20/00                 | 6.69                            |
| 1994 <i>IF</i>             | 0008019              | 111700AD              | 11/17/00                   | 11/19/00                 | 2.82                            |
| 1994*                      | 0008019              | 111700AD              | 11/17/00                   | 11/19/00                 | 2.93                            |
| 1995 <i>AI</i>             | 0008020              | 111700AD              | 11/17/00                   | 11/19/00                 | 3.53                            |
| 1995*                      | 0008020              | 111700AD              | 11/17/00                   | 11/19/00                 | 3.60                            |
| 1996 <i>F</i>              | 0008021              | 111700ADD             | 11/17/00                   | 11/20/00                 | 13.2                            |
| 1996*                      | 0008021              | 111700ADD             | 11/17/00                   | 11/20/00                 | 13.5                            |
| 1997 <i>AI</i>             | 0008022              | 111700AD              | 11/17/00                   | 11/19/00                 | 5.62                            |
| 1997*                      | 0008022              | 111700AD              | 11/17/00                   | 11/19/00                 | 5.58                            |
| 1998 <i>F</i>              | 0008023              | 111700AD              | 11/17/00                   | 11/19/00                 | 3.72                            |
| 1998*                      | 0008023              | 111700AD              | 11/17/00                   | 11/19/00                 | 3.86                            |
| 1999 <i>AI</i>             | 0008024              | 111700ADD             | 11/17/00                   | 11/20/00                 | 15.5                            |
| 1999*                      | 0008024              | 111700ADD             | 11/17/00                   | 11/20/00                 | 16.1                            |
| 2000 <i>IF</i>             | 0008025              | 111700AD              | 11/17/00                   | 11/19/00                 | 5.97                            |
| 2000*                      | 0008025              | 111700AD              | 11/17/00                   | 11/19/00                 | 6.10                            |
| <b>AVERAGE:</b>            |                      |                       |                            |                          | <b>9.64</b>                     |
| <b>STANDARD DEVIATION:</b> |                      |                       |                            |                          | <b>6.57</b>                     |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table X. Summary of PFOS in Juvenile Liver Samples at 0 ppm a.i.  
(Test Termination 12 Weeks)**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|---------------------|-----------|------------|-----------------|---------------|----------------------|
| 2301 ✓              | 0008086   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2301*               | 0008086   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2302 ✓              | 0008087   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2302*               | 0008087   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2303 ✓              | 0008088   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2303*               | 0008088   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2305 ✓              | 0008089   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2305*               | 0008089   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2307 ✓              | 0008090   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2307*               | 0008090   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2308 ✓              | 0008091   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2308*               | 0008091   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2314 ✓              | 0008092   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2314*               | 0008092   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2316 ✓              | 0008093   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2316*               | 0008093   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2317 ✓              | 0008094   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2317*               | 0008094   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2320 ✓              | 0008095   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| 2320*               | 0008095   | 112000A    | 11/20/00        | 11/20-21/00   | NQ                   |
| AVERAGE:            |           |            |                 |               | NQ                   |
| STANDARD DEVIATION: |           |            |                 |               | NQ                   |

\* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

**Table XI. Summary of PFOS in Juvenile Liver Samples at 1.8 ppm  
a.i. (Test Termination 12 Weeks)**

| Sponsor<br>ID              | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/g) |
|----------------------------|--------------|---------------|--------------------|------------------|-------------------------|
| 2322 <i>5</i>              | 0008096      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00454                 |
| 2322*                      | 0008096      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00458                 |
| 2323 <i>M</i>              | 0008097      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00124                 |
| 2323*                      | 0008097      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00129                 |
| 2325 <i>45</i>             | 0008098      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00150                 |
| 2325*                      | 0008098      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00154                 |
| 2326 <i>5</i>              | 0008099      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00236                 |
| 2326*                      | 0008099      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00245                 |
| 2328 <i>11</i>             | 0008100      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00167                 |
| 2328*                      | 0008100      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00165                 |
| 2330 <i>5</i>              | 0008101      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00185                 |
| 2330*                      | 0008101      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00182                 |
| 2331 <i>M</i>              | 0008102      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000719                |
| 2331*                      | 0008102      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000763                |
| 2332 <i>F</i>              | 0008103      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000425                |
| 2332*                      | 0008103      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000430                |
| 2333 <i>5</i>              | 0008104      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000969                |
| 2333*                      | 0008104      | 112000A       | 11/20/00           | 11/20-21/00      | 0.000966                |
| 2334 <i>M</i>              | 0008105      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00356                 |
| 2334*                      | 0008105      | 112000A       | 11/20/00           | 11/20-21/00      | 0.00355                 |
| <b>AVERAGE:</b>            |              |               |                    |                  | <b>0.00189</b>          |
| <b>STANDARD DEVIATION:</b> |              |               |                    |                  | <b>0.00126</b>          |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XII. Summary of PFOS in Juvenile Liver Samples at 6.2 ppm  
a.i. (Test Termination 12 Weeks)**

| Sponsor<br>ID              | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/g) |
|----------------------------|--------------|---------------|--------------------|------------------|-------------------------|
| 2335 F                     | 0008106      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00197                 |
| 2335*                      | 0008106      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00200                 |
| 2336 F                     | 0008107      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00377                 |
| 2336*                      | 0008107      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00378                 |
| 2337 F                     | 0008108      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00380                 |
| 2337*                      | 0008108      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00363                 |
| 2338 M                     | 0008109      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00555                 |
| 2338*                      | 0008109      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00535                 |
| 2339 M                     | 0008110      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00540                 |
| 2339*                      | 0008110      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00518                 |
| 2341 M                     | 0008111      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00306                 |
| 2341*                      | 0008111      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00313                 |
| 2342 F                     | 0008112      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00312                 |
| 2342*                      | 0008112      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00320                 |
| 2343 M                     | 0008113      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00329                 |
| 2343*                      | 0008113      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00332                 |
| 2344 F                     | 0008114      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00402                 |
| 2344*                      | 0008114      | 112100A       | 11/21/00           | 11/22-23/00      | 0.00405                 |
| 2346 F                     | 0008115      | 112100AD      | 11/21/00           | 11/27/00         | 0.0141                  |
| 2346*                      | 0008115      | 112100AD      | 11/21/00           | 11/27/00         | 0.0146                  |
| <b>AVERAGE:</b>            |              |               |                    |                  | <b>0.00482</b>          |
| <b>STANDARD DEVIATION:</b> |              |               |                    |                  | <b>0.00341</b>          |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XIII. Summary of PFOS in Juvenile Liver Samples at 17.6 ppm  
a.i. (Test Termination 12 Weeks)**

| <b>Sponsor<br/>ID</b>      | <b>Centre<br/>ID</b> | <b>Set<br/>Number</b> | <b>Extraction<br/>Date</b> | <b>Analysis<br/>Date</b> | <b>Analyte<br/>Found (µg/g)</b> |
|----------------------------|----------------------|-----------------------|----------------------------|--------------------------|---------------------------------|
| 2347 F                     | 0008116              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0383                          |
| 2347*                      | 0008116              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0370                          |
| 2348 F                     | 0008117              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0456                          |
| 2348*                      | 0008117              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0465                          |
| 2353 F                     | 0008118              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0234                          |
| 2353*                      | 0008118              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0227                          |
| 2354 M                     | 0008119              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0184                          |
| 2354*                      | 0008119              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0189                          |
| 2355 M                     | 0008120              | 112100A               | 11/21/00                   | 11/22-23/00              | 0.0110                          |
| 2355* M                    | 0008120              | 112100A               | 11/21/00                   | 11/22-23/00              | 0.0105                          |
| 2357 M                     | 0008121              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0616                          |
| 2357*                      | 0008121              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0629                          |
| 2358 F                     | 0008122              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0175                          |
| 2358*                      | 0008122              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0189                          |
| 2359 F                     | 0008123              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0211                          |
| 2359*                      | 0008123              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0211                          |
| 2360 M                     | 0008124              | 112100A               | 11/21/00                   | 11/22-23/00              | 0.00623                         |
| 2360*                      | 0008124              | 112100A               | 11/21/00                   | 11/22-23/00              | 0.00628                         |
| 2363 M                     | 0008125              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0172                          |
| 2363*                      | 0008125              | 112100AD              | 11/21/00                   | 11/27/00                 | 0.0191                          |
| <b>AVERAGE:</b>            |                      |                       |                            |                          | <b>0.0262</b>                   |
| <b>STANDARD DEVIATION:</b> |                      |                       |                            |                          | <b>0.0168</b>                   |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XIV. Summary of PFOS in Control Adult Serum Samples**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|----------------------------|-----------|------------|-----------------|---------------|-----------------------|
| E01-0263-21005 ✓           | 0103810   | 030701A    | 3/7/01          | 3/9/01        | 0.0463                |
| E01-0263-21005*            | 0103810   | 030701A    | 3/7/01          | 3/9/01        | 0.0455                |
| E01-0263-21006 ✓           | 0103811   | 030701A    | 3/7/01          | 3/9/01        | 0.00828               |
| E01-0263-21006*            | 0103811   | 030701A    | 3/7/01          | 3/9/01        | 0.00928               |
| E01-0263-21007 ✓           | 0103812   | 030701A    | 3/7/01          | 3/9/01        | 0.00954               |
| E01-0263-21007*            | 0103812   | 030701A    | 3/7/01          | 3/9/01        | 0.0103                |
| E01-0263-21008 ✓           | 0103813   | 030701A    | 3/7/01          | 3/9/01        | 0.00319               |
| E01-0263-21008*            | 0103813   | 030701A    | 3/7/01          | 3/9/01        | 0.00452               |
| E01-0263-21009 ✓           | 0103814   | 030701A    | 3/7/01          | 3/9/01        | 0.00423               |
| E01-0263-21009*            | 0103814   | 030701A    | 3/7/01          | 3/9/01        | 0.00616               |
| E01-0263-21025 ✓           | 0103830   | 030701B    | 3/7/01          | 3/9-10/01     | 0.00285               |
| E01-0263-21025*            | 0103830   | 030701B    | 3/7/01          | 3/9-10/01     | 0.00207               |
| E01-0263-21026 ✓           | 0103831   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| E01-0263-21026*            | 0103831   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| E01-0263-21027 ✓           | 0103832   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| E01-0263-21027*            | 0103832   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| E01-0263-21028 ✓           | 0103833   | 030701B    | 3/7/01          | 3/9-10/01     | 0.00428               |
| E01-0263-21028*            | 0103833   | 030701B    | 3/7/01          | 3/9-10/01     | 0.00265               |
| E01-0263-21029 ✓           | 0103834   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| E01-0263-21029*            | 0103834   | 030701B    | 3/7/01          | 3/9-10/01     | NQ                    |
| <b>AVERAGE:</b>            |           |            |                 |               | <b>0.00797</b>        |
| <b>STANDARD DEVIATION:</b> |           |            |                 |               | <b>0.0134</b>         |

\* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XV. Summary of PFOS in Adult Serum Samples at 0 ppm a.i.  
(Test Termination 19 Weeks)**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|---------------------|-----------|------------|-----------------|---------------|-----------------------|
| 1961 M              | 0008026   | 112100B    | 11/21/00        | 11/23/00      | 0.00605               |
| 1961*               | 0008026   | 112100B    | 11/21/00        | 11/23/00      | 0.00594               |
| 1962 F              | 0008027   | 112100B    | 11/21/00        | 11/23/00      | 0.00494               |
| 1962* M             | 0008027   | 112100B    | 11/21/00        | 11/23/00      | 0.00501               |
| 1963                | 0008028   | 112100B    | 11/21/00        | 11/23/00      | 0.00844               |
| 1963*               | 0008028   | 112100B    | 11/21/00        | 11/23/00      | 0.00825               |
| 1964 F              | 0008029   | 112100B    | 11/21/00        | 11/23/00      | 0.00798               |
| 1964*               | 0008029   | 112100B    | 11/21/00        | 11/23/00      | 0.00761               |
| 1965 M              | 0008030   | 112100B    | 11/21/00        | 11/23/00      | 0.0104                |
| 1965*               | 0008030   | 112100B    | 11/21/00        | 11/23/00      | 0.0103                |
| 1966 F              | 0008031   | 112100B    | 11/21/00        | 11/23/00      | 0.0358                |
| 1966*               | 0008031   | 112100B    | 11/21/00        | 11/23/00      | 0.0357                |
| 1967 M              | 0008032   | 112100BD   | 11/21/00        | 11/27-28/00   | 6.11                  |
| 1967*               | 0008032   | 112100BD   | 11/21/00        | 11/27-28/00   | 6.02                  |
| 1968 F              | 0008033   | 112100BD   | 11/21/00        | 11/27-28/00   | 3.80                  |
| 1968*               | 0008033   | 112100BD   | 11/21/00        | 11/27-28/00   | 3.81                  |
| 1969 M              | 0008034   | 112100BD   | 11/21/00        | 11/27-28/00   | 0.843                 |
| 1969*               | 0008034   | 112100BD   | 11/21/00        | 11/27-28/00   | 0.849                 |
| 1970 F              | 0008035   | 112100BD   | 11/21/00        | 11/27-28/00   | 1.41                  |
| 1970*               | 0008035   | 112100BD   | 11/21/00        | 11/27-28/00   | 1.38                  |
| AVERAGE:            |           |            |                 |               | 1.22                  |
| STANDARD DEVIATION: |           |            |                 |               | 2.03                  |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XVI. Summary of PFOS in Adult Serum Samples at 1.8 ppm a.i.  
(Test Termination 6 Weeks)**

