## STUDY TITLE

Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk 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-023-063
Centre Study Number: 023-063

Total Pages: 102

# GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-063, entitled "Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk 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 with the following exceptions.

1. §792.63. Maintenance and Calibration of Equipment. No equipment SOP was in place for the bead beater used in the extraction at the time of use.

Emily R. Decker

Emily R. Decker
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9/6/01
Date

Sean P. Gallagher

Sean Gallagher
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Date

# QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-063, entitled, "Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk for Analysis Using HPLC-Electrospray/Mass Spectrometry". All examined phases were reviewed 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.

| Phase | Date Inspected | Date Reported to Centre Management | Date Reported to Study Director and Sponsor Management |
|---|---|---|---|
| 1. Protocol Review | 3/30/01 | 4/9/01 | 5/7/01 |
| 2. Extraction, Fortification | 3/30/01 | 4/9/01 | 5/7/01 |
| 3. Raw Data Review | 4/5-9/01 | 4/18/01 | 5/7/01 |
| 4. Raw Data and Draft Report Review | 4/17-18/01 | 4/26/01 | 5/7/01 |
| 5. Final Report Review | 6/28/01 | 9/6/01 | 9/6/01 |

Angela Morgan
Quality Assurance Auditor

9/6/01
Date

# CERTIFICATION OF AUTHENTICITY

This report, for Centre Study Number 023-063, is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.
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# STUDY IDENTIFICATION

"Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk for Analysis Using HPLC-Electrospray/Mass Spectrometry,"

CENTRE PROTOCOL NUMBER: 00P-023-063

CENTRE STUDY NUMBER: 023-063

TYPE OF STUDY: Analytical

SAMPLE MATRIX: Mallard and Quail Egg Membrane, Albumen, and Yolk

TEST SUBSTANCE: Perfluorooctanesulfonate (PFOS)

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ANALYTICAL PHASE TIMETABLE:

| | |
|---|---|
| Study Initiation Date: | 03/26/01 |
| Experimental Start Date: | 03/26/01 |
| Experimental Termination Date: | 04/02/01 |
| 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:

| Name | Title |
|---|---|
| Emily Decker | Scientist |
| Dave Bell | Scientist |
| Mitra Arjmand | Technician |
| Ling Ling Liu | Technician |
| Yu Qiong Tan | Technician |
| Xiaoming Zhu | Technician |
| Lawrence Ord | Sample Custodian |
| Rickey Keller | Sample Custodian |
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# 1.0 SUMMARY

Centre Analytical Laboratories, Inc. (Centre) extracted mallard and quail egg membrane, albumen and yolk samples for the determination of perfluorooctanesulfonate (PFOS) according to protocol 00P-023-063 (Appendix A).

The limit of quantitation for each matrix was 10 ppb. The LOQ for each matrix was determined in a method validation study performed at Centre (Centre Study: 023-027). PFOS ranged from non-quantifiable (where non-quantifiable is defined as less than the concentration of the lowest calibration standard (0.1 ng/mL)) levels to 434 ng/g in the mallard membrane samples, from non-quantifiable levels to 3550 ng/g in the mallard albumen samples, and from non-quantifiable levels to 63,500 ng/g in the mallard yolk samples. PFOS ranged from non-quantifiable levels to 20,900 ng/g in the quail membrane samples, from non-quantifiable levels to 93.9 ng/g in the quail albumen samples, and from non-quantifiable levels to 65,500 ng/g in the quail yolk samples. All of these results are reported on a wet weight basis.

The average percent recoveries ± standard deviations for PFOS in mallard membrane, albumen, and yolk samples were 91% ± 6%, 91% ± 16%, and 93% ± 13%, respectively. The average percent recoveries ± standard deviations for PFOS in quail membrane, albumen, and yolk samples were 102% ± 11%, 85% ± 5%, and 96% ± 7%, respectively.

# 2.0 OBJECTIVE

The objective of this analytical phase of the study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of mallard and quail egg membrane, albumen, and yolk using the analytical method titled, "Determination of Perfluorooctanesulfonate in Egg Membrane, Ablumen and Yolk by LC/MS/MS".

# 3.0 INTRODUCTION

This report details the results of the analysis for the determination of PFOS in mallard and quail egg membrane, albumen, and yolk using the analytical method entitled, "Determination of Perfluorooctanesulfonate in Egg Membrane, Ablumen and Yolk by LC/MS/MS".

This analytical portion of the study was initiated on March 26, 2001, when the study director signed Centre protocol number 00P-023-063. The experimental start date was March 26, 2001, and the experimental termination date was April 2, 2001.

## 4.0 TEST SYSTEM

The control mallard and quail egg membrane, albumen, and yolk used for the matrix blanks and matrix fortifications were received on May 18, 2000 from Wildlife International Ltd., Easton, MD and stored frozen at Centre. The following Centre ID's were assigned to the different matrices:

| Matrix | Centre ID |
|---|---|
| Mallard Membrane | 0003802 |
| Mallard Albumen | 0003800 |
| Mallard Yolk | 0003799 |
| Quail Membrane | 0003798 |
| Quail Albumen | 0003796 |
| Quail Yolk | 0003795 |

On February 23, 2001, 308 quail egg samples were received. The samples were stored frozen until they were logged in by Centre personnel on February 26, 2001 and stored frozen. On February 24, 2001, 194 mallard egg samples were received. The samples were stored frozen until they were logged in by Centre personnel on March 1, 2001 and stored frozen. Each egg sample received was divided into membrane, albumen, yolk, and shell. Each component of the egg was given a unique Centre ID at the time of log-in. Although all of the egg samples were received at Centre, only those designated by the sponsor were extracted and analyzed.

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

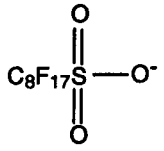
The analytical standard PFOS was received at Centre on June 3, 2000 from 3M Environmental Technology and Services.

Stability was not assessed in this study but the reference material was stored according to the certificate of analysis (frozen) and was used prior to expiration. The solubility of the reference material was not relevant to this study. The available information for the reference material is listed below.

| Compound | Centre Control No.. | TCR No. | Purity (%) | Expiration Date |
|---|---|---|---|---|
| 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 ($C_8F_{17}SO_3^-$)



**Note:** The neutral molecule and standard from which the PFOS (anion) is obtained, is potassium perfluorooctanesulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

## 6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Determination of Perfluorooctanesulfonate in Egg Membrane, Ablumen and Yolk by LC/MS/MS" was used for this study.

### 6.1 Extraction Procedure

**Egg membrane**

A 0.1 g sample was used for the extraction procedure. One milliliter of methanol was added to the sample along with three stainless steel beads. The sample was then placed on a bead beater set at position 7 for 5 minutes. The sample was then centrifuged on a mini-centrifuge at ~ 2000 rpm for ~ 2 minutes. Each sample was filtered and analyzed by LC/MS/MS electrospray.

**Egg albumen and yolk**

A 1.0 g sample was used for the extraction procedure. Twenty-five milliliters of methanol were added to the sample. The sample was placed on a wrist-action shaker for 15 minutes and then centrifuged at ~ 2000 rpm for ~ 10 minutes. A five milliliter aliquot of the sample was taken and 0.5 g of carbon was added. The sample was shaken then allowed to sit. Each sample was filtered and analyzed by LC/MS/MS electrospray.

### 6.2 Preparation of Standards and Fortification Solutions

Standard solutions were prepared on March 14, 2001 as specified in Centre Analytical Laboratories' protocol 00P-023-027. 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.

The 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 dilution of the 0.1 µg/mL and 0.01 µg/mL solutions in the following manner.

| Initial Conc. (µg/mL)[1] | 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 |

[1] of PFOS

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4°C ± 2°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 ~ 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 the results in several of the recoveries reported and the rest were less than the lowest calibration standard (0.0001 µg/mL).

### 6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.0001 µg/mL of PFOS.

## 6.5 Description of Instrument and Operating Conditions

Instrument: PE SCIEX API 3000 Biomolecular Mass Analyzer

Interface: SCIEX TurboIon 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 $C_8$ (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.

| Time | % A | % B |
|---|---|---|
| 0 | 60 | 40 |
| 1.0 | 0 | 100 |
| 7.0 | 0 | 100 |
| 7.5 | 60 | 40 |
| 11.0 | 60 | 40 |

Ions monitored:

| Analyte | Mode | Transition Monitored | Approximate Retention Time (min) |
|---|---|---|---|
| PFOS | negative | 499 → 99 | 4.40 |

**Tune File Parameters**

| Controls | Set |
|---|---|
| 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 |

| Gas Flows | Set |
|---|---|
| Nebulizer Gas | 12 |
| Curtain Gas | 14 |
| 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 weighted linear regression) by Analyst software using at least five concentrations of standards. The concentrations for mallard and quail egg membrane, albumen, and yolk (ng/g) were determined from the equations below.

Equation 1 calculated the amount of analyte found (in ng/mL, based on peak area) using the standard curve (linear regression parameters) generated by the Analyst software program. Then Equation 2 calculated the amount of analyte found in ng/g.

Equation 1:

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

Equation 2:

$$\text{Analyte found (ng/g)} = \frac{\text{anal. found (ng/mL)} \times \text{FV (mL)} \times \text{DF}}{\text{sample wt. (g)}}$$

Where:
FV = Final Volume
DF = Dilution Factor

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

Equation 3:

$$\text{Recovery (\%)} = \frac{\text{(anal. Found (ng/g) - avg. anal. in ctrl (ng/g)} \times 100}{\text{amount added (ng/g)}}$$

An example of a calculation using an actual sample follows:
Mallard albumen sample Centre ID 0003800 Spk A (Set: 032601A), fortified at 10 pbb with PFOS, where:

| | | |
|---|---|---|
| peak area | = | 3369 |
| intercept | = | 971 |
| slope | = | 6700 |
| dilution factor | = | 1 |
| ng/g added (fort level) | = | 10 ng/g |
| avg. amt in controls | = | 0 (Not quantifiable) |
| final volume | = | 25 mL |
| sample weight | = | 1.0038 g |

Note: Not quanitifiable is defined as less than the concentration of the lowest standard (0.1 ng/mL).

**From equation 1:**

$$\text{Analyte found (ng/mL)} = \frac{[3369 - 971]}{6700}$$

$$= 0.36 \text{ ng/mL}$$

**From equation 2:**

$$\text{Analyte found (ng/g)} = \frac{(0.36 \text{ ng/mL} \times 25 \text{ mL} \times 1)}{1.0038 \text{ (g)}}$$

$$= 8.97 \text{ ng/mL}$$

**From equation 3:**

$$\text{\% Recovery} = \frac{(8.97 \text{ ng/g} \times - 0 \text{ ng/g})}{10 \text{ ng/g}} \times 100$$

$$= 90\%$$

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the RAW DATA.

## 7.0 EXPERIMENTAL DESIGN

Each set of samples 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. Also, in each set, a Type I $H_2O$ blank was analyzed.

## 8.0 RESULTS

PFOS found in the reagent blanks are listed in **Table I.** The PFOS in the mallard membrane, albumen, and yolk blanks are given in **Tables II-IV.** The PFOS in the quail membrane, albumen, and yolk blanks are given in **Tables XXVI-XXVIII.** PFOS was detected in several of the control matrices, however few were significant enough to alter the recoveries reported.

Individual recoveries for PFOS in the mallard membrane, albumen, and yolk samples are detailed in **Tables V-VII.** The average percent recoveries ± standard deviations for PFOS in mallard membrane, albumen, and yolk samples were 91% ± 6%, 91% ± 16%, and 93% ± 13%, respectively. Individual recoveries for PFOS in the quail membrane, albumen, and yolk samples are detailed in **Table XXIX-XXXI.** The average percent recoveries ± standard deviations for PFOS in quail membrane, albumen, and yolk samples were 102% ± 11%, 85% ± 5%, and 96% ± 7%, respectively.

PFOS ranged from non-quantifiable (where non-quantifiable is defined as less than the concentration of the lowest calibration standard (0.1 ng/mL)) levels to 434 ng/g in the mallard membrane samples, from non-quantifiable levels to 3550 ng/g in the mallard albumen samples, and from non-quantifiable levels to 63,500 ng/g in the mallard yolk samples. These results are reported on a wet weight basis. Individual results are listed in **Tables VIII-XXV.** Some of the high levels of PFOS reported for some of the mallard egg membrane and egg albumen samples could be from possible contamination from the egg yolk or outer shell.

PFOS ranged from non-quantifiable levels to 20,900 ng/g in the quail membrane samples, from non-quantifiable levels to 93.9 ng/g in the quail albumen samples, and from non-quantifiable levels to 65,500 ng/g in the quail yolk samples. These results are reported on a wet weight basis. Individual results are listed in **Tables XXXII-IL.** Some of the high levels of PFOS reported for some of the quail egg membrane and egg albumen samples could be from possible contamination from the egg yolk or outer shell. The Sponsor Representative also informed Centre that the quail outer shells were not washed prior to separation of the internal components.

The average percent moistures for the mallard and quail egg membrane, albumen, and yolk samples are given in **Tables L-LV.**

## 9.0 CONCLUSIONS

The mallard and quail egg membrane, albumen, and yolk samples were successfully extracted and analyzed according to protocol 00P-023-063.

## 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

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | Reagent Blank A | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| na | Reagent Blank A* | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| na | Reagent Blank A | 032601B | 3/26/01 | 3/27/01 | NQ |
| na | Reagent Blank A* | 032601B | 3/26/01 | 3/27/01 | NQ |
| na | Reagent Blank A | 032601C | 3/26/01 | 3/27/01 | NQ |
| na | Reagent Blank A* | 032601C | 3/26/01 | 3/27/01 | NQ |
| na | Reagent Blank A | 032701A | 3/27/01 | 3/28/01 | NQ |
| na | Reagent Blank A* | 032701A | 3/27/01 | 3/28/01 | NQ |
| na | Reagent Blank A | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| na | Reagent Blank A* | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| na | Reagent Blank A | 032701C | 3/27/01 | 3/29/01 | NQ |
| na | Reagent Blank A* | 032701C | 3/27/01 | 3/29/01 | NQ |
| na | Reagent Blank A | 032801A | 3/28/01 | 3/30/01 | NQ |
| na | Reagent Blank A* | 032801A | 3/28/01 | 3/30/01 | NQ |
| na | Reagent Blank A | 032801B | 3/28/01 | 3/30/01 | NQ |
| na | Reagent Blank A* | 032801B | 3/28/01 | 3/30/01 | NQ |
| na | Reagent Blank A | 032901A | 3/29/01 | 3/31/01 | NQ |
| na | Reagent Blank A* | 032901A | 3/29/01 | 3/31/01 | NQ |
| na | Reagent Blank A | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| na | Reagent Blank A* | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| na | Reagent Blank A | 033001A | 3/30/01 | 4/1/01 | NQ |
| na | Reagent Blank A* | 033001A | 3/30/01 | 4/1/01 | NQ |
| na | Reagent Blank A | 033001B | 3/30/01 | 4/2/01 | NQ |
| na | Reagent Blank A* | 033001B | 3/30/01 | 4/2/01 | NQ |
| na | Reagent Blank A | 033001C | 3/30/01 | 4/2/01 | NQ |
| na | Reagent Blank A* | 033001C | 3/30/01 | 4/2/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | **STANDARD DEVIATION:** | | **NQ** |

* Duplicate injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table II. Summary of PFOS in Mallard Membrane Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003802 Blank A | 032601C | 3/26/01 | 3/27/01 | NQ |
| na | 0003802 Blank A* | 032601C | 3/26/01 | 3/27/01 | NQ |
| na | 0003802 Blank A | 032701C | 3/27/01 | 3/29/01 | NQ |
| na | 0003802 Blank A* | 032701C | 3/27/01 | 3/29/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table III. Summary of PFOS in Mallard Albumen Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003800 Blank A | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| na | 0003800 Blank A* | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| na | 0003800 Blank A | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| na | 0003800 Blank A* | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| na | 0003800 Blank A | 033001C | 3/30/01 | 4/2/01 | 6.03 |
| na | 0003800 Blank A* | 033001C | 3/30/01 | 4/2/01 | 6.59 |
| | | | | **AVERAGE:** | **2.94** |
| | | | | **STANDARD DEVIATION:** | **2.62** |

## Table IV. Summary of PFOS in Mallard Yolk Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003799 Blank A | 032601B | 3/26/01 | 3/27/01 | NQ |
| na | 0003799 Blank A* | 032601B | 3/26/01 | 3/27/01 | NQ |
| na | 0003799 Blank A | 032701A | 3/27/01 | 3/28/01 | 9.57 |
| na | 0003799 Blank A* | 032701A | 3/27/01 | 3/28/01 | 9.80 |
| | | | | **AVERAGE:** | **5.48** |
| | | | | **STANDARD DEVIATION:** | **4.87** |

* Duplicate injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

For residues recorded as NQ, half of the concentration of the lowest standard (1.25 ppb) was used to calculate the average and standard deviation.

## Table V. Summary of PFOS Recoveries in Mallard Membrane

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| na | 0003802 Spk A | 032601C | 3/26/01 | 3/27/01 | 10 | 86 |
| na | 0003802 Spk A* | 032601C | 3/26/01 | 3/27/01 | 10 | 87 |
| na | 0003802 Spk B | 032601C | 3/26/01 | 3/27/01 | 100 | 87 |
| na | 0003802 Spk B* | 032601C | 3/26/01 | 3/27/01 | 100 | 81 |
| na | 0003802 Spk A | 032701C | 3/27/01 | 3/29/01 | 10 | 97 |
| na | 0003802 Spk A* | 032701C | 3/27/01 | 3/29/01 | 10 | 94 |
| na | 0003802 Spk B | 032701C | 3/27/01 | 3/29/01 | 97 | 95 |
| na | 0003802 Spk B* | 032701C | 3/27/01 | 3/29/01 | 97 | 98 |
| | | | | | **AVERAGE:** | **91** |
| | | | | | **STANDARD DEVIATION:** | **6** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **7** |

## Table VI. Summary of PFOS Recoveries in Mallard Albumen

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| na | 0003800 Spk A | 032601A | 3/26/01 | 3/26-27/01 | 10 | 89 |
| na | 0003800 Spk A* | 032601A | 3/26/01 | 3/26-27/01 | 10 | 91 |
| na | 0003800 Spk B | 032601A | 3/26/01 | 3/26-27/01 | 99 | 94 |
| na | 0003800 Spk B* | 032601A | 3/26/01 | 3/26-27/01 | 99 | 92 |
| na | 0003800 Spk A | 032701B | 3/27/01 | 3/28-29/01 | 10 | 107 |
| na | 0003800 Spk A* | 032701B | 3/27/01 | 3/28-29/01 | 10 | 105 |
| na | 0003800 Spk B | 032701B | 3/27/01 | 3/28-29/01 | 99 | 101 |
| na | 0003800 Spk B* | 032701B | 3/27/01 | 3/28-29/01 | 99 | 99 |
| na | 0003800 Spk A | 033001C | 3/30/01 | 4/2/01 | 10 | 59 |
| na | 0003800 Spk A* | 033001C | 3/30/01 | 4/2/01 | 10 | 61 |
| na | 0003800 Spk B | 033001C | 3/30/01 | 4/2/01 | 98474 | 99 |
| na | 0003800 Spk B* | 033001C | 3/30/01 | 4/2/01 | 98474 | 99 |
| | | | | | **AVERAGE:** | **91** |
| | | | | | **STANDARD DEVIATION:** | **16** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **17** |

* Duplicate Injection

LOQ = 10 ppb

## Table VII. Summary of PFOS Recoveries in Mallard Yolk

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| Na | 0003799 Spk A | 032601B | 3/26/01 | 3/27/01 | 10 | 104 |
| Na | 0003799 Spk A* | 032601B | 3/26/01 | 3/27/01 | 10 | 104 |
| Na | 0003799 Spk B | 032601B | 3/26/01 | 3/27/01 | 98 | 99 |
| Na | 0003799 Spk B* | 032601B | 3/26/01 | 3/27/01 | 98 | 99 |
| Na | 0003799 Spk A | 032701A | 3/27/01 | 3/28/01 | 10 | 71 |
| Na | 0003799 Spk A* | 032701A | 3/27/01 | 3/28/01 | 10 | 73 |
| Na | 0003799 Spk B | 032701A | 3/27/01 | 3/28/01 | 97 | 98 |
| Na | 0003799 Spk B* | 032701A | 3/27/01 | 3/28/01 | 97 | 94 |
| | | | | | **AVERAGE:** | **93** |
| | | | | | **STANDARD DEVIATION:** | **13** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **14** |

## Table VIII. Summary of PFOS for Week 1 Mallard Membrane Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20852 | 0103200 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20852* | 0103200 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20853 | 0103204 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20853* | 0103204 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20854 | 0103208 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20854* | 0103208 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20842 | 0103160 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20842* | 0103160 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20843 | 0103164 | 032601C | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20843* | 0103164 | 032601C | 3/26/01 | 3/27/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table IX. Summary of PFOS for Week 1 Mallard Membrane Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20825 | 0103092 | 032601C | 3/26/01 | 3/27/01 | 168** |
| E01-0263-20825* | 0103092 | 032601C | 3/26/01 | 3/27/01 | 163** |
| E01-0263-20846 | 0103176 | 032601C | 3/26/01 | 3/27/01 | 46.8 |
| E01-0263-20846* | 0103176 | 032601C | 3/26/01 | 3/27/01 | 46.2 |
| E01-0263-20832 | 0103120 | 032601C | 3/26/01 | 3/27/01 | 27.1 |
| E01-0263-20832* | 0103120 | 032601C | 3/26/01 | 3/27/01 | 27.2 |
| E01-0263-20847 | 0103180 | 032601C | 3/26/01 | 3/27/01 | 33.1 |
| E01-0263-20847* | 0103180 | 032601C | 3/26/01 | 3/27/01 | 32.9 |
| E01-0263-20848 | 0103184 | 032601C | 3/26/01 | 3/27/01 | 53.4 |
| E01-0263-20848* | 0103184 | 032601C | 3/26/01 | 3/27/01 | 53.8 |
| | | | | **AVERAGE:** | **40.1** |
| | | | | **STANDARD DEVIATION:** | **11.2** |

## Table X. Summary of PFOS for Week 1 Mallard Membrane Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20856 | 0103216 | 032601C | 3/26/01 | 3/27/01 | 248** |
| E01-0263-20856* | 0103216 | 032601C | 3/26/01 | 3/27/01 | 264** |
| E01-0263-20850 | 0103192 | 032601C | 3/26/01 | 3/27/01 | 26.5 |
| E01-0263-20850* | 0103192 | 032601C | 3/26/01 | 3/27/01 | 28.6 |
| E01-0263-20857 | 0103220 | 032601C | 3/26/01 | 3/27/01 | 78.9 |
| E01-0263-20857* | 0103220 | 032601C | 3/26/01 | 3/27/01 | 79.0 |
| E01-0263-20851 | 0103196 | 032601C | 3/26/01 | 3/27/01 | 287** |
| E01-0263-20851* | 0103196 | 032601C | 3/26/01 | 3/27/01 | 281** |
| E01-0263-20858 | 0103224 | 032601C | 3/26/01 | 3/27/01 | 50.1 |
| E01-0263-20858* | 0103224 | 032601C | 3/26/01 | 3/27/01 | 49.2 |
| | | | | **AVERAGE:** | **52.1** |
| | | | | **STANDARD DEVIATION:** | **23.1** |

* Duplicate Injection

LOQ = 10 ppb

** Possible contamination by yolk or outer shell component of sample and as per Sponsor Representative request were not used to calculate the average and standard deviation.

## Table XI. Summary of PFOS for Week 1 Mallard Albumen Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20852 | 0103201 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20852* | 0103201 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20853 | 0103205 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20853* | 0103205 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20854 | 0103209 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20854* | 0103209 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20842 | 0103161 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20842* | 0103161 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20843 | 0103165 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| E01-0263-20843* | 0103165 | 032601A | 3/26/01 | 3/26-27/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XII. Summary of PFOS for Week 1 Mallard Albumen Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20825 | 0103093 | 032601A | 3/26/01 | 3/26-27/01 | 2.49 |
| E01-0263-20825* | 0103093 | 032601A | 3/26/01 | 3/26-27/01 | 2.75 |
| E01-0263-20846 | 0103177 | 032601A | 3/26/01 | 3/26-27/01 | 7.90 |
| E01-0263-20846* | 0103177 | 032601A | 3/26/01 | 3/26-27/01 | 7.89 |
| E01-0263-20832 | 0103121 | 032601A | 3/26/01 | 3/26-27/01 | 5.67 |
| E01-0263-20832* | 0103121 | 032601A | 3/26/01 | 3/26-27/01 | 5.79 |
| E01-0263-20847 | 0103181 | 032601A | 3/26/01 | 3/26-27/01 | 5.76 |
| E01-0263-20847* | 0103181 | 032601A | 3/26/01 | 3/26-27/01 | 5.58 |
| E01-0263-20848 | 0103185 | 032601A | 3/26/01 | 3/26-27/01 | 5.95 |
| E01-0263-20848* | 0103185 | 032601A | 3/26/01 | 3/26-27/01 | 6.29 |
| | | | | **AVERAGE:** | **5.63** |
| | | | | **STANDARD DEVIATION:** | **1.80** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XIII. Summary of PFOS for Week 1 Mallard Albumen Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20856 | 0103217 | 032601D | 3/26/01 | 3/27-28/01 | 3550** |
| E01-0263-20856* | 0103217 | 032601D | 3/26/01 | 3/27-28/01 | 3490** |
| E01-0263-20856 | 0103217 | 033001C | 3/30/01 | 4/2/01 | 1388** |
| E01-0263-20856* | 0103217 | 033001C | 3/30/01 | 4/2/01 | 1369** |
| E01-0263-20856 | 0103217 Dup | 033001C | 3/30/01 | 4/2/01 | 845** |
| E01-0263-20856* | 0103217 Dup | 033001C | 3/30/01 | 4/2/01 | 771** |
| E01-0263-20850 | 0103193 | 032601A | 3/26/01 | 3/26-27/01 | 24.7 |
| E01-0263-20850* | 0103193 | 032601A | 3/26/01 | 3/26-27/01 | 23.9 |
| E01-0263-20857 | 0103221 | 032601A | 3/26/01 | 3/26-27/01 | 23.8 |
| E01-0263-20857* | 0103221 | 032601A | 3/26/01 | 3/26-27/01 | 23.3 |
| E01-0263-20851 | 0103197 | 032601A | 3/26/01 | 3/26-27/01 | 78.0** |
| E01-0263-20851* | 0103197 | 032601A | 3/26/01 | 3/26-27/01 | 69.7** |
| E01-0263-20858 | 0103225 | 032601A | 3/26/01 | 3/26-27/01 | 19.7 |
| E01-0263-20858* | 0103225 | 032601A | 3/26/01 | 3/26-27/01 | 19.8 |
| | | | | **AVERAGE:** | **22.5** |
| | | | | **STANDARD DEVIATION:** | **2.20** |

## Table XIV. Summary of PFOS for Week 1 Mallard Yolk Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20852 | 0103198 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20852* | 0103198 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20853 | 0103202 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20853* | 0103202 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20854 | 0103206 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20854* | 0103206 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20842 | 0103158 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20842* | 0103158 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20843 | 0103162 | 032601B | 3/26/01 | 3/27/01 | NQ |
| E01-0263-20843* | 0103162 | 032601B | 3/26/01 | 3/27/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

** Possible contamination by yolk or outer shell component of sample and as per Sponsor Representative request were not used to calculate the average and standard deviation.

## Table XV. Summary of PFOS for Week 1 Mallard Yolk Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20825 | 0103090 | 032601D | 3/26/01 | 3/27-28/01 | 2470 |
| E01-0263-20825* | 0103090 | 032601D | 3/26/01 | 3/27-28/01 | 2580 |
| E01-0263-20846 | 0103174 | 032601D | 3/26/01 | 3/27-28/01 | 12500 |
| E01-0263-20846* | 0103174 | 032601D | 3/26/01 | 3/27-28/01 | 12100 |
| E01-0263-20832 | 0103118 | 032601D | 3/26/01 | 3/27-28/01 | 4010 |
| E01-0263-20832* | 0103118 | 032601D | 3/26/01 | 3/27-28/01 | 3890 |
| E01-0263-20847 | 0103178 | 032601D | 3/26/01 | 3/27-28/01 | 10500 |
| E01-0263-20847* | 0103178 | 032601D | 3/26/01 | 3/27-28/01 | 11100 |
| E01-0263-20848 | 0103182 | 032601D | 3/26/01 | 3/27-28/01 | 12100 |
| E01-0263-20848* | 0103182 | 032601D | 3/26/01 | 3/27-28/01 | 12100 |
| | | | | **AVERAGE:** | **8340** |
| | | | | **STANDARD DEVIATION:** | **4450** |

## Table XVI. Summary of PFOS for Week 1 Mallard Yolk Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20856 | 0103214 | 032601D | 3/26/01 | 3/27-28/01 | 30300 |
| E01-0263-20856* | 0103214 | 032601D | 3/26/01 | 3/27-28/01 | 30000 |
| E01-0263-20850 | 0103190 | 032601D | 3/26/01 | 3/27-28/01 | 40000 |
| E01-0263-20850* | 0103190 | 032601D | 3/26/01 | 3/27-28/01 | 43000 |
| E01-0263-20857 | 0103218 | 032601D | 3/26/01 | 3/27-28/01 | 57200 |
| E01-0263-20857* | 0103218 | 032601D | 3/26/01 | 3/27-28/01 | 56600 |
| E01-0263-20851 | 0103194 | 032601D | 3/26/01 | 3/27-28/01 | 51700 |
| E01-0263-20851* | 0103194 | 032601D | 3/26/01 | 3/27-28/01 | 53100 |
| E01-0263-20858 | 0103222 | 032601D | 3/26/01 | 3/27-28/01 | 61000 |
| E01-0263-20858* | 0103222 | 032601D | 3/26/01 | 3/27-28/01 | 62000 |
| | | | | **AVERAGE:** | **48500** |
| | | | | **STANDARD DEVIATION:** | **12000** |

* Duplicate Injection

LOQ = 10 ppb

## Table XVII. Summary of PFOS for Week 6 Mallard Membrane Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20993 | 0103764 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20993* | 0103764 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20994 | 0103768 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20994* | 0103768 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20995 | 0103772 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20995* | 0103772 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20975 | 0103692 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20975* | 0103692 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20997 | 0103780 | 032701C | 3/27/01 | 3/29/01 | NQ |
| E01-0263-20997* | 0103780 | 032701C | 3/27/01 | 3/29/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XVIII. Summary of PFOS for Week 6 Mallard Membrane Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20979 | 0103708 | 032701C | 3/27/01 | 3/29/01 | 70.8 |
| E01-0263-20979* | 0103708 | 032701C | 3/27/01 | 3/29/01 | 70.6 |
| E01-0263-21001 | 0103796 | 032701C | 3/27/01 | 3/29/01 | 55.3 |
| E01-0263-21001* | 0103796 | 032701C | 3/27/01 | 3/29/01 | 55.3 |
| E01-0263-20988 | 0103744 | 032701C | 3/27/01 | 3/29/01 | 95.7 |
| E01-0263-20988* | 0103744 | 032701C | 3/27/01 | 3/29/01 | 98.7 |
| E01-0263-20971 | 0103676 | 032701C | 3/27/01 | 3/29/01 | 169** |
| E01-0263-20971* | 0103676 | 032701C | 3/27/01 | 3/29/01 | 171** |
| E01-0263-20989 | 0103748 | 032701C | 3/27/01 | 3/29/01 | 83.8 |
| E01-0263-20989* | 0103748 | 032701C | 3/27/01 | 3/29/01 | 88.2 |
| | | | | **AVERAGE:** | **77.3** |
| | | | | **STANDARD DEVIATION:** | **17.0** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

** Possible contamination by yolk or outer shell component of sample and as per Sponsor Representative request were not used to calculate the average and standard deviation.