| Sponsor ID                  | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|-----------------------------|-----------|------------|-----------------|---------------|-----------------------|
| E01-0263-21010 <sup>M</sup> | 0103815   | 030701AD   | 3/7/01          | 3/10/01       | 12.8                  |
| E01-0263-21010*             | 0103815   | 030701AD   | 3/7/01          | 3/10/01       | 12.1                  |
| E01-0263-21011 <sup>M</sup> | 0103816   | 030701AD   | 3/7/01          | 3/10/01       | 18.2                  |
| E01-0263-21011*             | 0103816   | 030701AD   | 3/7/01          | 3/10/01       | 20.1                  |
| E01-0263-21012 <sup>M</sup> | 0103817   | 030701AD   | 3/7/01          | 3/10/01       | 22.5                  |
| E01-0263-21012*             | 0103817   | 030701AD   | 3/7/01          | 3/10/01       | 24.0                  |
| E01-0263-21013 <sup>M</sup> | 0103818   | 030701AD   | 3/7/01          | 3/10/01       | 20.4                  |
| E01-0263-21013*             | 0103818   | 030701AD   | 3/7/01          | 3/10/01       | 20.4                  |
| E01-0263-21014 <sup>M</sup> | 0103819   | 030701AD   | 3/7/01          | 3/10/01       | 10.6                  |
| E01-0263-21014*             | 0103819   | 030701AD   | 3/7/01          | 3/10/01       | 10.6                  |
| E01-0263-21030 <sup>F</sup> | 0103835   | 030701BD   | 3/7/01          | 3/10-11/01    | 0.870                 |
| E01-0263-21030*             | 0103835   | 030701BD   | 3/7/01          | 3/10-11/01    | 0.854                 |
| E01-0263-21031 <sup>F</sup> | 0103836   | 030701BD   | 3/7/01          | 3/10-11/01    | 6.70                  |
| E01-0263-21031*             | 0103836   | 030701BD   | 3/7/01          | 3/10-11/01    | 6.43                  |
| E01-0263-21032 <sup>F</sup> | 0103837   | 030701BD   | 3/7/01          | 3/10-11/01    | 0.443                 |
| E01-0263-21032*             | 0103837   | 030701BD   | 3/7/01          | 3/10-11/01    | 0.468                 |
| E01-0263-21033 <sup>F</sup> | 0103838   | 030701BD   | 3/7/01          | 3/10-11/01    | 8.88                  |
| E01-0263-21033*             | 0103838   | 030701BD   | 3/7/01          | 3/10-11/01    | 8.38                  |
| E01-0263-21034 <sup>F</sup> | 0103839   | 030701BD   | 3/7/01          | 3/10-11/01    | 1.68                  |
| E01-0263-21034*             | 0103839   | 030701BD   | 3/7/01          | 3/10-11/01    | 1.74                  |
| <b>AVERAGE:</b>             |           |            |                 |               | <b>10.4</b>           |
| <b>STANDARD DEVIATION:</b>  |           |            |                 |               | <b>8.15</b>           |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XVII. Summary of PFOS in Adult Serum Samples at 6.2 ppm a.i.  
(Test Termination 6 Weeks)**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|---------------------|-----------|------------|-----------------|---------------|-----------------------|
| E01-0263-21015 ✓    | 0103820   | 030701AD   | 3/7/01          | 3/10/01       | 47.1                  |
| E01-0263-21015*     | 0103820   | 030701AD   | 3/7/01          | 3/10/01       | 44.7                  |
| E01-0263-21016 ✓    | 0103821   | 030701AD   | 3/7/01          | 3/10/01       | 40.4                  |
| E01-0263-21016*     | 0103821   | 030701AD   | 3/7/01          | 3/10/01       | 35.5                  |
| E01-0263-21017 ✓    | 0103822   | 030701AD   | 3/7/01          | 3/10/01       | 45.5                  |
| E01-0263-21017*     | 0103822   | 030701AD   | 3/7/01          | 3/10/01       | 44.4                  |
| E01-0263-21018 ✓    | 0103823   | 030701AD   | 3/7/01          | 3/10/01       | 23.5                  |
| E01-0263-21018*     | 0103823   | 030701AD   | 3/7/01          | 3/10/01       | 24.7                  |
| E01-0263-21019 ✓    | 0103824   | 030701AD   | 3/7/01          | 3/10/01       | 20.3                  |
| E01-0263-21019*     | 0103824   | 030701AD   | 3/7/01          | 3/10/01       | 20.8                  |
| E01-0263-21035 ✓    | 0103840   | 030701BD   | 3/7/01          | 3/10-11/01    | 17.8                  |
| E01-0263-21035*     | 0103840   | 030701BD   | 3/7/01          | 3/10-11/01    | 17.3                  |
| E01-0263-21036 ✓    | 0103841   | 030701BD   | 3/7/01          | 3/10-11/01    | 2.83                  |
| E01-0263-21036*     | 0103841   | 030701BD   | 3/7/01          | 3/10-11/01    | 2.87                  |
| E01-0263-21037 ✓    | 0103842   | 030701BD   | 3/7/01          | 3/10-11/01    | 3.48                  |
| E01-0263-21037*     | 0103842   | 030701BD   | 3/7/01          | 3/10-11/01    | 4.12                  |
| E01-0263-21038 ✓    | 0103843   | 030701BD   | 3/7/01          | 3/10-11/01    | 61.9                  |
| E01-0263-21038*     | 0103843   | 030701BD   | 3/7/01          | 3/10-11/01    | 60.3                  |
| E01-0263-21039 ✓    | 0103844   | 030701BD   | 3/7/01          | 3/10-11/01    | 3.69                  |
| E01-0263-21039*     | 0103844   | 030701BD   | 3/7/01          | 3/10-11/01    | 2.79                  |
| AVERAGE:            |           |            |                 |               | 26.2                  |
| STANDARD DEVIATION: |           |            |                 |               | 20.0                  |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XVIII. Summary of PFOS in Adult Serum Samples at 17.6 ppm  
a.i. (Test Termination 19 Weeks)**

| Sponsor<br>ID       | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/mL) |
|---------------------|--------------|---------------|--------------------|------------------|--------------------------|
| 1991 M              | 0008036      | 112100BDD     | 11/21/00           | 11/28/00         | 41.0                     |
| 1991*               | 0008036      | 112100BDD     | 11/21/00           | 11/28/00         | 41.1                     |
| 1992 F              | 0008037      | 112100BDD     | 11/21/00           | 11/28/00         | 47.0                     |
| 1992*               | 0008037      | 112100BDD     | 11/21/00           | 11/28/00         | 49.5                     |
| 1993 M              | 0008038      | 112100BDD     | 11/21/00           | 11/28/00         | 199                      |
| 1993*               | 0008038      | 112100BDD     | 11/21/00           | 11/28/00         | 198                      |
| 1994 F              | 0008039      | 112100BDD     | 11/21/00           | 11/28/00         | 93.9                     |
| 1994*               | 0008039      | 112100BDD     | 11/21/00           | 11/28/00         | 93.0                     |
| 1995 M              | 0008040      | 112100BDD     | 11/21/00           | 11/28/00         | 182                      |
| 1995*               | 0008040      | 112100BDD     | 11/21/00           | 11/28/00         | 180                      |
| 1996 F              | 0008041      | 112100BDD     | 11/21/00           | 11/28/00         | 218                      |
| 1996*               | 0008041      | 112100BDD     | 11/21/00           | 11/28/00         | 217                      |
| 1997 M              | 0008042      | 112100BDD     | 11/21/00           | 11/28/00         | 214                      |
| 1997*               | 0008042      | 112100BDD     | 11/21/00           | 11/28/00         | 212                      |
| 1998 F              | 0008043      | 112100BDD     | 11/21/00           | 11/28/00         | 156                      |
| 1998*               | 0008043      | 112100BDD     | 11/21/00           | 11/28/00         | 156                      |
| 1999 M              | 0008044      | 112100BDD     | 11/21/00           | 11/28/00         | 156                      |
| 1999*               | 0008044      | 112100BDD     | 11/21/00           | 11/28/00         | 161                      |
| 2000 F              | 0008045      | 112100BDD     | 11/21/00           | 11/28/00         | 200                      |
| 2000*               | 0008045      | 112100BDD     | 11/21/00           | 11/28/00         | 205                      |
| AVERAGE:            |              |               |                    |                  | 151                      |
| STANDARD DEVIATION: |              |               |                    |                  | 65.0                     |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XIX. Summary of PFOS in Adult Serum Samples at 17.6 ppm  
a.i. (6 Week Samples)**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|----------------------------|-----------|------------|-----------------|---------------|-----------------------|
| E01-0263-21020 M           | 0103825   | 030801AD   | 3/7/01          | 3/11-12/01    | 18.6                  |
| E01-0263-21020*            | 0103825   | 030801AD   | 3/7/01          | 3/11-12/01    | 18.1                  |
| E01-0263-21021 M           | 0103826   | 030701AD   | 3/7/01          | 3/10/01       | 152                   |
| E01-0263-21021*            | 0103826   | 030701AD   | 3/7/01          | 3/10/01       | 151                   |
| E01-0263-21022 M           | 0103827   | 030701AD   | 3/7/01          | 3/10/01       | 156                   |
| E01-0263-21022*            | 0103827   | 030701AD   | 3/7/01          | 3/10/01       | 157                   |
| E01-0263-21023 M           | 0103828   | 030701AD   | 3/7/01          | 3/10/01       | 137                   |
| E01-0263-21023*            | 0103828   | 030701AD   | 3/7/01          | 3/10/01       | 138                   |
| E01-0263-21024 M           | 0103829   | 030801AD   | 3/7/01          | 3/11-12/01    | 42.2                  |
| E01-0263-21024*            | 0103829   | 030801AD   | 3/7/01          | 3/11-12/01    | 39.1                  |
| E01-0263-21040 E           | 0103845   | 030701BD   | 3/7/01          | 3/10-11/01    | 55.9                  |
| E01-0263-21040*            | 0103845   | 030701BD   | 3/7/01          | 3/10-11/01    | 62.1                  |
| E01-0263-21041 F           | 0103846   | 030701BD   | 3/7/01          | 3/10-11/01    | 49.8                  |
| E01-0263-21041*            | 0103846   | 030701BD   | 3/7/01          | 3/10-11/01    | 43.2                  |
| E01-0263-21042 F           | 0103847   | 030701BD   | 3/7/01          | 3/10-11/01    | 15.8                  |
| E01-0263-21042*            | 0103847   | 030701BD   | 3/7/01          | 3/10-11/01    | 17.7                  |
| E01-0263-21043 F           | 0103848   | 030701BD   | 3/7/01          | 3/10-11/01    | 18.5                  |
| E01-0263-21043*            | 0103848   | 030701BD   | 3/7/01          | 3/10-11/01    | 17.3                  |
| E01-0263-21044 ✓           | 0103849   | 030701BD   | 3/7/01          | 3/10-11/01    | 14.1                  |
| E01-0263-21044*            | 0103849   | 030701BD   | 3/7/01          | 3/10-11/01    | 14.4                  |
| <b>AVERAGE:</b>            |           |            |                 |               | <b>65.9</b>           |
| <b>STANDARD DEVIATION:</b> |           |            |                 |               | <b>57.5</b>           |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XX. Summary of PFOS in Juvenile Serum Samples at 0 ppm a.i. (Test Termination 12 Weeks)**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|---------------------|-----------|------------|-----------------|---------------|-----------------------|
| 2301 $\square$      | 0008126   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2301*               | 0008126   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2302 $\mathcal{F}$  | 0008127   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2302* $\mathcal{I}$ | 0008127   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2303 $\mathcal{F}$  | 0008128   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00458               |
| 2303*               | 0008128   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00453               |
| 2305 $\mathcal{M}$  | 0008129   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2305*               | 0008129   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2307 $\mathcal{H}$  | 0008130   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00278               |
| 2307*               | 0008130   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00272               |
| 2308 $\mathcal{M}$  | 0008131   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2308*               | 0008131   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2314 $\mathcal{H}$  | 0008132   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2314*               | 0008132   | 112700A    | 11/27/00        | 11/28-29/00   | NQ                    |
| 2316 $\mathcal{M}$  | 0008133   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00381               |
| 2316*               | 0008133   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00379               |
| 2317 $\mathcal{F}$  | 0008134   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00626               |
| 2317*               | 0008134   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00601               |
| 2320 $\mathcal{F}$  | 0008135   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00141               |
| 2320*               | 0008135   | 112700A    | 11/27/00        | 11/28-29/00   | 0.00153               |
| AVERAGE:            |           |            |                 |               | 0.00190               |
| STANDARD DEVIATION: |           |            |                 |               | 0.00222               |

\* Duplicate injection

NQ = Not Quantifiable (A peak was detected at the corresponding analyte retention time but was less than the lowest concentration of the calibration standards (0.0001 µg/mL))

For values recorded as NQ, 0.00005 µg/mL (half the value of the lowest calibration standard, 0.0001 µg/mL) was used to calculate the average and standard deviation.