## Table XIX. Summary of PFOS for Week 6 Mallard Membrane Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20991 | 0103756 | 032701CD | 3/27/01 | 3/29/01 | 434 |
| E01-0263-20991* | 0103756 | 032701CD | 3/27/01 | 3/29/01 | 431 |
| E01-0263-20990 | 0103752 | 032701CD | 3/27/01 | 3/29/01 | 365 |
| E01-0263-20990* | 0103752 | 032701CD | 3/27/01 | 3/29/01 | 370 |
| E01-0263-21002 | 0103800 | 032701CD | 3/27/01 | 3/29/01 | 347 |
| E01-0263-21002* | 0103800 | 032701CD | 3/27/01 | 3/29/01 | 339 |
| E01-0263-21003 | 0103804 | 032701CD | 3/27/01 | 3/29/01 | 306 |
| E01-0263-21003* | 0103804 | 032701CD | 3/27/01 | 3/29/01 | 307 |
| E01-0263-21004 | 0103808 | 032701CD | 3/27/01 | 3/29/01 | 259 |
| E01-0263-21004* | 0103808 | 032701CD | 3/27/01 | 3/29/01 | 261 |
| | | | | **AVERAGE:** | **342** |
| | | | | **STANDARD DEVIATION:** | **61.2** |

## Table XX. Summary of PFOS for Week 6 Mallard Albumen Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20993 | 0103765 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20993* | 0103765 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20994 | 0103769 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20994* | 0103769 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20995 | 0103773 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20995* | 0103773 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20975 | 0103693 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20975* | 0103693 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20997 | 0103781 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| E01-0263-20997* | 0103781 | 032701B | 3/27/01 | 3/28-29/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XXI. Summary of PFOS for Week 6 Mallard Albumen Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20979 | 0103709 | 032701B | 3/27/01 | 3/28-29/01 | 5.83 |
| E01-0263-20979* | 0103709 | 032701B | 3/27/01 | 3/28-29/01 | 6.23 |
| E01-0263-21001 | 0103797 | 032701B | 3/27/01 | 3/28-29/01 | 7.92 |
| E01-0263-21001* | 0103797 | 032701B | 3/27/01 | 3/28-29/01 | 8.04 |
| E01-0263-20988 | 0103745 | 032701B | 3/27/01 | 3/28-29/01 | 6.56 |
| E01-0263-20988* | 0103745 | 032701B | 3/27/01 | 3/28-29/01 | 6.26 |
| E01-0263-20971 | 0103677 | 032701B | 3/27/01 | 3/28-29/01 | 7.02 |
| E01-0263-20971* | 0103677 | 032701B | 3/27/01 | 3/28-29/01 | 6.81 |
| E01-0263-20989 | 0103749 | 032701B | 3/27/01 | 3/28-29/01 | 8.72 |
| E01-0263-20989* | 0103749 | 032701B | 3/27/01 | 3/28-29/01 | 8.33 |
| | | | | **AVERAGE:** | **7.19** |
| | | | | **STANDARD DEVIATION:** | **0.987** |

## Table XXII. Summary of PFOS for Week 6 Mallard Albumen Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20991 | 0103757 | 032701B | 3/27/01 | 3/28-29/01 | 25.3 |
| E01-0263-20991* | 0103757 | 032701B | 3/27/01 | 3/28-29/01 | 24.9 |
| E01-0263-20990 | 0103753 | 032701B | 3/27/01 | 3/28-29/01 | 14.1 |
| E01-0263-20990* | 0103753 | 032701B | 3/27/01 | 3/28-29/01 | 14.7 |
| E01-0263-21002 | 0103801 | 032701B | 3/27/01 | 3/28-29/01 | 19.7 |
| E01-0263-21002* | 0103801 | 032701B | 3/27/01 | 3/28-29/01 | 19.4 |
| E01-0263-21003 | 0103805 | 032701B | 3/27/01 | 3/28-29/01 | 23.6 |
| E01-0263-21003* | 0103805 | 032701B | 3/27/01 | 3/28-29/01 | 24.0 |
| E01-0263-21004 | 0103809 | 032701B | 3/27/01 | 3/28-29/01 | 19.1 |
| E01-0263-21004* | 0103809 | 032701B | 3/27/01 | 3/28-29/01 | 17.2 |
| | | | | **AVERAGE:** | **20.2** |
| | | | | **STANDARD DEVIATION:** | **4.12** |

* Duplicate Injection

LOQ = 10 ppb

## Table XXIII. Summary of PFOS for Week 6 Mallard Yolk Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20993 | 0103762 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20993* | 0103762 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20994 | 0103766 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20994* | 0103766 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20995 | 0103770 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20995* | 0103770 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20975 | 0103690 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20975* | 0103690 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20997 | 0103778 | 032701A | 3/27/01 | 3/28/01 | NQ |
| E01-0263-20997* | 0103778 | 032701A | 3/27/01 | 3/28/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XXIV. Summary of PFOS for Week 6 Mallard Yolk Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20979 | 0103706 | 032701AD | 3/27/01 | 3/29/01 | 16100 |
| E01-0263-20979* | 0103706 | 032701AD | 3/27/01 | 3/29/01 | 15700 |
| E01-0263-21001 | 0103794 | 032701AD | 3/27/01 | 3/29/01 | 9860 |
| E01-0263-21001* | 0103794 | 032701AD | 3/27/01 | 3/29/01 | 10400 |
| E01-0263-20988 | 0103742 | 032701AD | 3/27/01 | 3/29/01 | 11200 |
| E01-0263-20988* | 0103742 | 032701AD | 3/27/01 | 3/29/01 | 10600 |
| E01-0263-20971 | 0103674 | 032701AD | 3/27/01 | 3/29/01 | 16800 |
| E01-0263-20971* | 0103674 | 032701AD | 3/27/01 | 3/29/01 | 16900 |
| E01-0263-20989 | 0103746 | 032701AD | 3/27/01 | 3/29/01 | 27200 |
| E01-0263-20989* | 0103746 | 032701AD | 3/27/01 | 3/29/01 | 26600 |
| | | | | **AVERAGE:** | **16100** |
| | | | | **STANDARD DEVIATION:** | **6330** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XXV. Summary of PFOS for Week 6 Mallard Yolk Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0263-20991 | 0103754 | 032701AD | 3/27/01 | 3/29/01 | 41900 |
| E01-0263-20991* | 0103754 | 032701AD | 3/27/01 | 3/29/01 | 40700 |
| E01-0263-20990 | 0103750 | 032701AD | 3/27/01 | 3/29/01 | 63000 |
| E01-0263-20990* | 0103750 | 032701AD | 3/27/01 | 3/29/01 | 63500 |
| E01-0263-21002 | 0103798 | 032701AD | 3/27/01 | 3/29/01 | 57500 |
| E01-0263-21002* | 0103798 | 032701AD | 3/27/01 | 3/29/01 | 57300 |
| E01-0263-21003 | 0103802 | 032701AD | 3/27/01 | 3/29/01 | 43100 |
| E01-0263-21003* | 0103802 | 032701AD | 3/27/01 | 3/29/01 | 43600 |
| E01-0263-21004 | 0103806 | 032701AD | 3/27/01 | 3/29/01 | 46600 |
| E01-0263-21004* | 0103806 | 032701AD | 3/27/01 | 3/29/01 | 49800 |
| | | | | **AVERAGE:** | **50700** |
| | | | | **STANDARD DEVIATION:** | **8870** |

## Table XXVI. Summary of PFOS in Quail Membrane Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003798 Blank A | 032901A | 3/29/01 | 3/31/01 | NQ |
| na | 0003798 Blank A* | 032901A | 3/29/01 | 3/31/01 | NQ |
| na | 0003798 Blank A | 033001BR | 3/30/01 | 4/2/01 | NQ |
| na | 0003798 Blank A* | 033001BR | 3/30/01 | 4/2/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XXVII. Summary of PFOS in Quail Albumen Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003796 Blank A | 032801B | 3/28/01 | 3/30/01 | NQ |
| na | 0003796 Blank A* | 032801B | 3/28/01 | 3/30/01 | NQ |
| na | 0003796 Blank A | 033001A | 3/30/01 | 4/1/01 | NQ |
| na | 0003796 Blank A* | 033001A | 3/30/01 | 4/1/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XXVIII. Summary of PFOS in Quail Yolk Blanks

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| na | 0003795 Blank A | 032801A | 3/28/01 | 3/30/01 | NQ |
| na | 0003795 Blank A* | 032801A | 3/28/01 | 3/30/01 | NQ |
| na | 0003795 Blank A | 032901B | 3/29/01 | 3/31-4/1/01 | ND |
| na | 0003795 Blank A* | 032901B | 3/29/01 | 3/31-4/1/01 | ND |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XXIX. Summary of PFOS Recoveries in Quail Membrane

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| na | 0003798 Spk A | 032901A | 3/29/01 | 3/31/01 | 10 | 102 |
| na | 0003798 Spk A* | 032901A | 3/29/01 | 3/31/01 | 10 | 104 |
| na | 0003798 Spk B | 032901A | 3/29/01 | 3/31/01 | 986 | 88 |
| na | 0003798 Spk B* | 032901A | 3/29/01 | 3/31/01 | 986 | 85 |
| na | 0003798 Spk A | 033001BR | 3/30/01 | 4/2/01 | 10 | 114 |
| na | 0003798 Spk A* | 033001BR | 3/30/01 | 4/2/01 | 10 | 116 |
| na | 0003798 Spk B | 033001B | 3/30/01 | 4/2/01 | 98 | 108 |
| na | 0003798 Spk B* | 033001B | 3/30/01 | 4/2/01 | 98 | 101 |
| | | | | | **AVERAGE:** | **102** |
| | | | | | **STANDARD DEVIATION:** | **11** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **11** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
ND = No peak detected.
LOQ = 10 ppb

## Table XXX. Summary of PFOS Recoveries in Quail Albumen

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| na | 0003796 Spk A | 032801B | 3/28/01 | 3/30/01 | 10 | 82 |
| na | 0003796 Spk A* | 032801B | 3/28/01 | 3/30/01 | 10 | 79 |
| na | 0003796 Spk B | 032801B | 3/28/01 | 3/30/01 | 9963 | 80 |
| na | 0003796 Spk B* | 032801B | 3/28/01 | 3/30/01 | 9963 | 83 |
| na | 0003796 Spk A | 033001A | 3/30/01 | 4/1/01 | 10 | 82 |
| na | 0003796 Spk A* | 033001A | 3/30/01 | 4/1/01 | 10 | 88 |
| na | 0003796 Spk B | 033001A | 3/30/01 | 4/1/01 | 99 | 93 |
| na | 0003796 Spk B* | 033001A | 3/30/01 | 4/1/01 | 99 | 91 |
| | | | | | **AVERAGE:** | **85** |
| | | | | | **STANDARD DEVIATION:** | **5** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **6** |

## Table XXXI. Summary of PFOS Recoveries in Quail Yolk

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Amt Added (ppb) | % Recovery |
|---|---|---|---|---|---|---|
| na | 0003795 Spk A | 032801A | 3/28/01 | 3/30/01 | 10 | 102 |
| na | 0003795 Spk A* | 032801A | 3/28/01 | 3/30/01 | 10 | 104 |
| na | 0003795 Spk B | 032801A | 3/28/01 | 3/30/01 | 9623 | 98 |
| na | 0003795 Spk B* | 032801A | 3/28/01 | 3/30/01 | 9623 | 101 |
| na | 0003795 Spk A | 032901B | 3/29/01 | 3/31-4/1/01 | 10 | 87 |
| na | 0003795 Spk A* | 032901B | 3/29/01 | 3/31-4/1/01 | 10 | 87 |
| na | 0003795 Spk B | 032901D | 3/29/01 | 4/1-2/01 | 98834 | 94 |
| na | 0003795 Spk B* | 032901D | 3/29/01 | 4/1-2/01 | 98834 | 93 |
| | | | | | **AVERAGE:** | **96** |
| | | | | | **STANDARD DEVIATION:** | **7** |
| | | | | | **RELATIVE STANDARD DEVIATION:** | **7** |

* Duplicate Injection

LOQ = 10 ppb

## Table XXXII. Summary of PFOS for Week 1 Quail Membrane Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20506 | 0101987 | 032901A | 3/29/01 | 3/31/01 | 5.20** |
| E01-0259-20506* | 0101987 | 032901A | 3/29/01 | 3/31/01 | 5.57** |
| E01-0259-20507 | 0101991 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20507* | 0101991 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20520 | 0102043 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20520* | 0102043 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20521 | 0102047 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20521* | 0102047 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20509 | 0101999 | 032901A | 3/29/01 | 3/31/01 | NQ |
| E01-0259-20509* | 0101999 | 032901A | 3/29/01 | 3/31/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XXXIII. Summary of PFOS for Week 1 Quail Membrane Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20526 | 0102067 | 032901D | 3/29/01 | 4/1-2/01 | 18800** |
| E01-0259-20526* | 0102067 | 032901D | 3/29/01 | 4/1-2/01 | 17900** |
| E01-0259-20514 | 0102019 | 032901A | 3/29/01 | 3/31/01 | 127 |
| E01-0259-20514* | 0102019 | 032901A | 3/29/01 | 3/31/01 | 129 |
| E01-0259-20527 | 0102071 | 032901A | 3/29/01 | 3/31/01 | 123 |
| E01-0259-20527* | 0102071 | 032901A | 3/29/01 | 3/31/01 | 116 |
| E01-0259-20515 | 0102023 | 032901A | 3/29/01 | 3/31/01 | 172 |
| E01-0259-20515* | 0102023 | 032901A | 3/29/01 | 3/31/01 | 172 |
| E01-0259-20528 | 0102075 | 032901D | 3/29/01 | 4/1-2/01 | 11500** |
| E01-0259-20528* | 0102075 | 032901D | 3/29/01 | 4/1-2/01 | 11500** |
| | | | | **AVERAGE:** | **140** |
| | | | | **STANDARD DEVIATION:** | **25.3** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

** Possible contamination by yolk or outer shell component of sample since outer shell was not washed prior to separation of internal components. As per Sponsor Representative request these values were not used to calculate the average and standard deviation.

For residues recorded as NQ, half of the concentration of the lowest standard (0.5 ppb) was used to calculate the average and standard deviation.

## Table XXXIV. Summary of PFOS for Week 1 Quail Membrane Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20529 | 0102079 | 032901D | 3/29/01 | 4/1-2/01 | 20900 |
| E01-0259-20529* | 0102079 | 032901D | 3/29/01 | 4/1-2/01 | 20400 |
| E01-0259-20531 | 0102087 | 032901D | 3/29/01 | 4/1-2/01 | 11000 |
| E01-0259-20531* | 0102087 | 032901D | 3/29/01 | 4/1-2/01 | 11100 |
| E01-0259-20532 | 0102091 | 032901D | 3/29/01 | 4/1-2/01 | 14300 |
| E01-0259-20532* | 0102091 | 032901D | 3/29/01 | 4/1-2/01 | 13900 |
| E01-0259-20518 | 0102035 | 032901D | 3/29/01 | 4/1-2/01 | 12500 |
| E01-0259-20518* | 0102035 | 032901D | 3/29/01 | 4/1-2/01 | 12600 |
| E01-0259-20533 | 0102095 | 032901D | 3/29/01 | 4/1-2/01 | 6880 |
| E01-0259-20533* | 0102095 | 032901D | 3/29/01 | 4/1-2/01 | 6990 |
| | | | | **AVERAGE:** | **13100** |
| | | | | **STANDARD DEVIATION:** | **4730** |

## Table XXXV. Summary of PFOS for Week 1 Quail Albumen Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20506 | 0101988 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20506* | 0101988 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20507 | 0101992 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20507* | 0101992 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20520 | 0102044 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20520* | 0102044 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20521 | 0102048 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20521* | 0102048 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20509 | 0102000 | 032801B | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20509* | 0102000 | 032801B | 3/28/01 | 3/30/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XXXVI. Summary of PFOS for Week 1 Quail Albumen Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20526 | 0102068 | 032801B | 3/28/01 | 3/30-31/01 | 6.03 |
| E01-0259-20526* | 0102068 | 032801B | 3/28/01 | 3/30-31/01 | 5.43 |
| E01-0259-20514 | 0102020 | 032801B | 3/28/01 | 3/30-31/01 | 2.43 |
| E01-0259-20514* | 0102020 | 032801B | 3/28/01 | 3/30-31/01 | 2.78 |
| E01-0259-20527 | 0102072 | 032801B | 3/28/01 | 3/30-31/01 | 2.94 |
| E01-0259-20527* | 0102072 | 032801B | 3/28/01 | 3/30-31/01 | 3.48 |
| E01-0259-20515 | 0102024 | 032801B | 3/28/01 | 3/30-31/01 | 5.87 |
| E01-0259-20515* | 0102024 | 032801B | 3/28/01 | 3/30-31/01 | 6.08 |
| E01-0259-20528 | 0102076 | 032801B | 3/28/01 | 3/30-31/01 | 93.9** |
| E01-0259-20528* | 0102076 | 032801B | 3/28/01 | 3/30-31/01 | 93.7** |
| | | | | **AVERAGE:** | **4.38** |
| | | | | **STANDARD DEVIATION:** | **1.61** |

## Table XXXVII. Summary of PFOS for Week 1 Quail Albumen Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20529 | 0102080 | 032801B | 3/28/01 | 3/30-31/01 | 17.9 |
| E01-0259-20529* | 0102080 | 032801B | 3/28/01 | 3/30-31/01 | 17.3 |
| E01-0259-20531 | 0102088 | 032801B | 3/28/01 | 3/30-31/01 | 9.97 |
| E01-0259-20531* | 0102088 | 032801B | 3/28/01 | 3/30-31/01 | 10.5 |
| E01-0259-20532 | 0102092 | 032801B | 3/28/01 | 3/30-31/01 | 10.7 |
| E01-0259-20532* | 0102092 | 032801B | 3/28/01 | 3/30-31/01 | 11.0 |
| E01-0259-20518 | 0102036 | 032801B | 3/28/01 | 3/30-31/01 | 32.3 |
| E01-0259-20518* | 0102036 | 032801B | 3/28/01 | 3/30-31/01 | 31.6 |
| E01-0259-20533 | 0102096 | 032801B | 3/28/01 | 3/30-31/01 | 9.07 |
| E01-0259-20533* | 0102096 | 032801B | 3/28/01 | 3/30-31/01 | 10.1 |
| | | | | **AVERAGE:** | **16.0** |
| | | | | **STANDARD DEVIATION:** | **8.92** |

* Duplicate Injection

LOQ = 10 ppb

## Table XXXVIII. Summary of PFOS for Week 1 Quail Yolk Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20506 | 0101985 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20506* | 0101985 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20507 | 0101989 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20507* | 0101989 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20520 | 0102041 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20520* | 0102041 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20521 | 0102045 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20521* | 0102045 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20509 | 0101997 | 032801A | 3/28/01 | 3/30/01 | NQ |
| E01-0259-20509* | 0101997 | 032801A | 3/28/01 | 3/30/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XXXIX. Summary of PFOS for Week 1 Quail Yolk Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20526 | 0102065 | 032801AD | 3/28/01 | 3/31/01 | 17500 |
| E01-0259-20526* | 0102065 | 032801AD | 3/28/01 | 3/31/01 | 17500 |
| E01-0259-20514 | 0102017 | 032801AD | 3/28/01 | 3/31/01 | 8530 |
| E01-0259-20514* | 0102017 | 032801AD | 3/28/01 | 3/31/01 | 8410 |
| E01-0259-20527 | 0102069 | 032801AD | 3/28/01 | 3/31/01 | 12100 |
| E01-0259-20527* | 0102069 | 032801AD | 3/28/01 | 3/31/01 | 12200 |
| E01-0259-20515 | 0102021 | 032801AD | 3/28/01 | 3/31/01 | 13800 |
| E01-0259-20515* | 0102021 | 032801AD | 3/28/01 | 3/31/01 | 13800 |
| E01-0259-20528 | 0102073 | 032801AD | 3/28/01 | 3/31/01 | 11700 |
| E01-0259-20528* | 0102073 | 032801AD | 3/28/01 | 3/31/01 | 10900 |
| | | | | **AVERAGE:** | **12600** |
| | | | | **STANDARD DEVIATION:** | **3150** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XL. Summary of PFOS for Week 1 Quail Yolk Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20529 | 0102077 | 032801AD | 3/28/01 | 3/31/01 | 42500 |
| E01-0259-20529* | 0102077 | 032801AD | 3/28/01 | 3/31/01 | 41200 |
| E01-0259-20531 | 0102085 | 032801AD | 3/28/01 | 3/31/01 | 33200 |
| E01-0259-20531* | 0102085 | 032801AD | 3/28/01 | 3/31/01 | 31100 |
| E01-0259-20532 | 0102089 | 032801AD | 3/28/01 | 3/31/01 | 37400 |
| E01-0259-20532* | 0102089 | 032801AD | 3/28/01 | 3/31/01 | 38900 |
| E01-0259-20518 | 0102033 | 032801AD | 3/28/01 | 3/31/01 | 38100 |
| E01-0259-20518* | 0102033 | 032801AD | 3/28/01 | 3/31/01 | 38300 |
| E01-0259-20533 | 0102093 | 032801AD | 3/28/01 | 3/31/01 | 43000 |
| E01-0259-20533* | 0102093 | 032801AD | 3/28/01 | 3/31/01 | 43600 |
| | | | | **AVERAGE:** | **38700** |
| | | | | **STANDARD DEVIATION:** | **4130** |

## Table XLI. Summary of PFOS for Week 6 Quail Membrane Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20735 | 0102903 | 033001B | 3/30/01 | 4/2/01 | NQ |
| E01-0259-20735* | 0102903 | 033001B | 3/30/01 | 4/2/01 | NQ |
| E01-0259-20736 | 0102907 | 033001B | 3/30/01 | 4/2/01 | 9.85** |
| E01-0259-20736* | 0102907 | 033001B | 3/30/01 | 4/2/01 | 8.93** |
| E01-0259-20737 | 0102911 | 033001B | 3/30/01 | 4/2/01 | 15.6** |
| E01-0259-20737* | 0102911 | 033001B | 3/30/01 | 4/2/01 | 14.4** |
| E01-0259-20720 | 0102843 | 033001B | 3/30/01 | 4/2/01 | 21.8** |
| E01-0259-20720* | 0102843 | 033001B | 3/30/01 | 4/2/01 | 24.4** |
| E01-0259-20738 | 0102915 | 033001B | 3/30/01 | 4/2/01 | 6.51** |
| E01-0259-20738* | 0102915 | 033001B | 3/30/01 | 4/2/01 | 6.30** |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

** Possible contamination by yolk or outer shell component of sample since outer shell was not washed prior to separation of internal components. As per Sponsor Representative request these values were not used to calculate the average and standard deviation.

## Table XLII. Summary of PFOS for Week 6 Quail Membrane Samples at 6.2 ppm ai

| ID | ID | Number | Date | Date | Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20742 | 0102931 | 033001B | 3/30/01 | 4/2/01 | 120 |
| E01-0259-20742* | 0102931 | 033001B | 3/30/01 | 4/2/01 | 119 |
| E01-0259-20743 | 0102935 | 033001B | 3/30/01 | 4/2/01 | 213 |
| E01-0259-20743* | 0102935 | 033001B | 3/30/01 | 4/2/01 | 208 |
| E01-0259-20744 | 0102939 | 033001B | 3/30/01 | 4/2/01 | 123 |
| E01-0259-20744* | 0102939 | 033001B | 3/30/01 | 4/2/01 | 116 |
| E01-0259-20745 | 0102943 | 033001B | 3/30/01 | 4/2/01 | 109 |
| E01-0259-20745* | 0102943 | 033001B | 3/30/01 | 4/2/01 | 116 |
| E01-0259-20746 | 0102947 | 033001B | 3/30/01 | 4/2/01 | 134 |
| E01-0259-20746* | 0102947 | 033001B | 3/30/01 | 4/2/01 | 134 |
| | | | | **AVERAGE:** | **139** |
| | | | | **STANDARD DEVIATION:** | **38.4** |

## Table XLIII. Summary of PFOS for Week 6 Quail Membrane Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20747 | 0102951 | 033001BD | 3/30/01 | 4/2/01 | 405 |
| E01-0259-20747* | 0102951 | 033001BD | 3/30/01 | 4/2/01 | 389 |
| E01-0259-20748 | 0102955 | 033001BD | 3/30/01 | 4/2/01 | 318 |
| E01-0259-20748* | 0102955 | 033001BD | 3/30/01 | 4/2/01 | 318 |
| E01-0259-20749 | 0102959 | 033001BD | 3/30/01 | 4/2/01 | 394 |
| E01-0259-20749* | 0102959 | 033001BD | 3/30/01 | 4/2/01 | 400 |
| E01-0259-20750 | 0102963 | 033001B | 3/30/01 | 4/2/01 | 262 |
| E01-0259-20750* | 0102963 | 033001B | 3/30/01 | 4/2/01 | 250 |
| E01-0259-20751 | 0102967 | 033001B | 3/30/01 | 4/2/01 | 186 |
| E01-0259-20751* | 0102967 | 033001B | 3/30/01 | 4/2/01 | 185 |
| | | | | **AVERAGE:** | **311** |
| | | | | **STANDARD DEVIATION:** | **86.6** |

* Duplicate Injection

LOQ = 10 ppb

## Table XLIV. Summary of PFOS for Week 6 Quail Albumen Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20735 | 0102904 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20735* | 0102904 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20736 | 0102908 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20736* | 0102908 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20737 | 0102912 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20737* | 0102912 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20720 | 0102844 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20720* | 0102844 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20738 | 0102916 | 033001A | 3/30/01 | 4/1/01 | NQ |
| E01-0259-20738* | 0102916 | 033001A | 3/30/01 | 4/1/01 | NQ |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

## Table XLV. Summary of PFOS for Week 6 Quail Albumen Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20742 | 0102932 | 033001A | 3/30/01 | 4/1/01 | 5.34 |
| E01-0259-20742* | 0102932 | 033001A | 3/30/01 | 4/1/01 | 5.93 |
| E01-0259-20743 | 0102936 | 033001A | 3/30/01 | 4/1/01 | 6.19 |
| E01-0259-20743* | 0102936 | 033001A | 3/30/01 | 4/1/01 | 6.91 |
| E01-0259-20744 | 0102940 | 033001A | 3/30/01 | 4/1/01 | 6.82 |
| E01-0259-20744* | 0102940 | 033001A | 3/30/01 | 4/1/01 | 6.82 |
| E01-0259-20745 | 0102944 | 033001A | 3/30/01 | 4/1/01 | 5.26 |
| E01-0259-20745* | 0102944 | 033001A | 3/30/01 | 4/1/01 | 5.15 |
| E01-0259-20746 | 0102948 | 033001A | 3/30/01 | 4/1/01 | 3.56 |
| E01-0259-20746* | 0102948 | 033001A | 3/30/01 | 4/1/01 | 4.34 |
| | | | | **AVERAGE:** | **5.63** |
| | | | | **STANDARD DEVIATION:** | **1.12** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
LOQ = 10 ppb

## Table XLVI. Summary of PFOS for Week 6 Quail Albumen Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20747 | 0102952 | 033001A | 3/30/01 | 4/1/01 | 9.81 |
| E01-0259-20747* | 0102952 | 033001A | 3/30/01 | 4/1/01 | 9.84 |
| E01-0259-20748 | 0102956 | 033001A | 3/30/01 | 4/1/01 | 13.2 |
| E01-0259-20748* | 0102956 | 033001A | 3/30/01 | 4/1/01 | 13.1 |
| E01-0259-20749 | 0102960 | 033001A | 3/30/01 | 4/1/01 | 14.6 |
| E01-0259-20749* | 0102960 | 033001A | 3/30/01 | 4/1/01 | 14.2 |
| E01-0259-20750 | 0102964 | 033001A | 3/30/01 | 4/1/01 | 16.3 |
| E01-0259-20750* | 0102964 | 033001A | 3/30/01 | 4/1/01 | 16.7 |
| E01-0259-20751 | 0102968 | 033001A | 3/30/01 | 4/1/01 | 15.6 |
| E01-0259-20751* | 0102968 | 033001A | 3/30/01 | 4/1/01 | 15.4 |
| | | | | **AVERAGE:** | **13.9** |
| | | | | **STANDARD DEVIATION:** | **2.44** |

## Table XLVII. Summary of PFOS for Week 6 Quail Yolk Control Samples

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20735 | 0102901 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20735* | 0102901 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20736 | 0102905 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20736* | 0102905 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20737 | 0102909 | 032901B | 3/29/01 | 3/31-4/1/01 | 51.5** |
| E01-0259-20737* | 0102909 | 032901B | 3/29/01 | 3/31-4/1/01 | 51.1** |
| E01-0259-20720 | 0102841 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20720* | 0102841 | 032901B | 3/29/01 | 3/31-4/1/01 | NQ |
| E01-0259-20738 | 0102913 | 032901B | 3/29/01 | 3/31-4/1/01 | ND |
| E01-0259-20738* | 0102913 | 032901B | 3/29/01 | 3/31-4/1/01 | ND |
| | | | | **AVERAGE:** | **NQ** |
| | | | | **STANDARD DEVIATION:** | **NQ** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))
ND = No peak detected
LOQ = 10 ppb

** Possible contamination by outer shell component of sample since outer shell was not washed prior to separation of internal components. As per Sponsor Representative request these values were not used to calculate the average and standard deviation.