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XXI. Summary of PFOS in Juvenile Serum Samples at 1.8 ppm  
a.i. (Test Termination 12 Weeks)**

| Sponsor<br>ID              | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/mL) |
|----------------------------|--------------|---------------|--------------------|------------------|--------------------------|
| 2322 F                     | 0008136      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0464                   |
| 2322*                      | 0008136      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0472                   |
| 2323 M                     | 0008137      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0274                   |
| 2323*                      | 0008137      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0281                   |
| 2325 F                     | 0008138      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0497                   |
| 2325*                      | 0008138      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0487                   |
| 2326 F                     | 0008139      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0160                   |
| 2326*                      | 0008139      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0156                   |
| 2328 M                     | 0008140      | 112700D       | 11/27/00           | 11/29-30/00      | 0.249                    |
| 2328*                      | 0008140      | 112700D       | 11/27/00           | 11/29-30/00      | 0.250                    |
| 2330 F                     | 0008141      | 112700A       | 11/27/00           | 11/28-29/00      | 0.00788                  |
| 2330*                      | 0008141      | 112700A       | 11/27/00           | 11/28-29/00      | 0.00747                  |
| 2331 M                     | 0008142      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0101                   |
| 2331*                      | 0008142      | 112700A       | 11/27/00           | 11/28-29/00      | 0.00998                  |
| 2332 F                     | 0008143      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0113                   |
| 2332*                      | 0008143      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0110                   |
| 2333 F                     | 0008144      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0339                   |
| 2333*                      | 0008144      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0339                   |
| 2334 M                     | 0008145      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0554                   |
| 2334*                      | 0008145      | 112700A       | 11/27/00           | 11/28-29/00      | 0.0555                   |
| <b>AVERAGE:</b>            |              |               |                    |                  | <b>0.0507</b>            |
| <b>STANDARD DEVIATION:</b> |              |               |                    |                  | <b>0.0701</b>            |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XXII. Summary of PFOS in Juvenile Serum Samples at 6.2 ppm  
a.i. (Test Termination 12 Weeks)**

| Sponsor<br>ID       | Centre<br>ID | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Analyte<br>Found (µg/mL) |
|---------------------|--------------|---------------|--------------------|------------------|--------------------------|
| 2335 F              | 0008146      | 112700B       | 11/27/00           | 11/29/00         | 0.0159                   |
| 2335*               | 0008146      | 112700B       | 11/27/00           | 11/29/00         | 0.0162                   |
| 2336 F              | 0008147      | 112700D       | 11/27/00           | 11/29-30/00      | 0.118                    |
| 2336*               | 0008147      | 112700D       | 11/27/00           | 11/29-30/00      | 0.124                    |
| 2337 F              | 0008148      | 112700D       | 11/27/00           | 11/29-30/00      | 0.181                    |
| 2337*               | 0008148      | 112700D       | 11/27/00           | 11/29-30/00      | 0.183                    |
| 2338 M              | 0008149      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0584                   |
| 2338*               | 0008149      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0592                   |
| 2339 M              | 0008150      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0889                   |
| 2339*               | 0008150      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0840                   |
| 2341 M              | 0008151      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0556                   |
| 2341*               | 0008151      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0558                   |
| 2342 F              | 0008152      | 112700B       | 11/27/00           | 11/29/00         | 0.0380                   |
| 2342*               | 0008152      | 112700B       | 11/27/00           | 11/29/00         | 0.0383                   |
| 2343 M              | 0008153      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0700                   |
| 2343*               | 0008153      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0717                   |
| 2344 F              | 0008154      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0676                   |
| 2344*               | 0008154      | 112700D       | 11/27/00           | 11/29-30/00      | 0.0688                   |
| 2346 F              | 0008155      | 112700B       | 11/27/00           | 11/29/00         | 0.0371                   |
| 2346*               | 0008155      | 112700B       | 11/27/00           | 11/29/00         | 0.0373                   |
| AVERAGE:            |              |               |                    |                  | 0.0734                   |
| STANDARD DEVIATION: |              |               |                    |                  | 0.0466                   |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XXIII. Summary of PFOS in Juvenile Serum Samples at 17.6 ppm a.i. (Test Termination 12 Weeks)**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/mL) |
|---------------------|-----------|------------|-----------------|---------------|-----------------------|
| 2347 F              | 0008156   | 112700D    | 11/27/00        | 11/29-30/00   | 0.490                 |
| 2347*               | 0008156   | 112700D    | 11/27/00        | 11/29-30/00   | 0.496                 |
| 2348 F              | 0008157   | 112700D    | 11/27/00        | 11/29-30/00   | 0.150                 |
| 2348*               | 0008157   | 112700D    | 11/27/00        | 11/29-30/00   | 0.156                 |
| 2353 F              | 0008158   | 112700D    | 11/27/00        | 11/29-30/00   | 0.894                 |
| 2353*               | 0008158   | 112700D    | 11/27/00        | 11/29-30/00   | 0.908                 |
| 2354 M              | 0008159   | 112700D    | 11/27/00        | 11/29-30/00   | 0.591                 |
| 2354*               | 0008159   | 112700D    | 11/27/00        | 11/29-30/00   | 0.621                 |
| 2355 M              | 0008160   | 112700D    | 11/27/00        | 11/29-30/00   | 0.399                 |
| 2355*               | 0008160   | 112700D    | 11/27/00        | 11/29-30/00   | 0.384                 |
| 2357 M              | 0008161   | 112700D    | 11/27/00        | 11/29-30/00   | 0.547                 |
| 2357*               | 0008161   | 112700D    | 11/27/00        | 11/29-30/00   | 0.547                 |
| 2358 F              | 0008162   | 112700D    | 11/27/00        | 11/29-30/00   | 0.512                 |
| 2358*               | 0008162   | 112700D    | 11/27/00        | 11/29-30/00   | 0.517                 |
| 2359 F              | 0008163   | 112700D    | 11/27/00        | 11/29-30/00   | 0.402                 |
| 2359*               | 0008163   | 112700D    | 11/27/00        | 11/29-30/00   | 0.404                 |
| 2360 M              | 0008164   | 112700D    | 11/27/00        | 11/29-30/00   | 0.521                 |
| 2360*               | 0008164   | 112700D    | 11/27/00        | 11/29-30/00   | 0.514                 |
| 2363 M              | 0008165   | 112700D    | 11/27/00        | 11/29-30/00   | 0.439                 |
| 2363*               | 0008165   | 112700D    | 11/27/00        | 11/29-30/00   | 0.434                 |
| AVERAGE:            |           |            |                 |               | 0.496                 |
| STANDARD DEVIATION: |           |            |                 |               | 0.184                 |

\* Duplicate injection

LOQ = 0.01 µg/g for liver and 0.01 µg/mL for serum

**Table XXIV. Summary of Percent Moistures for Mallard Liver Samples**

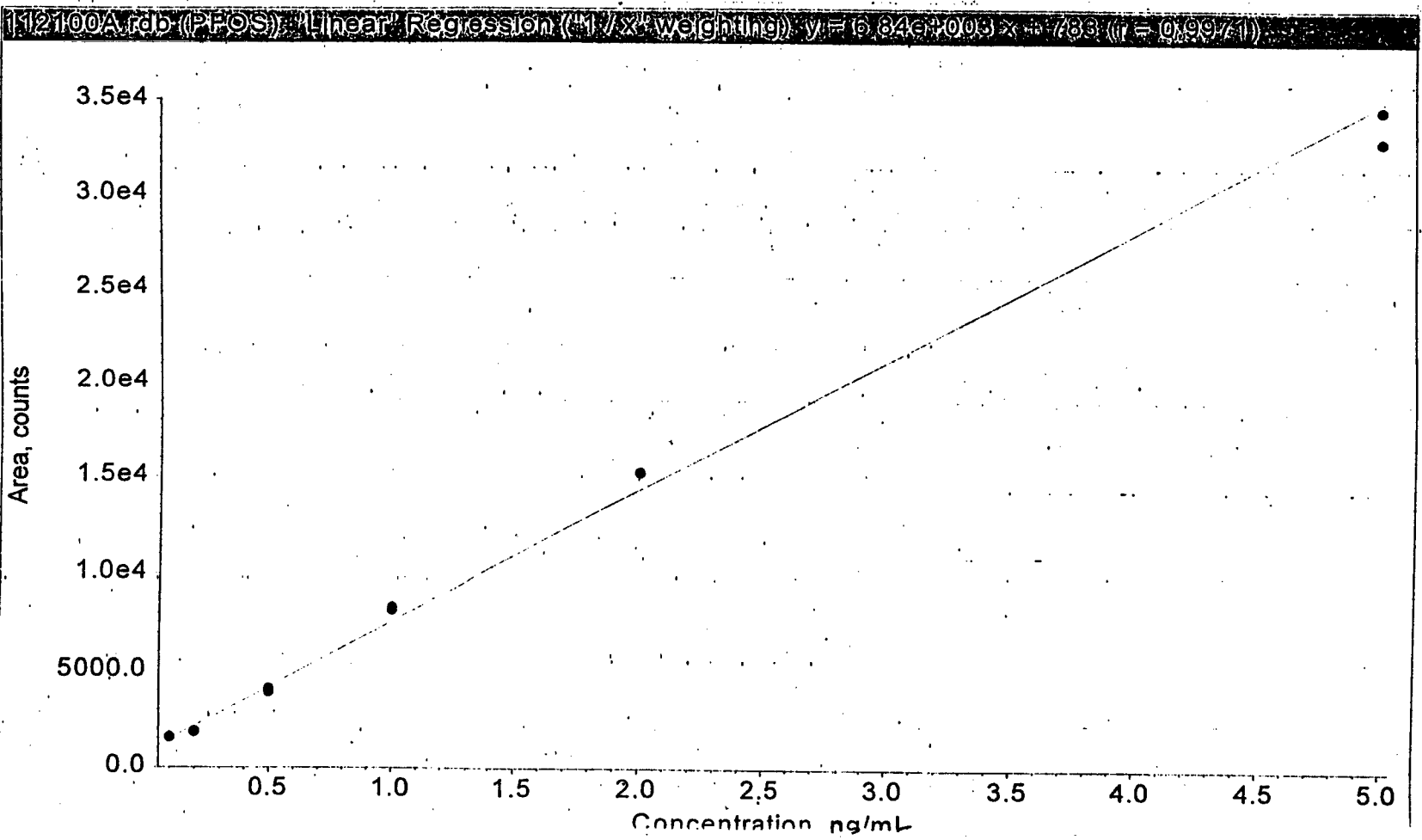
| <b>Sponsor<br/>ID</b> | <b>Centre<br/>ID</b> | <b>Average<br/>% Moisture</b> |
|-----------------------|----------------------|-------------------------------|
| 1961                  | 0008006              | 69.7                          |
| 1962                  | 0008007              | 73.4                          |
| 1963                  | 0008008              | 75.7                          |
| 1964                  | 0008009              | 69.2                          |
| 1965                  | 0008010              | 71.9                          |
| 1966                  | 0008011              | 75.0                          |
| 1967                  | 0008012              | 72.8                          |
| 1968                  | 0008013              | 70.4                          |
| 1969                  | 0008014              | 69.5                          |
| 1970                  | 0008015              | 72.9                          |
| 1991                  | 0008016              | 75.3                          |
| 1992                  | 0008017              | 71.8                          |
| 1993                  | 0008018              | 71.9                          |
| 1994                  | 0008019              | 71.8                          |
| 1995                  | 0008020              | 71.5                          |
| 1996                  | 0008021              | 72.4                          |
| 1997                  | 0008022              | 70.5                          |
| 1998                  | 0008023              | 71.3                          |
| 1999                  | 0008024              | 69.8                          |
| 2000                  | 0008025              | 72.0                          |
| 2301                  | 0008086              | 76.6                          |
| 2302                  | 0008087              | 77.3                          |
| 2303                  | 0008088              | 72.7                          |
| 2305                  | 0008089              | 72.5                          |
| 2307                  | 0008090              | 72.3                          |
| 2308                  | 0008091              | 72.0                          |
| 2314                  | 0008092              | 75.0                          |
| 2316                  | 0008093              | 69.6                          |
| 2317                  | 0008094              | 71.8                          |
| 2320                  | 0008095              | 71.6                          |
| 2322                  | 0008096              | 71.6                          |
| 2323                  | 0008097              | 72.5                          |
| 2325                  | 0008098              | 73.2                          |
| 2326                  | 0008099              | 72.6                          |
| 2328                  | 0008100              | 72.0                          |
| 2330                  | 0008101              | 68.9                          |
| 2331                  | 0008102              | 71.4                          |
| 2332                  | 0008103              | 71.7                          |
| 2333                  | 0008104              | 69.8                          |
| 2334                  | 0008105              | 68.1                          |
| 2335                  | 0008106              | 71.7                          |