## Table XLVIII. Summary of PFOS for Week 6 Quail Yolk Samples at 6.2 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20742 | 0102929 | 032901D | 3/29/01 | 4/1-2/01 | 38700 |
| E01-0259-20742* | 0102929 | 032901D | 3/29/01 | 4/1-2/01 | 40500 |
| E01-0259-20743 | 0102933 | 032901D | 3/29/01 | 4/1-2/01 | 37100 |
| E01-0259-20743* | 0102933 | 032901D | 3/29/01 | 4/1-2/01 | 36100 |
| E01-0259-20744 | 0102937 | 032901D | 3/29/01 | 4/1-2/01 | 23600 |
| E01-0259-20744* | 0102937 | 032901D | 3/29/01 | 4/1-2/01 | 23600 |
| E01-0259-20745 | 0102941 | 032901D | 3/29/01 | 4/1-2/01 | 40000 |
| E01-0259-20745* | 0102941 | 032901D | 3/29/01 | 4/1-2/01 | 40600 |
| E01-0259-20746 | 0102945 | 032901D | 3/29/01 | 4/1-2/01 | 24600 |
| E01-0259-20746* | 0102945 | 032901D | 3/29/01 | 4/1-2/01 | 25800 |
| | | | | **AVERAGE:** | **33100** |
| | | | | **STANDARD DEVIATION:** | **7610** |

## Table IL. Summary of PFOS for Week 6 Quail Yolk Samples at 17.6 ppm ai

| Sponsor ID | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (ppb) |
|---|---|---|---|---|---|
| E01-0259-20747 | 0102949 | 032901D | 3/29/01 | 4/1-2/01 | 53900 |
| E01-0259-20747* | 0102949 | 032901D | 3/29/01 | 4/1-2/01 | 54400 |
| E01-0259-20748 | 0102953 | 032901D | 3/29/01 | 4/1-2/01 | 65500 |
| E01-0259-20748* | 0102953 | 032901D | 3/29/01 | 4/1-2/01 | 64800 |
| E01-0259-20749 | 0102957 | 032901D | 3/29/01 | 4/1-2/01 | 46400 |
| E01-0259-20749* | 0102957 | 032901D | 3/29/01 | 4/1-2/01 | 46500 |
| E01-0259-20750 | 0102961 | 032901D | 3/29/01 | 4/1-2/01 | 59400 |
| E01-0259-20750* | 0102961 | 032901D | 3/29/01 | 4/1-2/01 | 59300 |
| E01-0259-20751 | 0102965 | 032901D | 3/29/01 | 4/1-2/01 | 56800 |
| E01-0259-20751* | 0102965 | 032901D | 3/29/01 | 4/1-2/01 | 57800 |
| | | | | **AVERAGE:** | **56500** |
| | | | | **STANDARD DEVIATION:** | **6510** |

* Duplicate Injection

NQ = Not Quantifiable (less than the concentration of the lowest standard (0.1 ng/mL))

## Table L. Summary of Moisture Determinations for Mallard Membrane Samples

| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0263-20852 | 0103200 | 80.0 |
| E01-0263-20853 | 0103204 | 55.0 |
| E01-0263-20854 | 0103208 | 75.0 |
| E01-0263-20842 | 0103160 | 80.0 |
| E01-0263-20843 | 0103164 | 80.0 |
| E01-0263-20825 | 0103092 | 70.0 |
| E01-0263-20846 | 0103176 | 70.0 |
| E01-0263-20832 | 0103120 | 75.0 |
| E01-0263-20847 | 0103180 | 65.0 |
| E01-0263-20848 | 0103184 | 60.0 |
| E01-0263-20856 | 0103216 | 75.0 |
| E01-0263-20850 | 0103192 | 50.0 |
| E01-0263-20857 | 0103220 | 85.0 |
| E01-0263-20851 | 0103196 | 85.0 |
| E01-0263-20858 | 0103224 | 85.0 |
| E01-0263-20993 | 0103764 | 75.0 |
| E01-0263-20994 | 0103768 | 71.4 |
| E01-0263-20995 | 0103772 | 80.0 |
| E01-0263-20975 | 0103692 | 65.0 |
| E01-0263-20997 | 0103780 | 80.0 |
| E01-0263-20979 | 0103708 | 75.0 |
| E01-0263-21001 | 0103796 | 65.0 |
| E01-0263-20988 | 0103744 | 75.0 |
| E01-0263-20971 | 0103676 | 90.0 |
| E01-0263-20989 | 0103748 | 80.0 |
| E01-0263-20991 | 0103756 | 75.0 |
| E01-0263-20990 | 0103752 | 70.0 |
| E01-0263-21002 | 0103800 | 70.0 |
| E01-0263-21003 | 0103804 | 75.0 |
| E01-0263-21004 | 0103808 | 65.0 |

## Table LI. Summary of Moisture Determinations for Mallard Albumen Samples

| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0263-20852 | 0103201 | 90.0 |
| E01-0263-20853 | 0103205 | 88.0 |
| E01-0263-20854 | 0103209 | 87.0 |
| E01-0263-20842 | 0103161 | 85.0 |
| E01-0263-20843 | 0103165 | 87.0 |
| E01-0263-20825 | 0103093 | 82.0 |
| E01-0263-20846 | 0103177 | 84.0 |
| E01-0263-20832 | 0103121 | 86.0 |
| E01-0263-20847 | 0103181 | 89.0 |
| E01-0263-20848 | 0103185 | 88.0 |
| E01-0263-20856 | 0103217 | 85.0 |
| E01-0263-20850 | 0103193 | 85.0 |
| E01-0263-20857 | 0103221 | 84.0 |
| E01-0263-20851 | 0103197 | 83.0 |
| E01-0263-20858 | 0103225 | 85.0 |
| E01-0263-20993 | 0103765 | 88.0 |
| E01-0263-20994 | 0103769 | 85.0 |
| E01-0263-20995 | 0103773 | 85.0 |
| E01-0263-20975 | 0103693 | 84.0 |
| E01-0263-20997 | 0103781 | 90.0 |
| E01-0263-20979 | 0103709 | 88.0 |
| E01-0263-21001 | 0103797 | 84.0 |
| E01-0263-20988 | 0103745 | 87.0 |
| E01-0263-20971 | 0103677 | 83.0 |
| E01-0263-20989 | 0103749 | 87.0 |
| E01-0263-20991 | 0103757 | 84.0 |
| E01-0263-20990 | 0103753 | 86.0 |
| E01-0263-21002 | 0103801 | 83.0 |
| E01-0263-21003 | 0103805 | 82.0 |
| E01-0263-21004 | 0103809 | 87.0 |

## Table LII. Summary of Moisture Determinations for Mallard Yolk Samples

| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0263-20852 | 0103198 | 44.0 |
| E01-0263-20853 | 0103202 | 45.0 |
| E01-0263-20854 | 0103206 | 48.0 |
| E01-0263-20842 | 0103158 | 45.0 |
| E01-0263-20843 | 0103162 | 43.0 |
| E01-0263-20825 | 0103090 | 54.0 |
| E01-0263-20846 | 0103174 | 49.0 |
| E01-0263-20832 | 0103118 | 43.0 |
| E01-0263-20847 | 0103178 | 43.0 |
| E01-0263-20848 | 0103182 | 48.0 |
| E01-0263-20856 | 0103214 | 45.0 |
| E01-0263-20850 | 0103190 | 50.0 |
| E01-0263-20857 | 0103218 | 49.0 |
| E01-0263-20851 | 0103194 | 48.0 |
| E01-0263-20858 | 0103222 | 45.0 |
| E01-0263-20993 | 0103762 | 45.0 |
| E01-0263-20994 | 0103766 | 44.0 |
| E01-0263-20995 | 0103770 | 59.0 |
| E01-0263-20975 | 0103690 | 45.0 |
| E01-0263-20997 | 0103778 | 46.0 |
| E01-0263-20979 | 0103706 | 45.0 |
| E01-0263-21001 | 0103794 | 48.0 |
| E01-0263-20988 | 0103742 | 45.0 |
| E01-0263-20971 | 0103674 | 48.0 |
| E01-0263-20989 | 0103746 | 50.0 |
| E01-0263-20991 | 0103754 | 56.0 |
| E01-0263-20990 | 0103750 | 42.0 |
| E01-0263-21002 | 0103798 | 51.0 |
| E01-0263-21003 | 0103802 | 44.9 |
| E01-0263-21004 | 0103806 | 44.0 |

## Table LIII. Summary of Moisture Determinations for Quail Membrane Samples

| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0259-20507 | 0101991 | 45.0 |
| E01-0259-20509 | 0101999 | 65.0 |
| E01-0259-20526 | 0102067 | 65.0 |
| E01-0259-20738 | 0102915 | 60.0 |
| E01-0259-20742 | 0102931 | 60.0 |
| E01-0259-20745 | 0102943 | 68.3 |
| E01-0259-20748 | 0102955 | 70.0 |
| E01-0259-20750 | 0102963 | 68.3 |

The percent moisture was determined for eight out of the 30 samples received because there was not enough sample available for the other samples.

# Table LIV. Summary of Moisture Determinations for Quail Albumen Samples

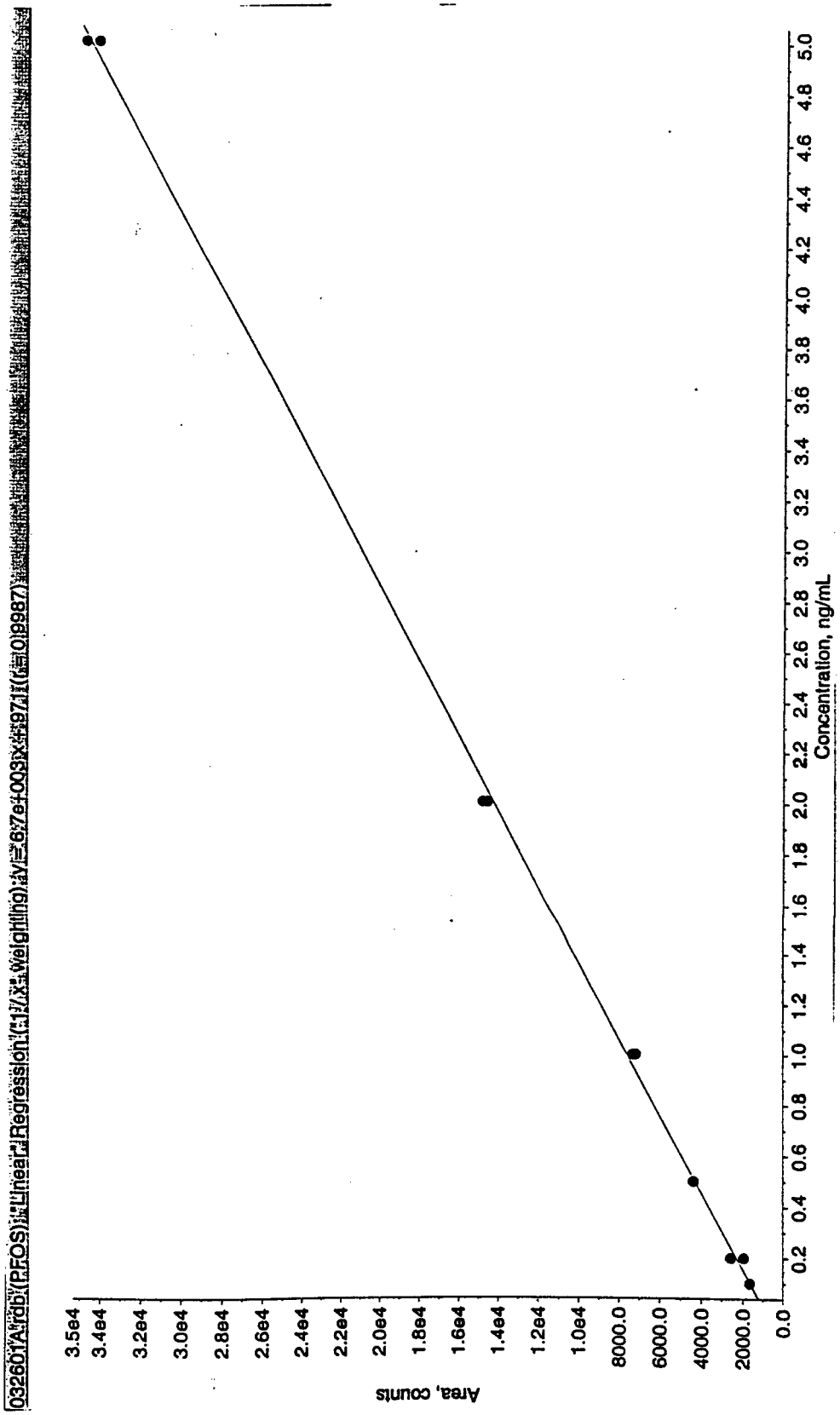
| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0259-20506 | 0101988 | 87.0 |
| E01-0259-20507 | 0101992 | 79.0 |
| E01-0259-20520 | 0102044 | 89.0 |
| E01-0259-20521 | 0102048 | 88.0 |
| E01-0259-20509 | 0102000 | 85.2 |
| E01-0259-20526 | 0102068 | 87.0 |
| E01-0259-20514 | 0102020 | 88.0 |
| E01-0259-20527 | 0102072 | 86.0 |
| E01-0259-20515 | 0102024 | 89.0 |
| E01-0259-20528 | 0102076 | 87.0 |
| E01-0259-20529 | 0102080 | 89.0 |
| E01-0259-20531 | 0102088 | 88.0 |
| E01-0259-20532 | 0102092 | 88.0 |
| E01-0259-20518 | 0102036 | 87.0 |
| E01-0259-20533 | 0102096 | 87.0 |
| E01-0259-20735 | 0102904 | 88.0 |
| E01-0259-20736 | 0102908 | 86.0 |
| E01-0259-20737 | 0102912 | 87.0 |
| E01-0259-20720 | 0102844 | 87.0 |
| E01-0259-20738 | 0102916 | 87.0 |
| E01-0259-20742 | 0102932 | 86.0 |
| E01-0259-20743 | 0102936 | 82.0 |
| E01-0259-20744 | 0102940 | 84.0 |
| E01-0259-20745 | 0102944 | 87.0 |
| E01-0259-20746 | 0102948 | 86.0 |
| E01-0259-20747 | 0102952 | 89.0 |
| E01-0259-20748 | 0102956 | 86.0 |
| E01-0259-20749 | 0102960 | 85.0 |
| E01-0259-20750 | 0102964 | 87.0 |
| E01-0259-20751 | 0102968 | 86.0 |

## Table LV. Summary of Moisture Determinations for Quail Yolk Samples

| Sponsor ID | Centre ID | Average % Moisture |
|---|---|---|
| E01-0259-20506 | 0101985 | 48.0 |
| E01-0259-20507 | 0101999 | 43.0 |
| E01-0259-20520 | 0102041 | 46.0 |
| E01-0259-20521 | 0102045 | 45.0 |
| E01-0259-20509 | 0101997 | 45.0 |
| E01-0259-20526 | 0102065 | 49.0 |
| E01-0259-20514 | 0102017 | 49.0 |
| E01-0259-20527 | 0102069 | 47.0 |
| E01-0259-20515 | 0102021 | 54.0 |
| E01-0259-20528 | 0102073 | 47.0 |
| E01-0259-20529 | 0102077 | 47.0 |
| E01-0259-20531 | 0102085 | 41.0 |
| E01-0259-20532 | 0102089 | 49.0 |
| E01-0259-20518 | 0102033 | 50.0 |
| E01-0259-20533 | 0102093 | 47.0 |
| E01-0259-20735 | 0102901 | 47.0 |
| E01-0259-20736 | 0102905 | 46.0 |
| E01-0259-20737 | 0102909 | 47.0 |
| E01-0259-20720 | 0102841 | 41.0 |
| E01-0259-20738 | 0102913 | 43.0 |
| E01-0259-20742 | 0102929 | 44.0 |
| E01-0259-20743 | 0102933 | 43.0 |
| E01-0259-20744 | 0102937 | 45.0 |
| E01-0259-20745 | 0102941 | 53.0 |
| E01-0259-20746 | 0102945 | 46.0 |
| E01-0259-20747 | 0102949 | 47.0 |
| E01-0259-20748 | 0102953 | 46.0 |
| E01-0259-20749 | 0102957 | 45.0 |
| E01-0259-20750 | 0102961 | 45.0 |
| E01-0259-20751 | 0102965 | 43.0 |

# FIGURES

**Figure 1. Typical Calibration Curve for PFOS**



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

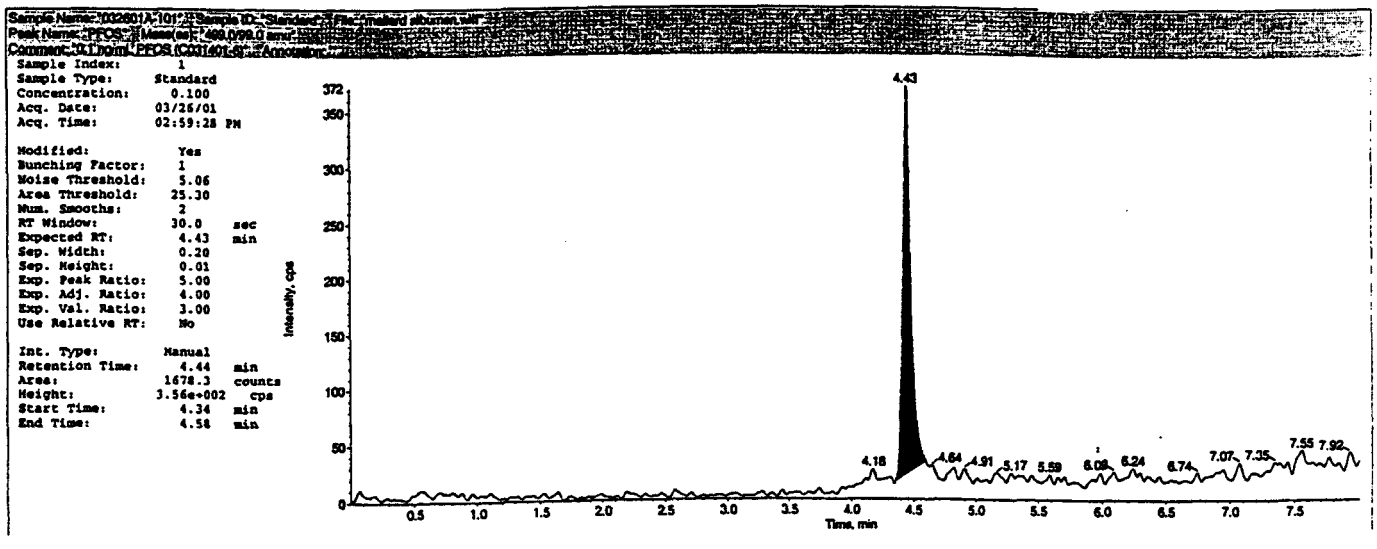
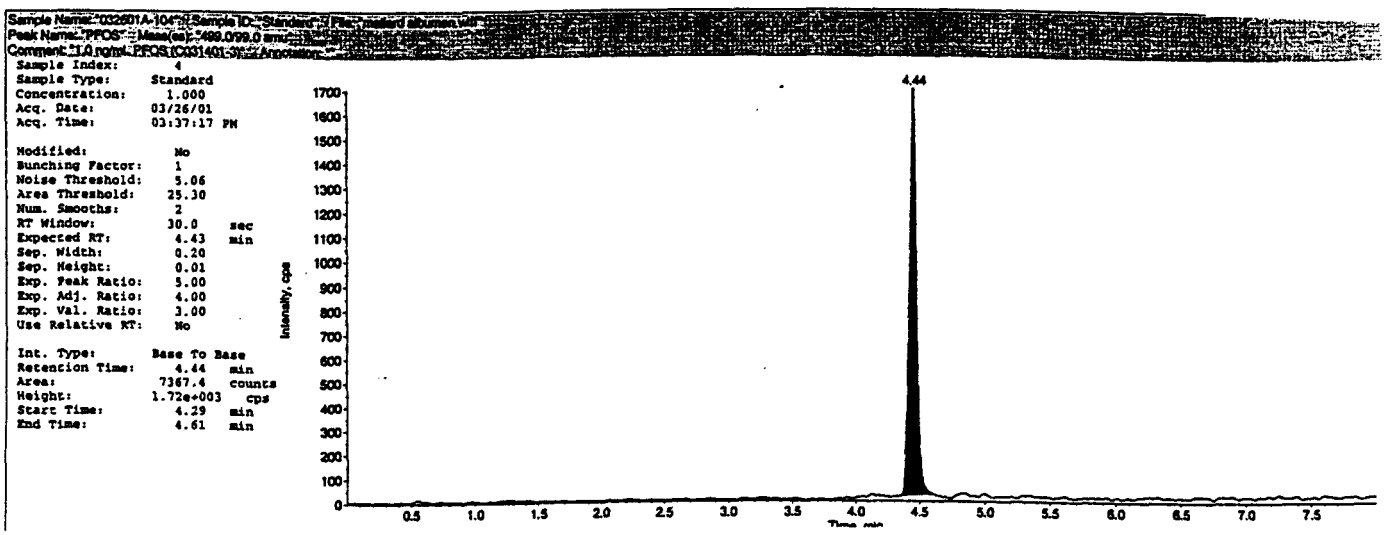


**Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS**



# Figure 4. Chromatogram Representing a Reagent Blank for PFOS (Centre ID Reagent Blank A, Set: 032601A)

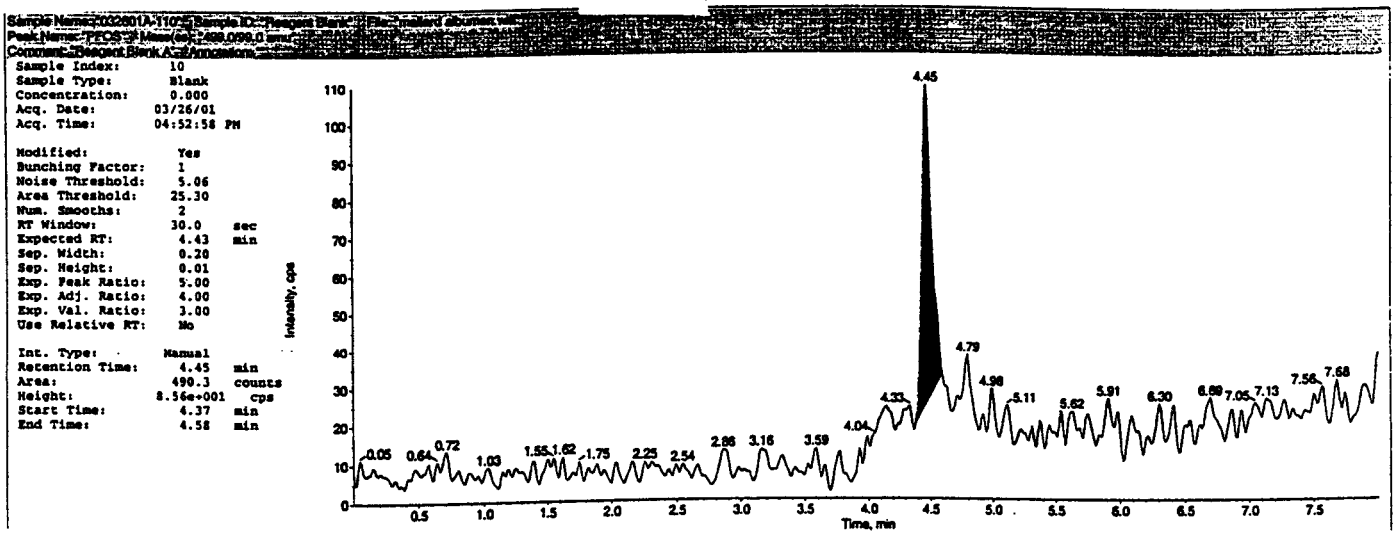


**Figure 5. Chromatogram Representing Control Mallard Membrane for PFOS (Centre ID: 0003802 Blank A, Set: 032601C)**

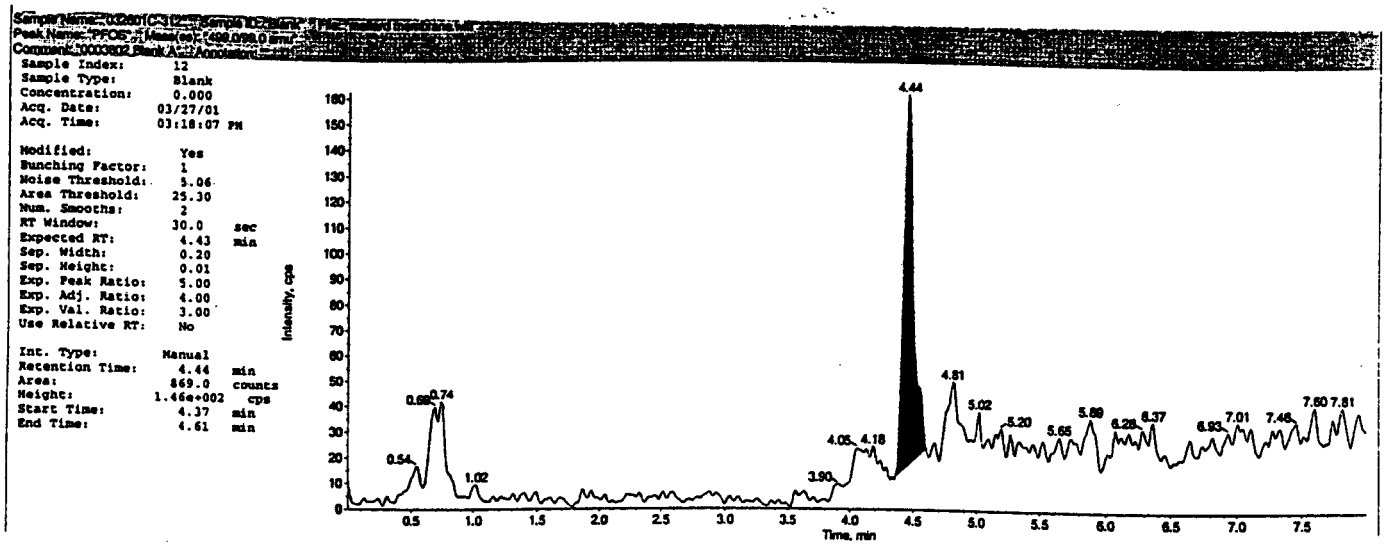


**Figure 6. Chromatogram Representing Control Mallard Membrane Fortified with 10 ppb of PFOS, (Centre ID: 0003802 Spk A Dup. Inj., Set: 032601C)**