**Table XXIV (cont'). Summary of Percent Moistures for Mallard Liver Samples**

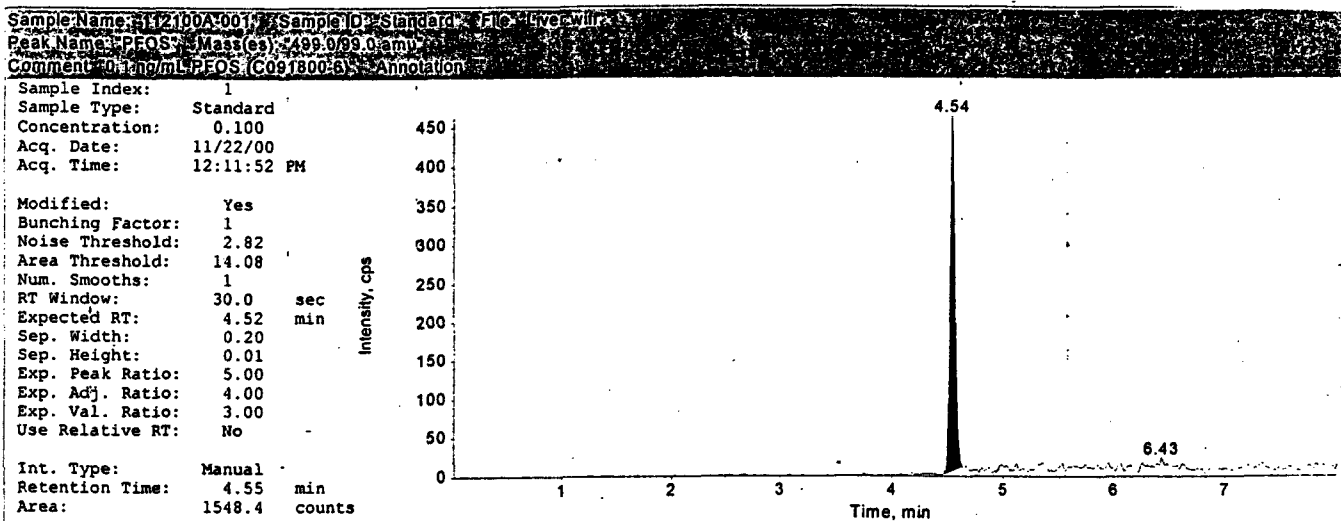
| <b>Sponsor<br/>ID</b> | <b>Centre<br/>ID</b> | <b>Average<br/>% Moisture</b> |
|-----------------------|----------------------|-------------------------------|
| 2336                  | 0008107              | 69.7                          |
| 2337                  | 0008108              | 69.7                          |
| 2338                  | 0008109              | 69.8                          |
| 2339                  | 0008110              | 70.6                          |
| 2341                  | 0008111              | 72.6                          |
| 2342                  | 0008112              | 78.2                          |
| 2343                  | 0008113              | 70.9                          |
| 2344                  | 0008114              | 69.2                          |
| 2346                  | 0008115              | 70.9                          |
| 2347                  | 0008116              | 69.7                          |
| 2348                  | 0008117              | 70.3                          |
| 2353                  | 0008118              | 73.2                          |
| 2354                  | 0008119              | 68.5                          |
| 2355                  | 0008120              | 67.8                          |
| 2357                  | 0008121              | 74.5                          |
| 2358                  | 0008122              | 72.0                          |
| 2359                  | 0008123              | 73.6                          |
| 2360                  | 0008124              | 69.0                          |
| 2363                  | 0008125              | 74.1                          |
| E01-0263-21065        | 0103870              | 73.8                          |
| E01-0263-21066        | 0103871              | 81.0                          |
| E01-0263-21067        | 0103872              | 82.4                          |
| E01-0263-21068        | 0103873              | 75.8                          |
| E01-0263-21069        | 0103874              | 78.2                          |
| E01-0263-21070        | 0103875              | 77.4                          |
| E01-0263-21071        | 0103876              | 77.5                          |
| E01-0263-21072        | 0103877              | 74.8                          |
| E01-0263-21073        | 0103878              | 77.5                          |
| E01-0263-21074        | 0103879              | 82.7                          |
| E01-0263-21075        | 0103880              | 71.7                          |
| E01-0263-21076        | 0103881              | 77.9                          |
| E01-0263-21077        | 0103882              | 77.5                          |
| E01-0263-21078        | 0103883              | 76.0                          |
| E01-0263-21079        | 0103884              | 63.8                          |
| E01-0263-21080        | 0103885              | 78.2                          |
| E01-0263-21081        | 0103886              | 73.6                          |
| E01-0263-21082        | 0103887              | 76.7                          |
| E01-0263-21083        | 0103888              | 81.4                          |
| E01-0263-21084        | 0103889              | 72.3                          |

# FIGURES

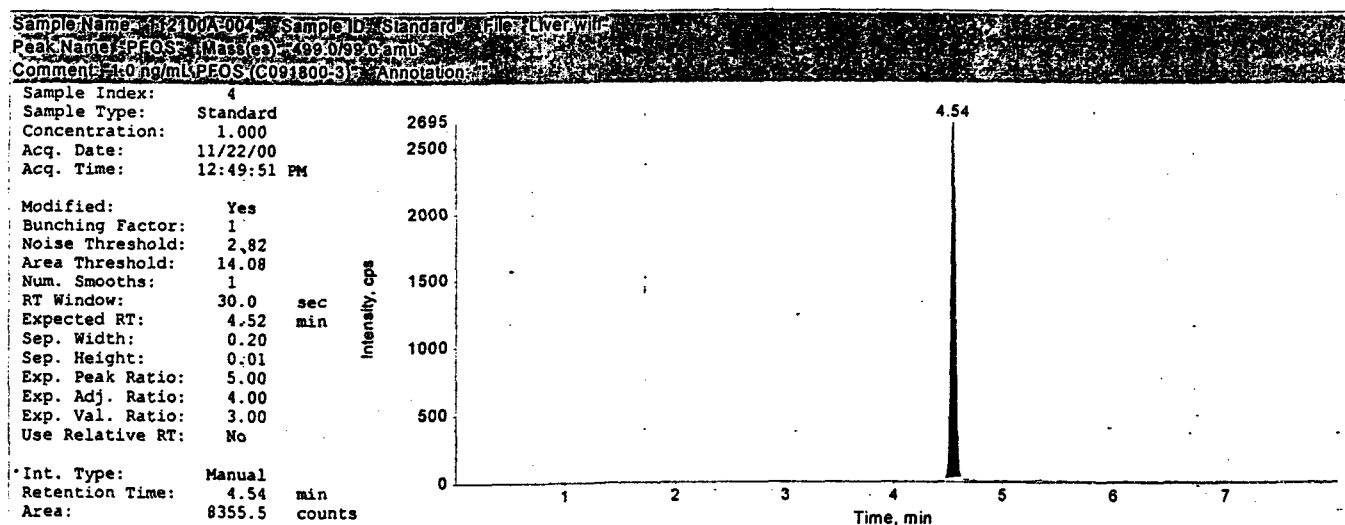
Figure 1. Typical Calibration Curve for PFOs



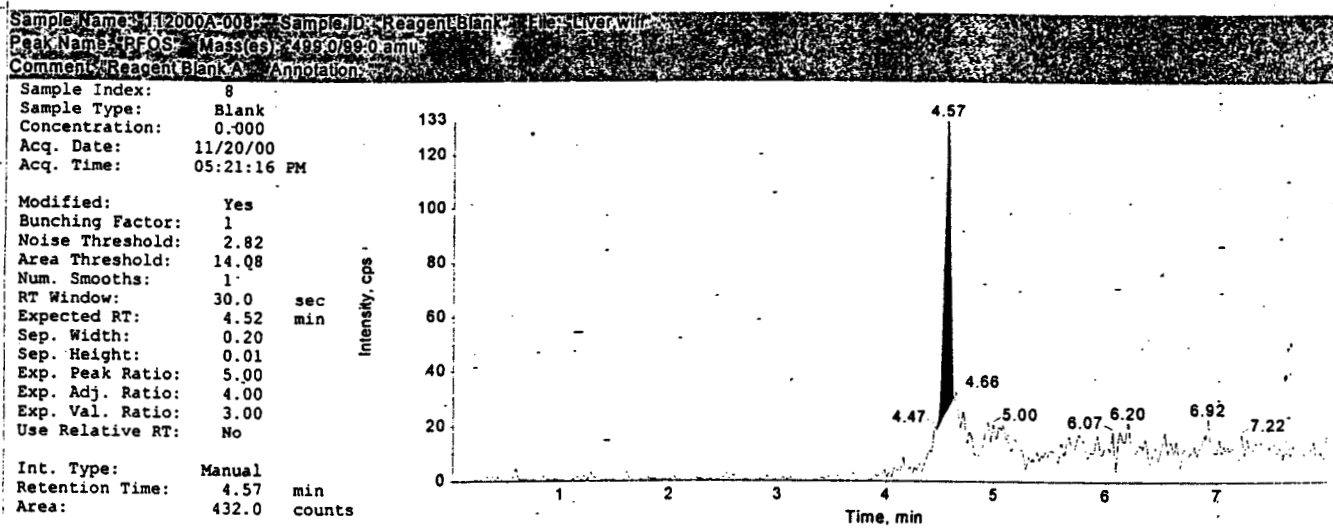
**Figure 2. Chromatogram Representing a 0.1 ng/mL standard for PFOS**



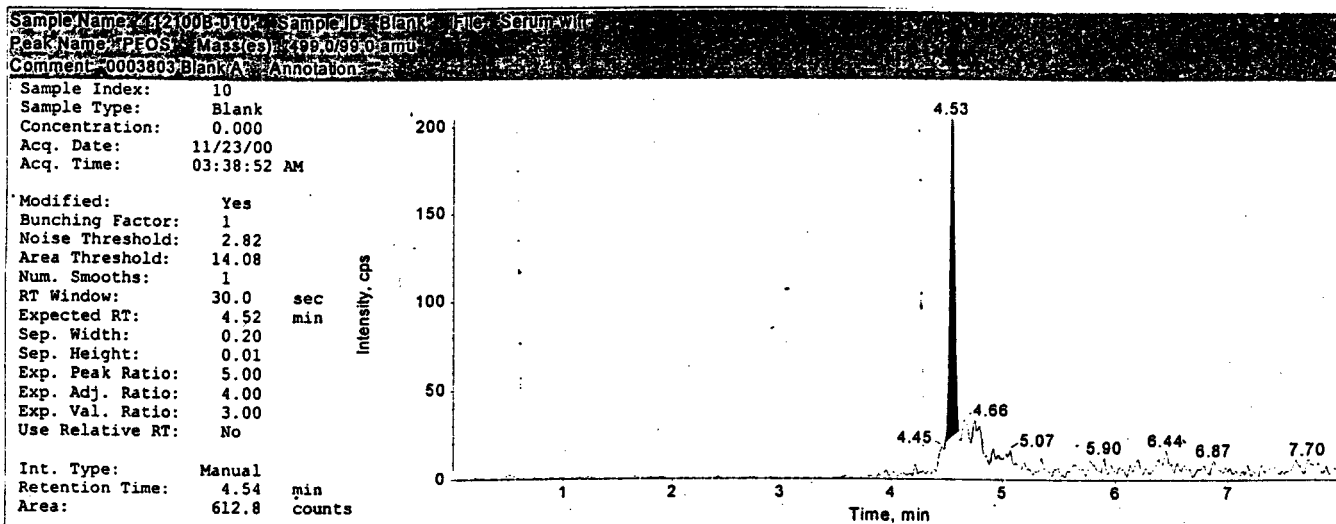
**Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS**



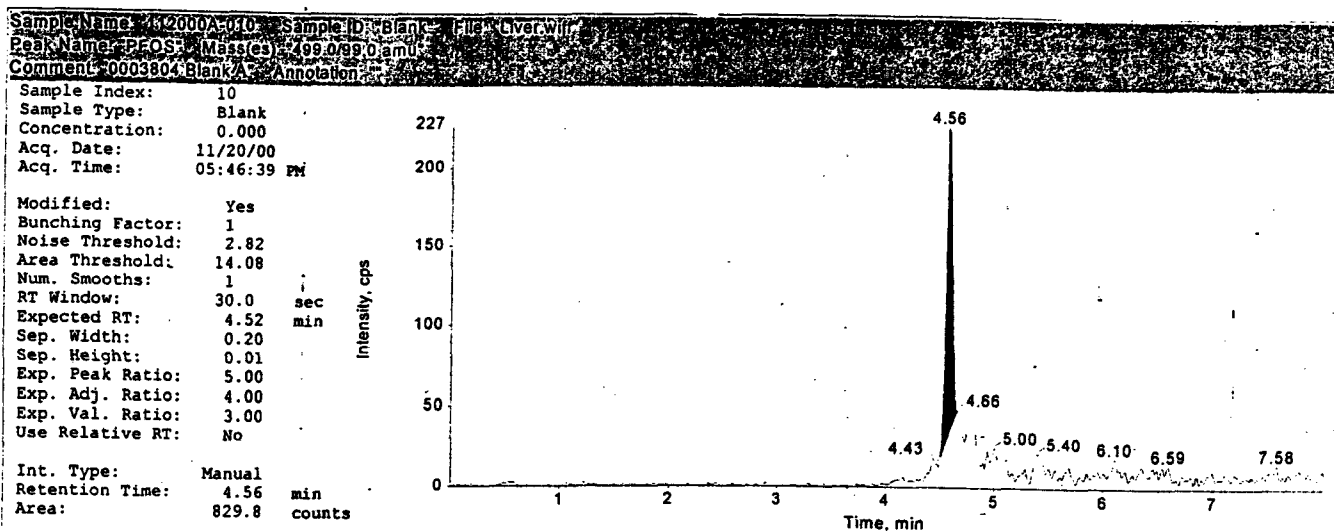
**Figure 4. Chromatogram Representing a Reagent Blank for PFOS  
(Centre ID Reagent Blank A, Set: 112000A)**



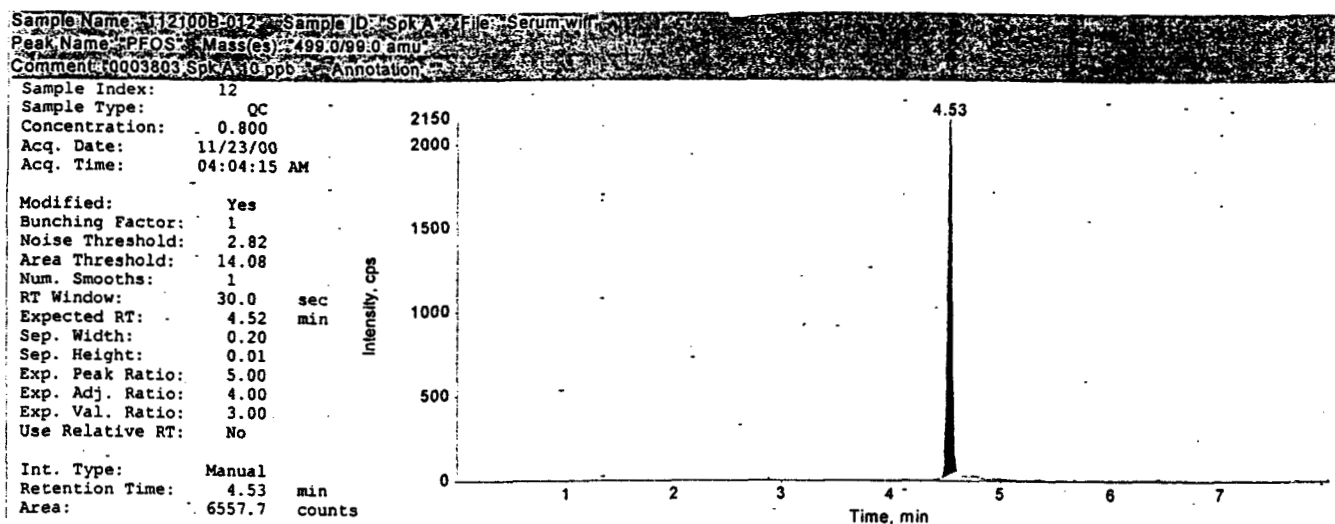
**Figure 5. Chromatogram Representing Control Mallard Serum for PFOS (Centre ID: 0003803 Blank A, Set: 112100B)**



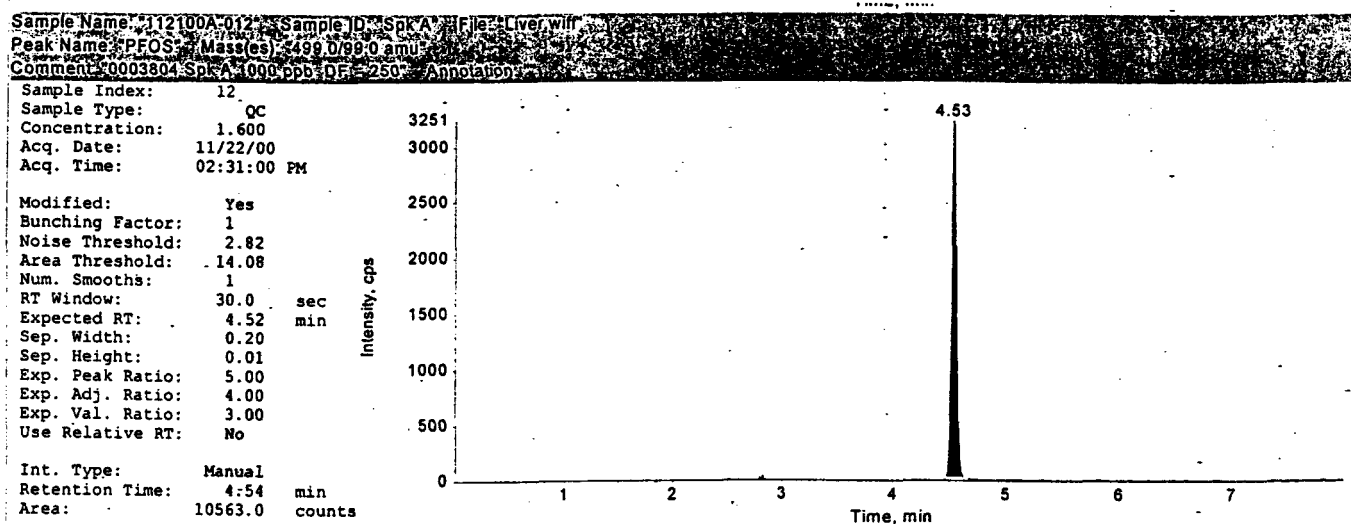
**Figure 6. Chromatogram Representing Control Mallard Liver for PFOS, (Centre ID: 0003804 Blank A, Set: 112000A)**



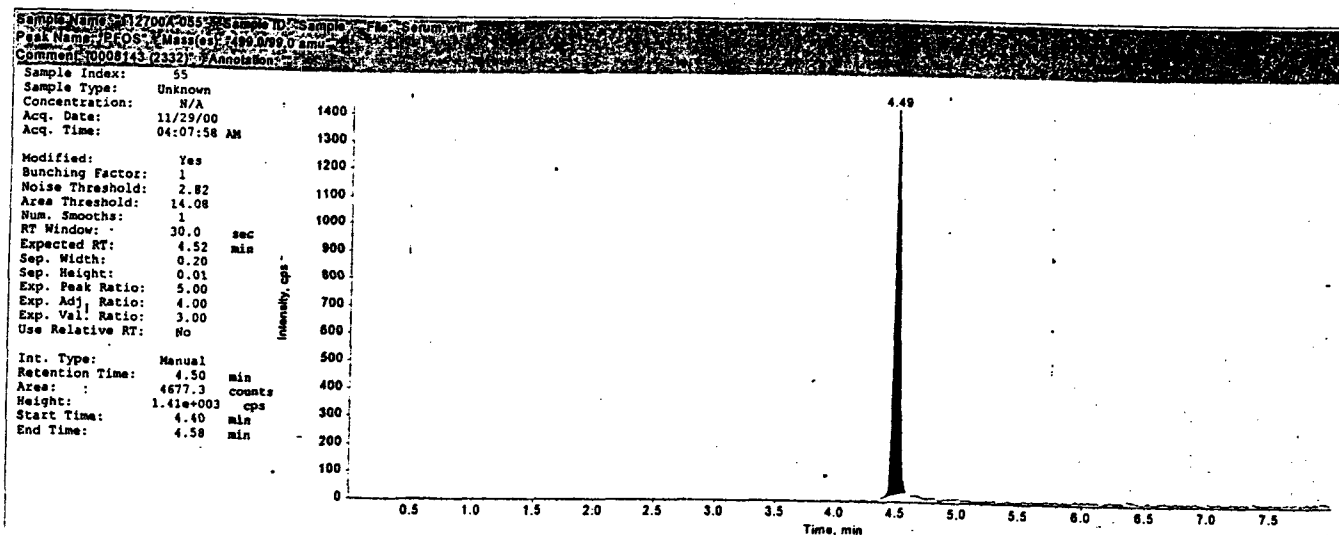
**Figure 7. Chromatogram Representing Control Mallard Serum  
fortified with 10 ppb of PFOS  
(Centre ID: 0003803 Spk A, Set: 112100B)**



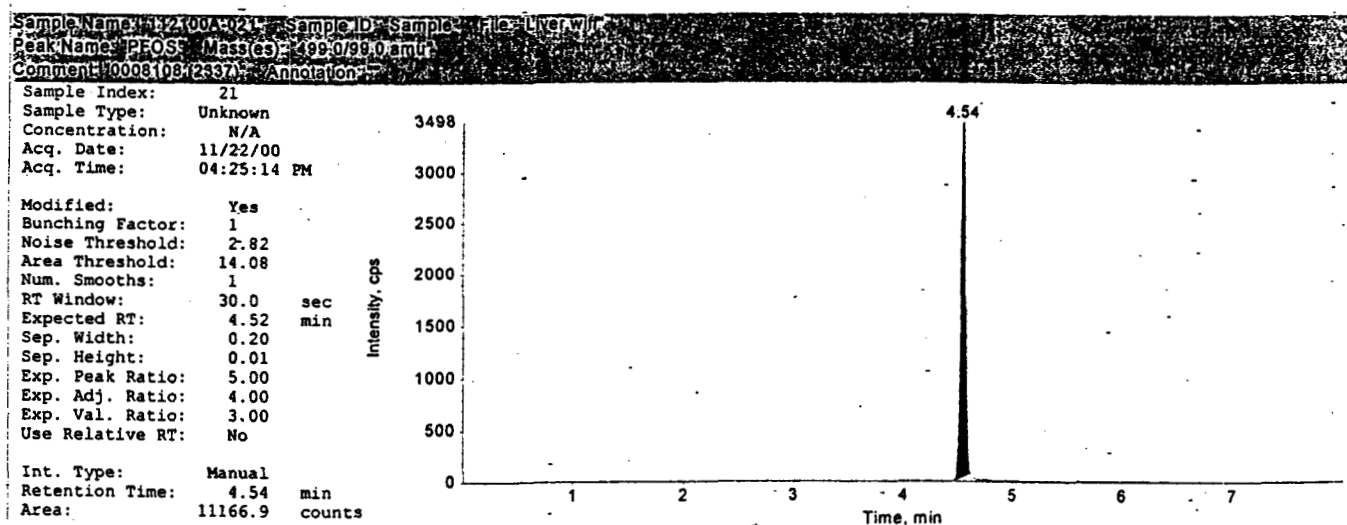
**Figure 8. Chromatogram Representing Control Mallard Liver  
Fortified at 1000 ppb with PFOS  
(Centre ID: 0003804 Spk A, Set: 112100A)**



**Figure 9. Chromatogram Representing Juvenile Mallard Serum Sample (Centre ID: 0008143, Sponsor ID: 2332, Set: 112700A)**



**Figure 10. Chromatogram Representing Juvenile Mallard Liver  
Sample (Centre ID: 0008108, Sponsor ID: 2337,  
Set: 112100A)**



# **APPENDIX A**

## **Study Protocol 00P-023-042 (Centre Study No. 023-042) and Amendments and Deviation**

Centre Protocol No. 00P-023-042

## STUDY PROTOCOL

### STUDY TITLE

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM  
MALLARD SERUM AND MALLARD LIVER FOR ANALYSIS USING HPLC-  
ELECTROSPRAY/MASS SPECTROMETRY

### SPONSOR

3M Environmental Technology and Safety Services  
Building 2-3E-09  
PO Box 33331  
St. Paul, MN 55133-3331

### DATA REQUIREMENTS

Analytical Method Requirements

### TESTING LABORATORY

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

### PROTOCOL IDENTIFICATION NUMBER

00P-023-042

Centre Protocol No. 00P-023-042

Title: EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM  
MALLARD SERUM AND MALLARD LIVER FOR ANALYSIS USING HPLC-  
ELECTROSPRAY/MASS SPECTROMETRY

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Study Title:

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM  
MALLARD SERUM AND MALLARD LIVER FOR ANALYSIS USING HPLC-  
ELECTROSPRAY/MASS SPECTROMETRY

1. **PURPOSE**

The purpose of this study is to analyze mallard serum samples and mallard liver samples for residues of perfluorooctanesulfonate (PFOS) using methods entitled, "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) and "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Liver For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-6.0) with the modifications listed in Section 8.

The Quality Assurance Unit of Centre Analytical Laboratories, Inc., will audit the study for compliance with EPA TSCA Good Laboratory Practice standards 40 CFR Part 792 (1).

2. **TEST MATERIALS**

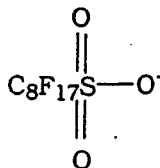
The following analytical standards will be used:

| Test Material | TCR Number  | Purity (%) | Expiration Date |
|---------------|-------------|------------|-----------------|
| PFOS          | TCR00017-46 | 97.9       | 08/31/01        |

Chemical name and structure of the compound is presented below.

**PFOS**

Chemical Name: Perfluorooctanesulfonate  
IUPAC Name: 1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptafluoro-, potassium salt  
CAS Number: 2795-39-3  
Molecular Weight: 499 (C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub><sup>-</sup>)



Note: The neutral molecule and standard form that PFOS (anion) is derived from is perfluorooctanesulfonate potassium salt [C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>K], molecular weight 538.

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A record of test and reference substance receipt, storage conditions, and a record of use will be maintained at Centre Analytical Laboratories, Inc. Forms documenting chain-of-custody and shipping records for tracking of the test substances will be included as part of the raw data package.

All standards/test substances and any prepared solutions must be identified with a unique label or number on the container or cross-referenced to the container.

### HAZARD INFORMATION

A current MSDS for the chemical(s) used in this study will be maintained at the testing facility.

#### 3. SPONSOR

3M Environmental Technology and Safety Services  
Building 2-3E-09  
PO Box 33331  
St. Paul, MN 55133-3331

STUDY DIRECTOR:  
Rochelle Robideau  
TELEPHONE: 651-778-7065  
FAX NUMBER: 651-778-6176

#### 4. TESTING FACILITY AND PERFORMING LABORATORY

Centre Analytical Laboratories, Inc. (Centre)  
3048 Research Drive  
State College, PA 16801

PRINCIPAL INVESTIGATOR:  
Emily R. Stauffer, Centre  
TELEPHONE: 814-231-8032  
FAX NUMBER: 814-231-1580

#### 5. PROPOSED EXPERIMENTAL TIME-FRAME

|                               |               |
|-------------------------------|---------------|
| Experimental Start Date       | Nov. 6, 2000  |
| Experimental Termination Date | Nov. 24, 2000 |
| Report Issued                 | Dec. 30, 2000 |

#### 6. JUSTIFICATION FOR THE SELECTION OF THE TEST SYSTEM

Mallard serum samples and liver samples collected by Wildlife International, Ltd. will be used as the test systems for this study. Mallards represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. Mallard habitats include ponds, lakes and marshes, and

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they are considered aquatic grazers. The use of these matrices for the study was to determine if PFOS was deposited in the liver and serum of mating adults that were fed PFOS.

## 7. SAMPLE PROCESSING, STORAGE AND IDENTIFICATION

No processing will be required for the serum samples. Due to the small amount of sample available, the liver samples will not be homogenized prior to extraction; however, they will be homogenized by lying the samples on a flat surface and mashing them by hand as best as possible.

Each sample will be assigned a unique sample identification number at Centre, which will be used for tracking and identification of the samples. The samples will be stored in a temperature-monitored freezer, maintained at  $< -10^{\circ}\text{C}$ , except when removed for extraction and analysis as described in the method. The samples will be kept isolated from the test substance during storage.

Sample receipt and storage location and conditions during the study will be documented. All samples and any resulting sample extracts will be identified with a unique label or sample number. Such identification will be either on the container or cross-referenced to the container.