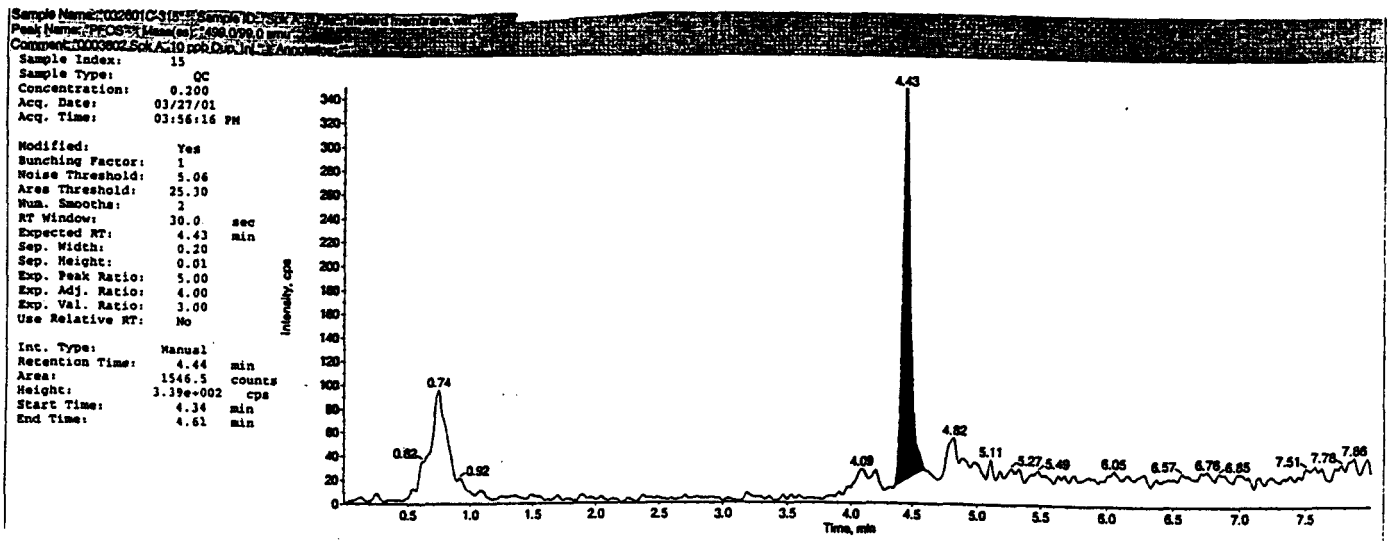
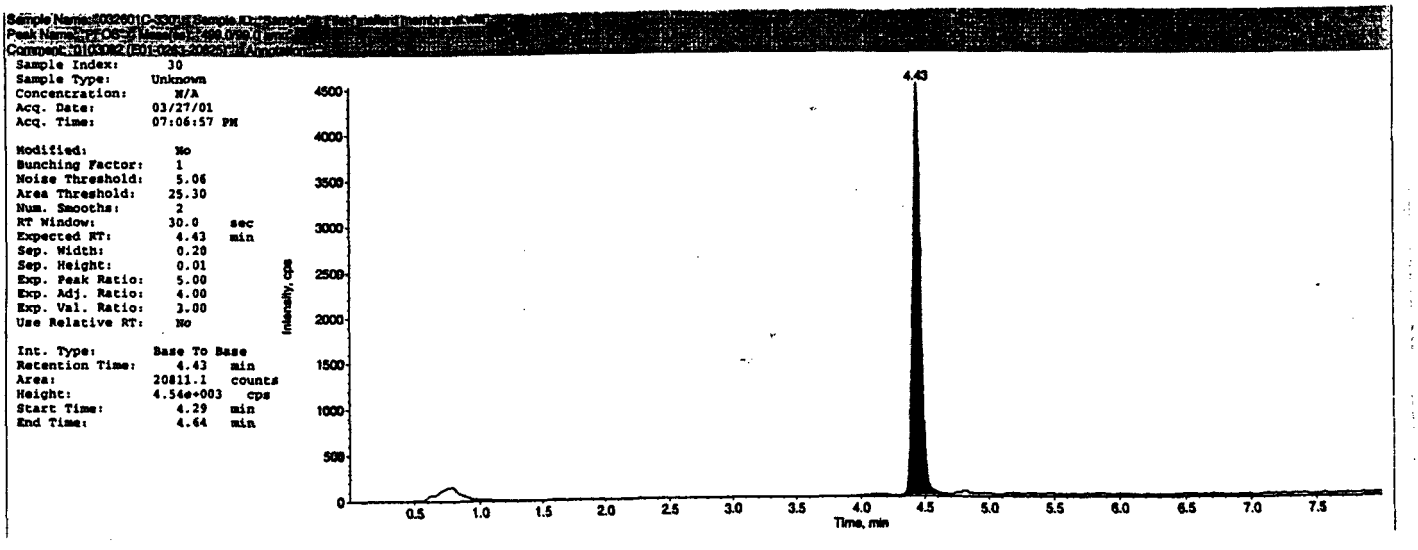


**Figure 7. Chromatogram Representing Mallard Membrane Sample from Week 1 at 6.2 ppm ai (E01-0263-20825) (Centre ID: 0103092, Set: 032601C)**



# APPENDIX A

## Study Protocol
## 00P-023-063
## (Centre Study No. 023-063)
## and
## Amendments and Deviations

Centre Protocol No. 00P-023-063

# ANALYTICAL PHASE PROTOCOL

**ANALYTICAL PHASE TITLE**

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM EGG MEMBRANE, YOLK AND ALBUMEN 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

**PERFORMING LABORATORY**

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

**PROTOCOL IDENTIFICATION NUMBER**

00P-023-063

Centre Protocol No. 00P-023-063

Title: EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM EGG MEMBRANE, YOLK AND ALBUMEN FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

TABLE OF CONTENTS

Centre Protocol No. 00P-023-063

Title:
EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM EGG MEMBRANE, YOLK AND ALBUMEN FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

## 1. PURPOSE

The purpose of this study is to analyze mallard and quail egg membrane, yolk, and albumen samples for residues of perfluorooctanesulfonate (PFOS) using method entitled, "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen, and Yolk by LC/MS/MS", Centre method number 00M-023-015.

The analytical phase will be conducted under EPA TSCA Good Laboratory Practice standards 40 CFR Part 792 (1).

## 2. REFERENCE MATERIAL

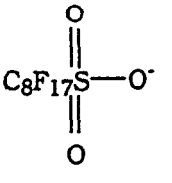
The following analytical standards will be used:

| 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-heptadecafluoro-, potassium salt
CAS Number: 2795-39-3
Molecular Weight: 499 ($C_8F_{17}SO_3^-$)



Note: The neutral molecule and standard form that PFOS (anion) is derived from is perfluorooctanesulfonate potassium salt [$C_8F_{17}SO_3K$], molecular weight 538.
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

Sponsor Representative:
Rochelle Robideau
TELEPHONE: 651-778-7065
FAX NUMBER: 651-778-6176

## 4. TESTING FACILITY

Wildlife International, Ltd.
8598 Commerce Drive
Easton, MD 21601

STUDY DIRECTOR:
Sean Gallagher
TELEPHONE: 410-822-8600
FAX NUMBER: 410-822-0632

## 5. 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

## 6. PROPOSED EXPERIMENTAL TIME-FRAME

| | |
|---|---|
| Analytical Start Date | Mar. 19, 2001 |
| Analytical Termination Date | Mar. 31, 2001 |
| Report Issued | April 1, 2001 |

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

Mallard and quail egg membrane, yolk, and albumen samples collected by Wildlife International, Ltd. will be used as the analytical samples for this phase. Mallards and quails represent wild bird populations and they are an EPA recommended species because they do well in a laboratory environment. Quail

habitats include bushy pastures, grassy roadsides, farmlands and open woodlands, and they are considered land grazers. Mallard habitats include ponds, lakes and marshes, and they are considered aquatic grazers. The use of these matrices for the study was to determine if PFOS was deposited in the egg membrane, yolk, and albumen of mating adults that were fed PFOS.

A complete description of the test system along with all of the in-life parameters is documented in Mallard Protocol No. 454/010600/MP/SUB454, Project No. 454-105 and Quail Protocol No. 454/010600/QP/SUB454, Project No. 454-104. This project is the analytical portion of those studies.

## 8. SAMPLE PROCESSING, STORAGE AND IDENTIFICATION

No processing will be required for the any of the samples.

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° 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.

## 9. ANALYTICAL METHOD

All samples will be analyzed according to the analytical method titled "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen, and Yolk by LC/MS/MS", Centre method number 00M-023-015

## 10. EXPERIMENTAL DESIGN

Samples obtained by Wildlife International Ltd. have been shipped to Centre Analytical Laboratories, Inc. for analysis. The are no known contaminants expected in the test system that would affect the results of this study.

Only those samples designated by the Sponsor will be extracted and analyzed at Centre according to method, "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen, and Yolk by LC/MS/MS", Centre method number 00M-023-015.

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. Fortification will be made to the matrix prior to extraction at levels ranging from the LOQ to above the highest residue found. The fortification solution containing the test/reference substance will be applied to the designated samples via a Hamilton syringe, micropipette, pipet, or equivalent, to maintain consistency and accuracy.

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.

The percent moisture will also be determined for each extracted sample if adequate sample remains after extraction. For each matrix, 0.1 g samples will be weighed and dried in an oven at ~ 105°C for ~ 12-14 hours. Percent moisture for each sample will be determined in duplicate when there is sufficient sample available.

## 11. 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 and Sponsor Representative. Planned changes to this protocol or to the analytical method will be made in writing as an amendment and approved by the Principal Investigator, the Study Director, and the Sponsor Representative. 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, Study Director, and Sponsor Representative. 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, Study Director, and Sponsor Representative and reviewed by QAU.

## 12. 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., µg/ml, 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.

## 13. 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 GLP standards are met. 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.

## 14. 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 report will be issued by Centre, approved and signed by the Study Director and the Sponsor Representative 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. Reference materials identified by name, lot, purity, and other characteristics
4. Name of performing laboratory and analytical start and termination 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 and Sponsor Representative, who will return the report with comments. Centre Analytical will make revisions and finalize the report with the approval of the Study Director and Sponsor Representative. The Centre QA unit will conduct an audit of the final draft report and data files to assure accuracy and GLP compliance. The report must be approved by the Study Director and Sponsor Representative 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 , Study Director and Sponsor Representative.

REPORT DISTRIBUTION:
Original: Study Director
Copy: Centre Archives

## 15. 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. 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.

## 16. REFERENCES

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

## 17. 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 — Date: 3/28/01

**Principal Investigator, Centre**

Emily Stauffer
Scientist — Date: 3/28/01

**Facility Management, Centre**

John Flaherty
Operations Manager — Date: 3/28/01

**Study Director, Wildlife International, Ltd.**

Sean Gallagher
Senior Biologist — Date: 3/26/01

**Sponsor, 3M**

Rochelle Robideau
Environmental Technologist — Date: 3/22/01

## APPENDIX: ANALYTICAL METHOD

A. "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen, and Yolk by LC/MS/MS", Centre method number 00M-023-015

TITLE

Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen and Yolk by LC/MS/MS

AUTHORS
Emily Stauffer and John Flaherty

DATE ISSUED

January 2, 2001

SPONSOR

3M Environmental Laboratory
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

PERFORMING LABORATORY

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

CENTRE STUDY NUMBER

023-015

CENTRE METHOD NUMBER

00M-023-015

TOTAL NUMBER OF PAGES

28

Centre Protocol No. 00P-023-063

Centre Method No: 00M-023-015

## MANAGEMENT APPROVAL

As per 40 CFR 792.3, method development is not required to be conducted in compliance with Good Laboratory Practices. However, the work was in conformance with applicable standard operating procedures and general GLP regulations.

Emily R Stauffer 3/6/01
Emily R Stauffer Date
Principal Investigator
Centre Analytical Laboratories, Inc.

John M. Flaherty 3/6/01
John Flaherty Date
Laboratory Manager
Centre Analytical Laboratories, Inc.

6-MAR-01
Richard A. Grazzini, Ph.D. Date
President
Centre Analytical Laboratories, Inc.

Reynelle Robideau 3/12/01
Reynelle Robideau Date
Sponsor Representative
3M Environmental Laboratory

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Centre Protocol No. 00P-023-063

Centre Method No: 00M-023-015

## LIST OF TABLES

## LIST OF FIGURES

## 1. SUMMARY

This document details a method of analysis for the residual determination of perfluorooctanesulfonate (PFOS) in egg membrane, albumen and yolk. The chemical formula of the analyte is given in Section 2 of this method.

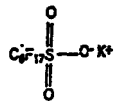
Perfluorooctanesulfonate is extracted from each matrix with methanol (MeOH). For egg membrane samples, the methanol extract is passed through a membrane filter. For egg albumen and yolk samples, a 0.5g of carbon is added to an aliquot of the methanol extract. Quantification of PFOS is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM).

For this method the LOQ is 10 ng/g (parts-per-billion). This is based on studies conducted with egg membrane, albumen, and yolk control samples during method development.

## 2. EXPERIMENTAL COMPOUNDS

The molecular structure of PFOS is given below:

PFOS
Molecular weight: 499 ($C_8F_{17}SO_3^-$)



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-heptadecafluoro-potassium salt
CAS #: 2795-39-3

Note: The neutral molecule and standard form which PFOS (anion) is derived from is perfluorooctanesulfonate potassium salt [$C_8F_{17}SO_3K$] (molecular weight 538).

## 3. CHEMICALS AND SUPPLIES

### 3.1. CHEMICALS

| Chemical | Grade | Source | Catalog No. |
|---|---|---|---|
| Carbon | 120/400 | Supelco | 57210-U |
| Methanol (MeOH) | HPLC | (VWR) J.T. Baker | JT9093-2 |
| Ammonium Acetate | Reagent | Aldrich Chemical | 111-87-5 |
| Water | Type I | Centre | |

(Type I water = electrical resistivity, minimum of 16.67 MΩ-cm at 25°C, from a Labconco™ waterpro workstation, based on ASTM classification)

### 3.2. STANDARDS

| Standard | Grade | Test Control Reference Number | Source |
|---|---|---|---|
| PFOS | Analytical | TCR-00017-46 | 3M Environmental Laboratory, St. Paul, MN |

### 3.3. Equipment and Supplies

| EQUIPMENT | SOURCE |
|---|---|
| Balance, 5 place analytical | Mettler |
| Balance, 1 place top-loading | Mettler |
| Mini Bead Beater-8 | Bio Spec Products |
| Wrist-action shaker | Burrell |
| Bench-top centrifuge | IEC |
| Mini-centrifuge -1201 | VWR |
| 50 mL disposable polypropylene centrifuge tubes | VWR |
| Stainless steel beads (cat # 11079) | Bio Spec Products |
| Micro-centrifuge tubes | VWR |
| Disposable pipettes, test tubes etc. | VWR |
| 15-mL disposable polypropylene centrifuge tubes | VWR |
| Whatman™ Anotop™ Filter (cat # 6809-1022) | VWR |
| 2-mL Eppendorf™ microcentrifuge tubes (cat# 22-36-335-2) | VWR |
| 2-mL clear HPLC vial kit (cat # 5181-3400) | HP |
| Standard lab equipment (class A pipettes and volumetric flasks, graduated cylinders, etc.) | |
| LC/MS/MS and HPLC systems | As described in Section 4.4.1. |

Note:

1. In order to avoid contamination, the use of disposable labware is highly recommended (containers, tubes, pipettes, etc.).
2. Teflon or teflon-lined containers or equipment, including teflon-lined HPLC vial caps, should not be used.
4. It is necessary to check the solvents (methanol) for the presence of contaminants by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
5. Use disposable micropipettes or pipettes to aliquot standard solutions and when preparing standards and samples for extraction.
6. Equivalent materials may be substituted for those specified in this method. However, the use of carbon from Supelco is strongly recommended.

### 3.4. Solutions

1. 50 mM ammonium acetate solution: Dissolve 3.85 g of ammonium acetate in 1 L of ASTM type I water. Store in an appropriate glass bottle at room temperature for up to 1 year.
2. 2 mM ammonium acetate solution: Dilute 40 mL of the 50 mM ammonium acetate solution in a liter of ASTM type I water, for mobile

phase A. Store in an appropriate glass bottle at room temperature for up to 1-year.

Note: The volumes shown are provided for guidance; alternative volumes may be prepared.

## 3.5. Preparation of Stock, Fortification, and Calibration Solutions

Analytical standards are prepared for two purposes. They are used to fortify untreated samples in order to determine analytical recovery and to calibrate the response of the detector used in the analysis.