## 8. ANALYTICAL METHOD

The serum samples will be analyzed according to the analytical method titled "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Serum For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-4.1) and the control liver samples will be analyzed according to the method titled "Extraction Of Potassium Perfluorooctanesulfonate Or Other Fluorochemical Compounds From Liver For Analysis Using HPLC-Electrospray/Mass Spectrometry" (3M method number ETS-8-6.0). The following modifications will be applied to each method:

1. There will be no surrogate standard used in the extraction procedure. The stock, fortification, and calibration standards will be prepared as follows:

### Stock Solution

Prepare a stock solution of PFOS at  $100\text{ }\mu\text{g/mL}$  by weighing out 10.0 mg of analytical standard (corrected for percent salt and percent purity). Adjust final volume to 100 mL with methanol in a 100-mL volumetric flask. Store this stock solution (in 125-mL LDPE bottles) in a refrigerator at  $2^{\circ}\text{C}$  to  $6^{\circ}\text{C}$  for a maximum period of 6 months from the date of preparation.

### Fortification Solutions

- a.  $1.0\text{ }\mu\text{g/mL}$  Fortification Solution – Pipette 1.0 mL of the  $100\text{ }\mu\text{g/mL}$  stock solution into a 100 mL volumetric flask. Bring up to volume with methanol.

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- b. 0.1 µg/mL Fortification Solution – Pipette 10.0 mL of the 1.0 µg/mL fortification solution into a 100-mL volumetric flask and bring up to volume with methanol.
- c. 0.01 µg/mL Fortification Solution – Pipette 10.0 mL of the 0.1 µg/mL fortification solution into a 100-mL volumetric flask and bring up to volume with methanol

Store all fortification standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

**Calibration Standards**

Prepare six LC/MS/MS calibration standards in methanol via dilution of the 0.1 µg/mL and the 0.01 µg/mL fortification solutions.

This is a typical example; additional concentrations may be prepared as needed.

| Initial Conc. (µg/mL) | Volume (mL) | Diluted to (mL) | Final Conc. (µg/mL) |
|-----------------------|-------------|-----------------|---------------------|
| 0.1                   | 5           | 100             | 0.005               |
| 0.1                   | 2           | 100             | 0.002               |
| 0.1                   | 1           | 100             | 0.001               |
| 0.01                  | 5           | 100             | 0.0005              |
| 0.01                  | 2           | 100             | 0.0002              |
| 0.01                  | 1           | 100             | 0.0001              |

Store all calibration standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

- The liver samples will not be processed to make the homogenate described in the method Section 12.2 to 12.12. Due to limited sample available, samples will be mashed on a flat surface by hand prior to extraction.
- The sample weight for liver will be 0.50 g ( $\pm$  0.05) and the sample volume for serum will be 100 µL. The amount of 0.5 M TBA added to the serum sample will be 0.5 mL and the amount of 0.25 M sodium carbonate/sodium bicarbonate buffer added will be 1.0 mL. Samples will be placed on a wrist-action shaker for ~ 20 min. at level 10 and then centrifuged for ~ 15 min @ ~3000 rpm. The samples will only be filtered with 0.2 µm nylon mesh filter if necessary. Samples will be reconstituted in a final volume of 1 mL of methanol for both matrices.

4. The samples will be analyzed under the following conditions:

**LC/MS/MS System and Operating Conditions (TurboIonSpray)**

Mass Spec: PE SCIEX API 3000 Biomolecular Mass Analyzer  
Interface: SCIEX TurboIon Spray Liquid Introduction Interface  
Harvard infusion pump  
Computer: Power Macintosh G3  
Software: PE SciexAnalyst 1.1  
Windows NT  
HPLC: Hewlett Packard (HP) Series 1100  
HP Quat Pump  
HP Vacuum Degasser  
HP Autosampler  
HP Column Oven

HPLC Column: Genesis C<sub>8</sub> (Jones Chromatography), 2.1 mm x 50 mm, 4µ  
Column Temp.: 35° C  
Injection Vol.: 10 µL  
Mobile Phase (A): 2 mM Ammonium Acetate in ASTM type I water  
Mobile Phase (B): Methanol  
Flow Rate: 0.3 mL/min.

| <u>Time</u> | <u>% A</u> | <u>% B</u> |
|-------------|------------|------------|
| 0           | 60         | 40         |
| 1.0         | 0          | 100        |
| 7.0         | 0          | 100        |
| 7.5         | 40         | 60         |
| 11.0        | 40         | 60         |

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g., 2.1 mm x 30 mm) and columns from different manufacturers (Keystone Betasil C<sub>18</sub> etc.) can be used, provided equivalent chromatography is obtained.

Ions monitored:

| <u>Analyte</u> | <u>Mode</u> | <u>Transition Monitored</u> | <u>Approximate Retention Time (min)</u> |
|----------------|-------------|-----------------------------|-----------------------------------------|
| PFOS           | negative    | 499 → 99                    | 4.20                                    |

## Centre Protocol No. 00P-023-042

On a day-to-day basis, the retention times may vary slightly depending on the batch of mobile phase, etc.

**Example Tune File Parameters**

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.

The mass spectrometer is tuned using a 0.5µg/mL PFOS solution, prepared via dilution of the stock solution in methanol. The solution is infused (using a "T" connector) at 10 µL/min into a 0.2 mL/min stream of mobile phase consisting of 40% methanol and 60% 2 mM ammonium acetate. The analytes are initially tuned for the parent ion and then tuned for the product ion. Once the instrument is tuned, the optimized parameters are saved as a "tune file". This tune file is then used during routine analysis. The tuning procedure may be repeated as necessary to ensure optimal sensitivity.

| <u>Controls</u>                 | <u>Set</u> |
|---------------------------------|------------|
| IS-Iospray                      | -4000.0    |
| OR-Orifice                      | -61.0      |
| RNG-Focus Ring                  | -270.0     |
| Q0-Quad 0 Rod Offset            | 10.0       |
| IQ1- Inter quad 1 lens          | 9.3        |
| ST-Stubbies                     | 15.0       |
| RO1-Quad1 Rod Offset            | 9.3        |
| IQ2-Inter quad 2 lens           | 20.0       |
| RO2-Quad 2 rod offset           | 84.0       |
| ST3-Stubbies                    | 100.0      |
| RO3- Quad 3 rod offset          | 86.0       |
| DF-CEM Deflection Plate         | 300.0      |
| CEM-Channel Electron Multiplier | 2800.0     |
|                                 |            |
| <u>Gas Flows</u>                | <u>Set</u> |
| Nebulizer Gas                   | 12         |
| Curtain Gas                     | 13         |
| Collision Gas                   | 4          |
| TIS Temperature                 | 350°C      |

**Calibration Procedures**

- Inject the same volume (between 10 to 20 µL) of each calibration standard (prepared in MeOH) into the LC/MS/MS.
- Use linear, 1/x weighted standard curves for quantification. Linear standard curves are generated for each set by linear regression using the appropriate software system. Any calibration standards falling outside  $\pm 30\%$ , based on its calculated concentration, must

Centre Protocol No. 00P-023-042

be excluded from the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 30% of the total number of standards injected.

- c. The correlation coefficient ( $r$ ) for calibration curves generated must be  $\geq 0.9925$  ( $r^2 \geq 0.985$ ). If calibration results fall outside these limits, then appropriate steps should be taken to adjust instrument operation, and the relevant set of samples must be reanalyzed.

**Sample Analysis**

Inject the same volume used for the calibration standards (between 10 to 20  $\mu\text{L}$ ) of each sample, fortification, control, etc. into the LC/MS/MS. Each sample must be analyzed in duplicate.

- a. Standards corresponding to at least six concentrations must be included in an analytical set.
- b. Inject an entire set of standards (six) at the beginning of the run and inject standards interspersed about every 5-10 samples. All sample injections must be bracketed by standard injections.
- c. Each set of samples analyzed (not to exceed 25) must include at least one reagent control (extraction solvents only), at least one matrix control, and two matrix control samples fortified at known concentrations and carried through the procedure to verify recovery.
- d. The concentration of each sample, fortification, control, etc. is determined from the standard curve based on the peak area of the analyte in all standards injected during a set. The standard responses must bracket responses of the residue found in the sample set. If necessary, dilute the samples and re-analyze to give a response within the standard curve range.
- e. Fortification recoveries within 80 to 120% are acceptable for fortifications at the LOQ level. Recoveries between 80 to 120% are acceptable for fortifications at levels greater than the LOQ. Failure to meet these criteria requires an investigation of cause and a full reanalysis of the affected samples.
- f. Samples in which no peaks are detected at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention times but are less than 3:1 signal:noise will be reported as NQ (not quantifiable).

- g. Background levels of analyte found in control/blank samples that correspond to values below the LOQ, but are still quantifiable, will be used to correct fortification recoveries.
- h. If samples are not loaded on the instrument to be analyzed the day they are extracted, samples must be stored refrigerated at approximately 2°C to 6°C until analysis and analyzed preferably within a week.

## 9. EXPERIMENTAL DESIGN

Samples obtained by Wildlife International Ltd. have been shipped to Centre Analytical Laboratories, Inc. for analysis.

All samples will be analyzed according to methods, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry" and "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" with the modifications detailed in Section 8.

Methods to control bias will include assay of untreated control samples, fortification of untreated control samples to obtain recovery data, and replicate analysis of fortified samples to provide an indication of reproducibility.

The average recovery and relative standard deviation of the fortified samples will be calculated. Also, the average residue found and standard deviation for each matrix will be calculated.

## 10. PROTOCOL AMENDMENTS AND DEVIATIONS

Any deviations from the protocol or from the analytical method as provided will be documented and reported promptly to the Study Director. Planned changes to this protocol or to the analytical method will be made in writing as an amendment and approved by the Principal Investigator and the Study Director. Any amendments or deviations will be appended to this protocol and included in the final report.

1. Protocol amendments: Planned changes to the approved protocol shall be documented by amendments that clearly describe the change, justification for the change, and impact on the study. Amendments will be signed and dated by the Principal Investigator and Study Director. Copies of amendments will be sent to the quality assurance unit.

2. Protocol deviations: Protocol deviations, which are one time and unplanned deviations from the protocol, shall be documented in the study records, noting the nature of the deviation, potential effect or impact on the study, and corrective action if required. Protocol deviations are signed and dated by the Principal Investigator and Study Director and reviewed by QAU.

## 11. RECORDS

Records to be maintained include, but are not limited the following (as appropriate):

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

All chromatograms will contain the following:

- a. Sample identification, date, arrow or other indication of the area of interest, and injection number corresponding to the run.
- b. Additionally, fortifications will include the fortification level of the analyte.
- c. Analytical standard chromatograms will additionally include the concentration (e.g.,  $\mu\text{g/ml}$ ,  $\text{ng/mL}$ , ppb, ppt, etc.).

Each data set will contain information on temperatures, flow rates, column parameters, gases, instrument parameters, and instrument type, etc. The Centre study number will also be recorded on the first chromatogram of each daily analytical run.

## 12. QUALITY ASSURANCE

Centre QA Unit will review the protocol, audit the study conduct and the study documentation (including raw data and final report) to ascertain that all QA/GLP procedures are adhered to. Centre QA Unit will inspect the study at intervals adequate to assure compliance to GLP's, and will report the findings of the audits to the Principal Investigator, Centre Management, and the Study Director.

### 13. DATA AND REPORT

1. All raw data and the original signed protocol will be maintained in the study file. This data includes the protocol amendments, protocol deviations, laboratory notebooks, analytical standard solution preparation, sample chain of custody sheets, sample work sheets, chromatograms, calibration curves, and any other appropriate data generated. Raw data not used will be stored in the study file. The reason for exclusion will be documented.
2. A final report will be issued by Centre, approved and signed by the Study Director and sufficient for submission to EPA. The report contents should include, but not limited to:
  1. Objectives and procedures stated in the protocol
  2. Analytical and statistical methods used
  3. Test materials identified by name, lot, purity, and other characteristics
  4. Name of testing facility and study initiation and completion dates
  5. Statement prepared and signed by the quality assurance unit
  6. Tables containing all applicable data
  7. All chromatographic and instrumental conditions
  8. A complete listing of Centre study personnel

Centre will send a copy of the draft report to the Study Director, who will return the report with comments. Centre Analytical will make revisions and finalize the report with the approval of the Study Director. The Centre QA unit will conduct an audit of the final draft report and data files to assure accuracy and GLP compliance. The final report must be approved by the Study Director prior to signature. A statement of QAU inspections and a statement of GLP compliance will be included in the final report.

Any corrections or additions to the final report shall be in the form of an amendment by the Principal Investigator. The amendment shall clearly identify the part of the final report that is being corrected and the justification for correction. The amendment shall be signed and dated by the Principal Investigator and the Study Director.

#### REPORT DISTRIBUTION:

Original: Study Director  
Copy: Centre Archives

### 14. ARCHIVE STATEMENT

Study records to be maintained: Records to be maintained for the study include all raw data, observations recorded during the conduct of the study, documentation, chromatograms, specimen tracking information, and study related correspondence.

Centre Protocol No. 00P-023-042

This includes a description of equipment used during the conduct of the study. All characterization data and any shipping records shall be retained.

Document archives: Upon completion of the study, the study records, protocol and amendments, and the final report and amendments shall be retained in the 3M document archives. If it is necessary to substitute a copy for an original record, it will be certified as an exact copy. Centre will retain facility-related originals and a copy of the final report and raw data package.

## 15. REFERENCES

- 1 U.S. Environmental Protection Agency. Toxic Substances Control Act (TSCA). Good Laboratory Practice Standards. Final Rule, 40 CFR Part 792.

Centre Protocol No. 00P-023-042

16. PROTOCOL APPROVAL

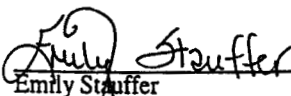
This protocol was audited by the Quality Assurance Unit of Centre Analytical Laboratories, Inc.

Quality Assurance Reviewer, Centre

  
\_\_\_\_\_  
Naomi Lovallo, Ph.D.  
Quality Assurance Auditor

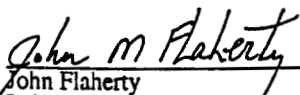
11/15/00  
Date

Principal Investigator, Centre

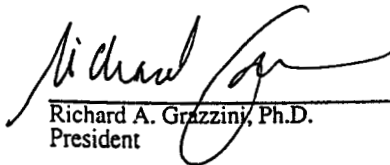
  
\_\_\_\_\_  
Emily Stauffer  
Scientist

11/15/00  
Date

Facility Management, Centre

  
\_\_\_\_\_  
John Flaherty  
Laboratory Manager

11/15/00  
Date

  
\_\_\_\_\_  
Richard A. Grazzini, Ph.D.  
President

15- NOV - 00  
Date

Study Director, 3M

  
\_\_\_\_\_  
Rochelle Robideau  
Environmental Technologist

11/10/00  
Date

**APPENDIX: ANALYTICAL METHODS**

- A. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- B. "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds From Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

Centre Protocol No. OOP-023-042

**3M ENVIRONMENTAL LABORATORY****METHOD****EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER  
FLUORO-CHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC-  
ELECTROSPRAY/MASS SPECTROMETRY**

Method Number: ETS-4.1

Adoption Date: 03/01/99

Revision Date: 4/27/99

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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.

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**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 autosials.

2.2 These sample extracts are analyzed following method ETS-8-5.1 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 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 ( $\pm 0.100$  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 T-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  $\mu$ L to 50  $\mu$ L

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2  $\mu$ m, 25 mm

7.13 Timer

7.14 Crimp cap anvils 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 ( $\text{Na}_2\text{CO}_3$ ), J.T. Baker or equivalent

8.5 Sodium bicarbonate ( $\text{NaHCO}_3$ ), J.T. Baker or equivalent

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 Fluorochemical standards

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

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

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- 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,  $C_8F_{17}SO_3H$ ) 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 ( $\mu\text{g/mL}$ ).
- 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,  $C_6F_5SO_3H$  into a 50 mL volumetric flask and record the actual weight.

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

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

### 9.0 SAMPLE HANDLING

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

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

### 10.0 QUALITY CONTROL

#### 10.1 Solvent Blanks, Method blanks and matrix blanks

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

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

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

#### 10.2 Matrix spikes

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

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

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

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

#### 10.3 Continuing calibration checks

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

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

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

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

#### 11.0 CALIBRATION AND STANDARDIZATION

##### 11.1 Prepare matrix calibration standards

- 11.1.1 Transfer 1 mL of serum to a 15 mL centrifuge tube.
- 11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.
- 11.1.3 While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.
- 11.1.4 Two 1 mL aliquots or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.
- 11.1.5 Refer to validation reports ETS-3-4.0 & ETS-3-5.0-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<br>(approx. conc.) | $\mu\text{L}$ | Approx. final conc. of<br>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.

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- 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 autoval or low-volume autoval when necessary.
- 12.20 Label the autoval 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:

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

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.

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**16.0 RECORDS**

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

**17.0 ATTACHMENTS**

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

**18.0 REFERENCES**

- 18.1 The validation report associated with this method is ETS-8-4.0 & 5.0-V-1  
18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

**19.0 AFFECTED DOCUMENTS**

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

**20.0 REVISIONS**

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

## Extraction Worksheet ETS-8-4.1

| Study #<br>Matrix<br>Box #<br>Wk/Day                                                  | Surrogate Std.<br>ppm actual ppm<br># | FC-Mix<br>approx. 0.5 ppm actual ppm<br># | FC-Mix<br>approx. 5 ppm actual ppm<br># | FC-Mix<br>approx. 50 ppm actual ppm<br># | Comments        |
|---------------------------------------------------------------------------------------|---------------------------------------|-------------------------------------------|-----------------------------------------|------------------------------------------|-----------------|
| Date Spiked / Analyst                                                                 |                                       |                                           |                                         |                                          |                 |
| CCV                                                                                   |                                       |                                           |                                         |                                          |                 |
| MS                                                                                    |                                       |                                           |                                         |                                          |                 |
| MSD                                                                                   |                                       |                                           |                                         |                                          |                 |
| Blank                                                                                 |                                       |                                           |                                         |                                          |                 |
| Surrogate Extraction Method                                                           | Sid #                                 | amount =                                  | ml.                                     |                                          | Date & Initials |
| Vortex 15 sec.                                                                        |                                       |                                           |                                         |                                          |                 |
| Pipette Matrix                                                                        | Volume                                | ml.                                       |                                         |                                          |                 |
| Pipette 1 mL of 0.5 M TBA, pH 10. pH =                                                | Sid #                                 |                                           |                                         |                                          |                 |
| Pipette 2 mL of 0.25 Na <sub>2</sub> CO <sub>3</sub> /0.25M NaHCO <sub>3</sub> buffer | Sid #                                 |                                           |                                         |                                          |                 |
| Dispense 5 mL of methyl-t-butyl ether                                                 | TN-A                                  |                                           |                                         |                                          |                 |
| Shake 20 min.                                                                         | Shaker speed:                         |                                           |                                         |                                          |                 |
| Centrifuge 20-25 min.                                                                 | Centrifuge speed:                     |                                           |                                         |                                          |                 |
| Remove a 4 mL aliquot of organic layer                                                |                                       |                                           |                                         |                                          |                 |
| Put on Nitrogen Evaporator to dryness                                                 | Temperature:                          |                                           |                                         |                                          |                 |
| Add methanol                                                                          | Volume                                | ml.                                       |                                         |                                          |                 |
| Vortex 30 sec.                                                                        |                                       |                                           |                                         |                                          |                 |
| Filter using a Jee B-D syringe with a 0.2um filter into a 1.5 mL autosample vial      |                                       |                                           |                                         |                                          |                 |

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MDL/LOQ values for rabbit serum

| Compound  | MDL (ppb) | LOQ (ppb) | Linear Calibration Range (LCR)<br>Approximate concentrations to be used for preparing the<br>Standard Calibration Curve |
|-----------|-----------|-----------|-------------------------------------------------------------------------------------------------------------------------|
| PFOS      | 1.74      | 5.55      | 5 ppb - 1000 ppb                                                                                                        |
| PFOSA     | 1.51      | 4.79      | 5 ppb - 1000 ppb                                                                                                        |
| PFOSAA    | 3.46      | 20.5      | 5 ppb - 1000 ppb                                                                                                        |
| EnFOSE-OH | 11.4      | 36.2      | 5 ppb - 1000 ppb                                                                                                        |
| M556      | 6.03      | 19.2      | 5 ppb - 1000 ppb                                                                                                        |
| PFOSFA    | 5.71      | 18.2      | 5 ppb - 1000 ppb                                                                                                        |

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

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

Attachment B: MDL/LOQ Summary

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## Compound: PFOS

| Rabbit Serum | Prepared range of standards (ppb) (ng/mL) | LCR from curve (ppb) (ng/mL) | % Recovery Range | RSD Range |
|--------------|-------------------------------------------|------------------------------|------------------|-----------|
| Full Range   | 0.995 - 978                               | 24.8 - 978                   | 83-108           | 4.67-11.0 |
| Low Curve    | 4.94 - 248                                | 4.94 - 248                   | 85-104           | 5.34-12.0 |
| High curve   | 97.8 - 978                                | 97.8 - 978                   | 85-106           | 4.84-9.80 |
| 1/X          | 0.995 - 978                               | 4.94 - 978                   | 94-111           | 4.60-10.5 |

## Compound: PFOSA

| Rabbit Serum | Prepared range of standards (ppb) (ng/mL) | LCR from curve (ppb) (ng/mL) | % Recovery Range | RSD Range |
|--------------|-------------------------------------------|------------------------------|------------------|-----------|
| Full Range   | 0.993 - 976                               | 4.93 - 976                   | 88-103           | 5.10-14.7 |
| Low Curve    | 4.93 - 97.6                               | 4.93 - 97.6                  | 87-105           | 9.25-14.7 |
| High curve   | 24.8 - 976                                | 24.8 - 978                   | 93-102           | 5.08-13.9 |
| 1/X          | 0.993 - 976                               | 4.93 - 976                   | 94-103           | 5.10-14.5 |

## Compound: PFOSAA

| Rabbit Serum | Prepared range of standards (ppb) (ng/mL) | LCR from curve (ppb) (ng/mL) | % Recovery Range | RSD Range |
|--------------|-------------------------------------------|------------------------------|------------------|-----------|
| Full Range   | 0.991 - 974                               | 24.7 - 974                   | 81-111           | 4.18-10.6 |
| Low Curve    | 4.92 - 247                                | 9.74 - 247                   | 97-107           | 6.38-21.8 |
| High curve   | 49.2 - 974                                | 97.4 - 974                   | 85-108           | 4.33-12.5 |
| 1/X          | 0.991 - 974                               | 9.74 - 974                   | 95-115           | 4.11-23.2 |

Attachment B: MDL/LOQ Summary

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## Compound: EtFOSE-OH

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 49.3 - 976                            | 77-110              | 11.2-25.5    |
| Low Curve    | 4.93 - 97.6                                     | 9.76 - 97.6                           | 97-107              | 14.1-21.3    |
| High curve   | 49.3 - 976                                      | 97.6 - 976                            | 90-109              | 11.5-19.6    |
| 1/X          | 0.993 - 493                                     | 9.76 - 976                            | 86-111              | 11.1-21.2    |

## Compound: PFOSEA

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 24.8 - 976                            | 96-106              | 10.1-16.2    |
| Low Curve    | 4.93 - 248                                      | 9.76 - 248                            | 91-110              | 11.8-19.5    |
| High curve   | 49.3 - 976                                      | 49.3 - 976                            | 86-106              | 10.2-18.2    |
| 1/X          | 0.993 - 976                                     | 9.76 - 976                            | 95-117              | 10.1-19.1    |

## Compound: M556

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 24.8 - 976                            | 88-106              | 4.82-17.9    |
| Low Curve    | 4.93 - 97.6                                     | 9.76 - 97.6                           | 100-105             | 5.95-18.2    |
| High curve   | 97.6 - 976                                      | 97.6 - 976                            | 81-111              | 5.11-9.74    |
| 1/X          | 0.993 - 976                                     | 9.76 - 976                            | 97-110              | 4.77-19.5    |

Attachment B: MDL/LOQ Summary

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Prep date(s):

Analyte(s):

Sample matrix:

100

Method/revision:

**Target analyte(s)**

FC mix std approx

FC mix std approx 5.00 ppm:

FC mix std approx 50.0 ppm

Surrogate std approx 100 ppm

1990

Actual concentrations of standards in the FC mix

Standard number:

Equipment number

Final solvent and TN:

Blank fluid/identifiers

| Calculated concentrations of standards in the sample matrix |                     |                     |                     |                     |                     |                   |                 |
|-------------------------------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|-------------------|-----------------|
| PFOs                                                        | PFOSA               | PFOSAA              | ElFOSE              | PFOSEA              | PFM556              | Surrogate         | All Am't spiked |
| Final conc<br>ng/mL                                         | Final conc<br>ng/mL | Final conc<br>ng/mL | Final conc<br>ng/mL | Final conc<br>ng/mL | Final conc<br>ng/mL | Std conc<br>ng/mL | ML              |
| 4.33                                                        | 5.00                | 5.24                | 4.94                | 5.01                | 5.13                | 100               | 0.005           |
| 9.76                                                        | 9.89                | 10.4                | 9.78                | 9.93                | 10.2                |                   |                 |
| 24.8                                                        | 25.1                | 26.3                | 24.8                | 25.2                | 25.8                | Surrogate         |                 |
| 49.3                                                        | 50.0                | 52.4                | 49.4                | 50.1                | 51.3                | Final conc:       |                 |
| 97.6                                                        | 98.9                | 104                 | 97.8                | 99.3                | 102                 | ng/mL             |                 |
| 248                                                         | 251                 | 263                 | 248                 | 252                 | 258                 | 500               |                 |
| 493                                                         | 500                 | 524                 | 494                 | 501                 | 513                 |                   |                 |
| 976                                                         | 989                 | 1038                | 978                 | 993                 | 1017                |                   |                 |
| 248                                                         | 251                 | 263                 | 248                 | 252                 | 258                 |                   |                 |
| 493                                                         | 500                 | 524                 | 494                 | 501                 | 513                 |                   |                 |
| 976                                                         | 989                 | 1038                | 978                 | 993                 | 1017                |                   |                 |

Validated ranges – approximate concentrations

|                            |                                       |           |           |           |           |
|----------------------------|---------------------------------------|-----------|-----------|-----------|-----------|
| <b>Serum</b>               | PFOS                                  | PFOSA     | PFOSAA    | PFOS-ON   | PFOSA     |
| <b>Rabbit</b>              | 5.00-1000                             | 5.00-1000 | 5.00-1000 | 5.00-1000 | 5.00-1000 |
| <b>Bovine</b>              | Estimates only: Use values for rabbit |           |           |           |           |
| <b>Rat</b>                 | Estimates only: Use values for rabbit |           |           |           |           |
| <b>Monkey &amp; Plasma</b> | Estimates only: Use values for rabbit |           |           |           |           |
| <b>Human</b>               | Estimates only: Use values for rabbit |           |           |           |           |

Centre Protocol No. 00P-023-042

# 3M ENVIRONMENTAL LABORATORY

## METHOD

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

Method Number: ETS-3-6.0

Adoption Date: 07/22/99

Revision Date: NR

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.

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ETS-3-6.0

Extraction of PFOS from Liver

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**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, EFOSE-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 µm 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 EFOSE-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

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Extraction of PFOS from Liver

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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 NaIgent 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  $\mu$ L to 50  $\mu$ L
- 7.12 Graduated pipettes
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2  $\mu$ m, 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 ( $\text{Na}_2\text{CO}_3$ ), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate ( $\text{NaHCO}_3$ ), 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

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- 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, C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>H) 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 flask 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 (Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>): Weigh approximately 26.5 g of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and 21.0 g of sodium bicarbonate (NaHCO<sub>3</sub>) 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,  $C_{12}F_{11}SO_3H$  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<sup>®</sup> 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.

**Table 1**  
Approximate Spiking Amounts for Calibration Standards

| Working Standard<br>(Approx. Conc.) | $\mu$ l | Approx. final conc. of<br>PFOS in liver |
|-------------------------------------|---------|-----------------------------------------|
| Blank                               |         | 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<sup>TM</sup> 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  $\mu$ m 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:

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

\* 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.

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**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 Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

**20.0 REVISIONS**

| Revision<br>Number | Reason For Revision | Revision<br>Date |
|--------------------|---------------------|------------------|
|--------------------|---------------------|------------------|

Attachment B: MDL/LOQ Values

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## MDL/LOQ values for rabbit liver

| Compound  | MDL (ppb) | LOQ (ppb) | Linear Calibration Range (LCR)<br>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                                                                                                    |
| PFOSAA    | 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                                   |

Attachment B: MDL/LOQ Values

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## 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                            | 31 - 1200                        | N/A                                  | N/A                                  | N/A                                   | N/A                                   |

Attachment C: Standard Calculations

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## Ion Pair Standard Curves - Tissue

Prep date(s):

Analyte(s):

Sample matrix:

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:

Standard number:

Equipment number:

Final solvent and TN:

Blank liver/identifier:

## Actual concentrations of standards in the FC mix

| PFOS     | PFOSA    | PFOSAA   | EtFOSE   | PFOSAA   | M556     | Std conc | All         | All     |
|----------|----------|----------|----------|----------|----------|----------|-------------|---------|
| Std conc | Std conc | Std conc | Std conc | Std conc | Std conc | ug/mL    | Am't spiked | Density |
| ug/mL    | ug/mL    | ug/mL    | ug/mL    | ug/mL    | ug/mL    |          | mL          | 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  | PFOSA | PFOSAA | EtFOSE | PFOSAA | M556  | Surrogate | All    |
|-------|-------|--------|--------|--------|-------|-----------|--------|
| Final | Final | Final  | Final  | Final  | Final | Std conc  | Am't   |
| conc  | conc  | conc   | conc   | conc   | conc  | ng/mL     | spiked |
| ng/g  | ng/g  | ng/g   | ng/g   | ng/g   | ng/g  |           | 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  |           |        |
| 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       | PFOSAA     |
|--------|------------------------------------|------------|------------|------------|------------|------------|
| 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. |            |            |            |            |            |

Attachment C: Standard Calculations

ETS-8.6.0  
Extraction of PFOS from Liver

Page 14 of 14



**Centre Analytical  
Laboratories, Inc.**

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|                                                                                                                                                                                                                                                     |                                            |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| <b>PROTOCOL AMENDMENT</b>                                                                                                                                                                                                                           | Page 1 of 1                                |
| Amendment Number: <u>1</u><br>Effective Date: <u>11/21/00</u>                                                                                                                                                                                       |                                            |
| Centre Study Number: <u>023-042</u>                                                                                                                                                                                                                 | Centre Protocol Number: <u>00P-023-042</u> |
| <b><u>DESCRIPTION OF AMENDED SECTION</u></b>                                                                                                                                                                                                        |                                            |
| 1. § 6: Justification for the Selection of the Test System                                                                                                                                                                                          |                                            |
| <b><u>AMENDED TO</u></b>                                                                                                                                                                                                                            |                                            |
| 1. Add the following:<br>A complete description of the test system along with all of the in-life parameters is documented in the sponsor's protocol no. 454/010600/MP/SUB454, Project No. 454-105 that is associated with this analytical protocol. |                                            |
| <b><u>RATIONALE</u></b>                                                                                                                                                                                                                             |                                            |
| 1. At the request of the sponsor, a description of the in-life parameters for the test system was not to be included in this protocol that covers the analytical phase for the analysis of mallard liver and serum.                                 |                                            |
| <b><u>IMPACT ON THE STUDY</u></b>                                                                                                                                                                                                                   |                                            |
| 1. No negative impact on the study.                                                                                                                                                                                                                 |                                            |
| _____<br>Principal Investigator Signature                                                                                                                                                                                                           | _____<br>Date <u>11/30/00</u>              |
| _____<br>Sponsor Representative (Study Director) Signature                                                                                                                                                                                          | _____<br>Date <u>12/8/00</u>               |
| Centre QAU Review <u>NC</u> <u>11/30/00</u><br>February 12, 1998/1                                                                                                                                                                                  |                                            |

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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|
| Page 1 of 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <b>PROTOCOL AMENDMENT</b><br><br>Amendment Number: 2<br>Effective Date: <u>11/29/00</u><br><br>Centre Study Number: <u>023-042</u> Centre Protocol Number: <u>00P-023-042</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <u>DESCRIPTION OF AMENDED SECTION</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| 1. § 9: Experimental Design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <u>AMENDED TO</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| 1. Add the following:<br>Moisture determination will be performed on all liver samples according to AOAC Official Method 950.46 Section B, (a), with the following modifications:<br>1. The sample weight will be ~ 0.5 g.<br>2. The drying time will be ~ 12-14 hours.                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <u>RATIONALE</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| 1. At the request of the sponsor, moisture determinations will be done for every liver sample. Due to limited sample size, the sample weight used will be ~0.5 g instead of ~10 g. The drying time required for such a small sample size does not have to be 16-18 hours, so the samples will be dried for ~ 12-14 hours                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <u>IMPACT ON THE STUDY</u>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| 1. No negative impact on the study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: bottom;"> <div style="text-align: center;"> <br/>           _____<br/>           Principal Investigator Signature         </div> </td> <td style="width: 50%; vertical-align: bottom;"> <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div> </td> </tr> <tr> <td style="vertical-align: bottom;"> <div style="text-align: center;"> <br/>           _____<br/>           Sponsor Representative (Study Director) Signature         </div> </td> <td style="vertical-align: bottom;"> <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div> </td> </tr> </table> | <div style="text-align: center;"> <br/>           _____<br/>           Principal Investigator Signature         </div> | <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div> | <div style="text-align: center;"> <br/>           _____<br/>           Sponsor Representative (Study Director) Signature         </div> | <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div> |
| <div style="text-align: center;"> <br/>           _____<br/>           Principal Investigator Signature         </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div>                |                                                                                                         |                                                                                                                                         |                                                                                                         |
| <div style="text-align: center;"> <br/>           _____<br/>           Sponsor Representative (Study Director) Signature         </div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | <div style="text-align: center;"> <u>9/6/01</u><br/>           _____<br/>           Date         </div>                |                                                                                                         |                                                                                                                                         |                                                                                                         |
| Centre QAU Review <u>9/6/01</u><br>February 12, 1998/1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                        |                                                                                                         |                                                                                                                                         |                                                                                                         |

NOTE: THIS AMENDMENT WAS RE-ISSUED ON 9/6/01 BECAUSE ORIGINAL WAS LOST SEE  
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## Centre Analytical Laboratories, Inc.

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### PROTOCOL AMENDMENT

Page 1 of 1

Amendment Number: 3

Effective Date: 03/22/01

Centre Study Number: 023-042

Centre Protocol Number: 00P-023-042

### DESCRIPTION OF AMENDED SECTION

- § 8: Sample Analysis (f)
- § 9: Experimental Design
- § 8: LC/MS/MS System and Operating Conditions (TurboIonSpray)

### AMENDED TO

- Change the last sentence to read the following:  
Samples in which peaks are detected at the corresponding analyte retention times but are less than the lowest concentration of the calibration standards (0.0001 µg/mL) will be reported as NQ (not quantifiable).
- Add the following:  
The following equations will be used for quantitation of the samples:  
  

$$1. \text{Analyte Found (ng/mL)} = (\text{peak area} - \text{intercept}) / \text{slope}$$

$$2. \text{Analyte Found (µg/g)} = \frac{(\text{analyte found (ng/mL)} \times \text{FV (mL)} \times \text{DF} \times \text{EV (mL)} \times 1000 \text{ ng}}{\text{AV (mL)} \times \text{sample wt. (g)} \times 1 \text{ µg}}$$

Where FV = final volume, DF = dilution factor, EV = extraction volume, AV = aliquot volume

$$3. \text{Recovery (\%)} = \frac{(\text{anal. found (µg/mL)} - \text{avg. analyte in ctrl (µg/mL)}) \times 1000 \text{ ng} \times 100\%}{\text{amount added (ng/mL)} \times 1 \text{ µg}}$$
- Change the computer listed to Dell UltraScan P1110

### RATIONALE

- 1-3. Clarify how the samples are quantitated and reported and the instruments used to acquire data.

### IMPACT ON THE STUDY

- 1-3. No negative impact on the study.

Emily R. Decker  
Principal Investigator Signature

9/6/01  
Date

Sean P. Gallagher  
Study Director Signature

9/6/01  
Date

Centre QAU Review 9/6/01

February 12, 1998/1

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DATE 9/6/01 EDD 9/6/01



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|                                                                                                                                                                                                                                                                                                       |                        |                                            |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|--------------------------------------------|
| <b>PROTOCOL AMENDMENT</b><br>Amendment Number: <u>4</u><br>Date of Occurrence: <u>08/24/01</u>                                                                                                                                                                                                        |                        | Page 1 of 1                                |
| Centre Study Number: <u>023-042</u>                                                                                                                                                                                                                                                                   |                        | Centre Protocol Number: <u>00P-023-042</u> |
| <u>DESCRIPTION OF AMENDED SECTION</u>                                                                                                                                                                                                                                                                 |                        |                                            |
| 1. § 3: SPONSOR                                                                                                                                                                                                                                                                                       |                        |                                            |
| <u>AMENDED TO</u>                                                                                                                                                                                                                                                                                     |                        |                                            |
| 1. Change the study director from Rochelle Robideau at 3M Environmental Technology and Safety Services to Sean Gallagher at Wildlife International, LTD, 8598 Commerce Drive, Easton, MD 21601, phone: 410-822-8600, fax: 410-822-0632.<br>2. Change Rochelle Robideau to the Sponsor Representative. |                        |                                            |
| <u>RATIONALE</u>                                                                                                                                                                                                                                                                                      |                        |                                            |
| 1-2. By request of Rochelle Robideau (fax dated 08/24/01 included in correspondence section of the raw data associated with this study)                                                                                                                                                               |                        |                                            |
| <u>IMPACT ON THE STUDY</u>                                                                                                                                                                                                                                                                            |                        |                                            |
| 1-2. No negative impact on the study.                                                                                                                                                                                                                                                                 |                        |                                            |
| <br>Testing Facility Management Signature                                                                                                                                                                                                                                                             | <u>8/30/01</u><br>Date |                                            |
| <br>Outgoing Study Director Signature                                                                                                                                                                                                                                                                 | <u>9/5/01</u><br>Date  |                                            |
| <br>Incoming Study Director Signature                                                                                                                                                                                                                                                                 | <u>8/31/01</u><br>Date |                                            |
| Centre QAU Review <u>BBK 8/30/01</u>                                                                                                                                                                                                                                                                  |                        | February 12, 1998/1                        |

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**Centre Analytical  
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### PROTOCOL DEVIATION

Deviation Number: 1

 Date of Occurrence: (1) 11/19/00 (2) 11/19/00 (3) 11/23/00 (4) 11/29-30/00 (5) 3/10/01  
 Centre Study Number: 023-042 Centre Protocol Number: 00P-023-042

#### DESCRIPTION OF DEVIATION

1. § 8 Sample Analysis (a) Page 9: Accepted recoveries of 79% and 77% for Centre sample 0003804 Spk A and its duplicate injection in Set 111700AD.
2. § 8 Calibration Procedures (b) Pages 8-9: Included a standard in the calibration curve that fell outside  $\pm 30\%$  of its calculated concentration in Set 111700AD (C091800-5).
3. § 8 Calibration Procedures (b) Pages 8-9: Excluded a standard from the calibration curve that fell within  $\pm 30\%$  of its calculated concentration in Set 112100B (C091800-3).
4. § 8 Calibration Procedures (b) Pages 8-9: Included a standard in the calibration curve that fell outside  $\pm 30\%$  of its calculated concentration in Set 112700D (C091800-6).
5. § 8 Sample Analysis (a) Page 9: Accepted recoveries of 79% and 77% for Centre sample 0003803 Spk A and its duplicate injection in Set 030701A.

#### ACTIONS TAKEN

i.e. amendment issued, SOP revision, etc.

 5 RE-ISSUED 9/6/01  
 1-7 Protocol deviation issued.
Recorded By/Date: Tracy R Decker 9/6/01

#### IMPACT ON THE STUDY

- 1.5. No negative impact on the study because the overall average of the spike recoveries for the study fell within the acceptable range of 80%-120%.
- 2.4. No negative impact on the study because the curve was still within acceptable linear requirements.
3. No negative impact on the study because the total number of standards excluded from the curve was less than 20% of the total number of standards injected.

Principal Investigator Signature

Study Director Signature

Sponsor Management Signature

Centre 023-042 9/6/01

CAL QAU Review

Date

Date

Date

February 12, 1998/2

NOTE: THIS AGREEMENT WAS RE-ISSUED ON 9/6/01 BECAUSE ORIGINAL WAS LOST SEE  
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