The analyst may vary the absolute volumes of the standards as long as the correct proportions of solute to solvent are maintained. The solutions cited below are given as an example; alternative concentrations may be prepared if needed.

### *3.5.1. Stock Solution*

Prepare stock solution of PFOS at 100 µg/mL by weighing out 10.0 mg of analytical standard (corrected for percent salt). 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°C to 6°C for a maximum period of 6 months from the date of preparation.

### *3.5.2. Fortification Solutions*

a. 1.0 µg/mL Fortification Solution – Pipette 1.0 mL of the 100 µg/mL stock solution into a 100 mL volumetric flask. Bring up to volume with methanol.

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.

### *3.5.3. Calibration Standards*

Prepare six LC/MS/MS calibration standards in methanol via dilution of the 0.1 µg/mL and 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 | 1 | 100 | 0.0001 |
| 0.01 | 0.5 | 100 | 0.00005 |

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.

## 4. METHOD

### 4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Weigh 1 g of matrix (0.1g for membrane). Fortify if needed
↓
Extract with MeOH
↓
Carbon clean up (except membrane)
↓
Filter
↓
Dilution
↓
LC/MS/MS analysis

### 4.2. SAMPLE PROCESSING

All samples are received frozen and will be kept frozen (below –10 °C) until time of the extraction.

### 4.3. SAMPLE EXTRACTION

NOTE: All egg sample matrices were separated prior to arrival at Centre.

*4.3.1 Egg Membrane*

a. Allow sample to thaw.

b. Weigh 0.1 g (± 0.005g) of sample into a 2-mL Eppendorf™ tube. Record the weight to the nearest 0.0001g. Fortify untreated control samples at this point for determination of method recovery.

c. Add 1 mL of MeOH and three stainless-steel beads, replace lid tightly and homogenize with bead beater set at position 7 (~2500 rpm) for 5 minutes. Centrifuge the tubes with mini-centrifuge at 2,000 rpm for ~ 2 minutes, then carefully transfer supernatant to a new 2-mL Eppendorf™ tube, filtering with Whatman™ Anotop™ 10 filter if necessary.

d. Make a 5X dilution by transferring 0.1 mL of filtrate to HPLC vials and adding 0.4 mL of methanol using disposable micropipettes (100-200µL).

e. Store the rest of the extract in a refrigerator at approximately 2°C to 6°C for future re-dilution or re-injection.

*4.3.2. Egg Yolk and Albumen*

a. Allow sample to thaw.

b. Weigh 1.0 g (± 0.05g) of sample into a 50-mL disposable polypropylene centrifuge tube. Record the weight to the nearest 0.0001g. Fortify untreated control samples at this point for determination of method recovery.

c. Add 25 mL of MeOH, replace lid tightly, and shake on a wrist-action shaker for 15 minutes. Centrifuge tubes at ~ 2,000 rpm for ~ 10 minutes.

d. Transfer about 5 mL of extract into a 15-mL disposable polypropylene centrifuge tube, add 0.5 g carbon, replace lid tightly, and shake by hand for about 10 seconds.

e. Let the tubes sit for ~ 2 minutes, carefully transfer ~ 2.0 mL of the sample into a 10-ml disposable syringe barrel connected to a Whatman™ Anotop™ 10 filter. Filter sample into two HPLC vials, one for injection and one for storage in a refrigerator at approximately 2°C to 6°C for future re-dilution or re-injection.

## 4.4. ANALYSIS BY LC/MS/MS

### *4.4.1: 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 $C_8$ (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.

| Time | % A | % B |
|---|---|---|
| 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 $C_{18}$ etc.) can be used, provided equivalent chromatography is obtained.

Ions monitored:

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

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

*4.4.2. 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.

| Controls | Set |
|---|---|
| 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 | 2400.0 |

| Gas Flows | Set |
|---|---|
| Nebulizer Gas | 12 |
| Curtain Gas | 13 |
| Collision Gas | 4 |
| TIS Temperature | 350°C |

*4.4.3. Calibration Procedures*

a. Inject the same volume (between 10 to 20 µL) of each calibration standard (prepared in MeOH) into the LC/MS/MS.

b. 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 ± 30%, based on its calculated concentration, must 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 ≥0.9925 ($r^2$≥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.

*4.4.4. Sample Analysis*

a. Inject the same volume used for the calibration standards (between 10 to 25 µL) of each sample, fortification, control, etc. into the LC/MS/MS.

b. Standards corresponding to at least six concentrations must be included in an analytical set.

c. 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 (see Section 4.5).

d. Each set of samples analyzed (not to exceed 25) must include at least one reagent control (method blank), one ASTM Type I water blank, at least one matrix control, and two matrix control samples fortified at known concentrations and carried through the procedure to verify recovery.

e. All samples must be analyzed with duplicate injections.

Note: The analysis performed during method development included fortifications at 10, 50 and 250 ng/g (ppb) for the analyte.

f. 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.

g. Fortifications that bracket the highest residue expected in each treated sample will be included with each sample set. If residues are found outside these limits, additional fortifications will be included in a subsequent sample set to establish that method recoveries are available for the analyte of interest at concentrations exceeding those in treated samples.

h. Fortification recoveries within 60 to 130% are acceptable for fortifications at the LOQ level. Recoveries between 70 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.

i. 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 the lowest standard will be reported as NQ (not quantifiable).

j. 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.

j. 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.

Recoveries from method development for all matrices can be found in Tables I-VI.

## 4.5. PERFORMANCE CRITERIA

The following two criteria must be met before the initial analysis of samples, especially when using different instrumentation set-ups than those cited in this method.

First Criterion - Inject a standard solution on the LC/MS/MS corresponding to the estimated LOQ (10 ppb LOQ is equivalent to a standard solution of 0.2 ng/mL) and obtain a signal to noise ratio of at least 9:1 relative to the reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion - Inject a set of standards ranging from at or below the LOQ, up to the highest concentration level. Generate a calibration curve for the analyte and obtain a linear regression with a coefficient of determination ($r^2$) of at least 0.985 for the analyte. Once this criterion has been demonstrated, samples may be analyzed with standards interspersed.

### 4.6. TIME REQUIRED FOR ANALYSIS

A set of 14 samples can be taken through the extraction procedure in approximately three hours by one person. The LC/MS/MS analysis (8-10 standards and 14 samples) will take approximately 6 hours.

## 5. CALCULATIONS

### 5.1 ANALYTE FOUND:

Calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve generated by the MacQuan software program using Equation 1.

Equation 1:

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

### 5.2 COMPONENT RESIDUE CONCENTRATION

Determine the component residue concentration using Equation 2

Equation 2:

$$\text{Residue found (ng/g)} = \frac{\text{(analyte found (ng/mL)} \times \text{DF} \times \text{FV (mL))}}{\text{sample weight (g)}}$$

Where DF = dilution factor and FV = final volume

Note: ng/g = ppb

### 5.3 PERCENT RECOVERY

Calculate the percent recovery for samples fortified with known amounts of analytes prior to extraction, from Equation 3.

Equation 3:

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

## 6. SAFETY

There are no unusual hazards associated with this method. The analyst should read the material safety data sheets for all reagents before performing this method. Normal laboratory precautions should be taken.

Centre Protocol No. 00P-023-063

Centre Method No: 00M-023-015

# TABLES

Centre Protocol No. 00P-023-063

Centre Method No: 00M-023-015

Table I: Summary of Recoveries for PFOS in Quail Egg Membrane

| Sample | Fort. Level (ppb) | % Recovery |
|---|---|---|
| 0003798 Matrix Blank A | 0 | NA |
| 0003798 Matrix Blank B | 0 | NA |
| 0003798 Matrix Blank C | 0 | NA |
| 0003798 Spk A | 10 | 88 |
| 0003798 Spk B | 10 | 95 |
| 0003798 Spk C | 10 | 92 |
| 0003798 Spk D | 50 | 92 |
| 0003798 Spk E | 50 | 95 |
| 0003798 Spk F | 50 | 96 |
| 0003798 Spk G | 250 | 93 |
| 0003798 Spk H | 250 | 95 |
| 0003798 Spk I | 250 | 93 |
| | AVERAGE: | 93 |
| | STANDARD DEVIATION: | 2 |
| | RELATIVE STANDARD DEVIATION: | 3 |

Table II: Summary of Recoveries for PFOS in Mallard Egg Membrane

| Sample | Fort. Level (ppb) | % Recovery |
|---|---|---|
| 0003802 Matrix Blank A | 0 | NA |
| 0003802 Matrix Blank B | 0 | NA |
| 0003802 Matrix Blank C | 0 | NA |
| 0003802 Spk A | 10 | 106 |
| 0003802 Spk B | 10 | 122 |
| 0003802 Spk C | 10 | 106 |
| 0003802 Spk D | 50 | 96 |
| 0003802 Spk E | 50 | 96 |
| 0003802 Spk F | 50 | 90 |
| 0003802 Spk G | 250 | 93 |
| 0003802 Spk H | 250 | 92 |
| 0003802 Spk I | 250 | 93 |
| | AVERAGE: | 99 |
| | STANDARD DEVIATION: | 10 |
| | RELATIVE STANDARD DEVIATION: | 10 |

NA = Not Applicable

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Table III: Summary of Recoveries for PFOS in Quail Egg Yolk

| Sample | Fort. Level (ng/g) | % Recovery |
|---|---|---|
| 0003795 Matrix Blank A | 0 | NA |
| 0003795 Matrix Blank B | 0 | NA |
| 0003795 Matrix Blank C | 0 | NA |
| 0003795 Spk A | 10 | 69 |
| 0003795 Spk B | 10 | 68 |
| 0003795 Spk C | 10 | 70 |
| 0003795 Spk D | 50 | 72 |
| 0003795 Spk E | 50 | 80 |
| 0003795 Spk F | 50 | 83 |
| 0003795 Spk G | 250 | 85 |
| 0003795 Spk H | 250 | 89 |
| 0003795 Sok I | 250 | 83 |
| | AVERAGE: | 78 |
| | STANDARD DEVIATION: | 9 |
| | RELATIVE STANDARD DEVIATION: | 11 |

Table IV: Summary of Recoveries for PFOS in Mallard Egg Yolk

| Sample | Fort. Level (ng/g) | % Recovery |
|---|---|---|
| 0003799 Matrix Blank A | 0 | NA |
| 0003799 Matrix Blank B | 0 | NA |
| 0003799 Matrix Blank C | 0 | NA |
| 0003799 Spk A | 10 | 83 |
| 0003799 Spk B | 10 | 82 |
| 0003799 Spk C | 10 | 100 |
| 0003799 Spk D | 50 | 85 |
| 0003799 Spk E | 50 | 88 |
| 0003799 Spk F | 50 | 90 |
| 0003799 Spk G | 250 | 87 |
| 0003799 Spk H | 250 | 85 |
| 0003799 Sok I | 250 | 88 |
| | AVERAGE: | 88 |
| | STANDARD DEVIATION: | 5 |
| | RELATIVE STANDARD DEVIATION: | 6 |

NA = Not Applicable

Table V: Summary of Recoveries for PFOS in Quail Egg Albumen

| Sample | Fort. Level (ppb) | % Recovery |
|---|---|---|
| 0003796 Matrix Blank A | 0 | NA |
| 0003796 Matrix Blank B | 0 | NA |
| 0003796 Matrix Blank C | 0 | NA |
| 0003796 Spk A | 10 | 79 |
| 0003796 Spk B | 10 | 93 |
| 0003796 Spk C | 10 | 88 |
| 0003796 Spk D | 50 | 96 |
| 0003796 Spk E | 50 | 95 |
| 0003796 Spk F | 50 | 90 |
| 0003796 Spk G | 250 | 97 |
| 0003796 Spk H | 250 | 102 |
| 0003796 Spk I | 250 | 102 |
| | AVERAGE: | 94 |
| | STANDARD DEVIATION: | 7 |
| | RELATIVE STANDARD DEVIATION: | 7 |

Table VI: Summary of Recoveries for PFOS in Mallard Egg Albumen

| Sample | Fort. Level (ppb) | % Recovery |
|---|---|---|
| 0003800 Matrix Blank A | 0 | NA |
| 0003800 Matrix Blank B | 0 | NA |
| 0003800 Matrix Blank C | 0 | NA |
| 0003800 Spk A | 10 | 93 |
| 0003800 Spk B | 10 | 85 |
| 0003800 Spk C | 10 | 85 |
| 0003800 Spk D | 50 | 96 |
| 0003800 Spk E | 50 | 100 |
| 0003800 Spk F | 50 | 100 |
| 0003800 Spk G | 250 | 98 |
| 0003800 Spk H | 250 | 102 |
| 0003800 Spk I | 250 | 101 |
| | AVERAGE: | 96 |
| | STANDARD DEVIATION: | 7 |
| | RELATIVE STANDARD DEVIATION: | 7 |

NA = Not Applicable

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Centre Method No: 00M-023-015

# FIGURES

Figure 1: Representative Chromatogram of a Quail Egg Membrane Control

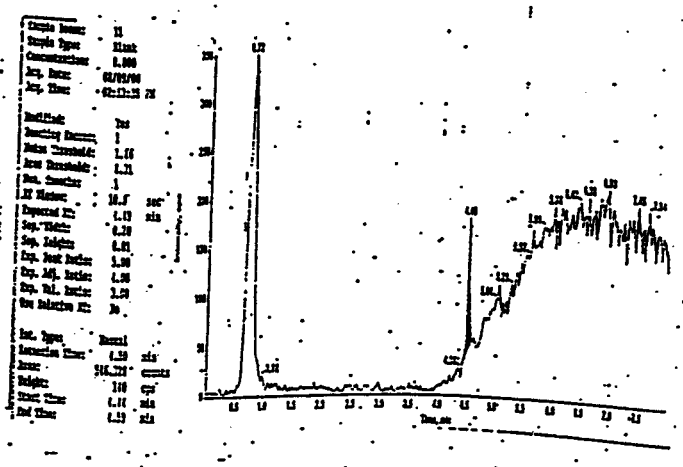

Figure 2: Representative Chromatogram of a Mallard Egg Membrane Control

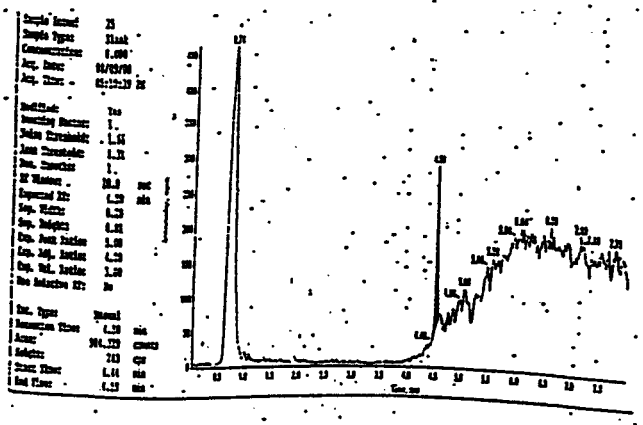

Figure 3: Representative Chromatogram of a Quail Egg Yolk Control

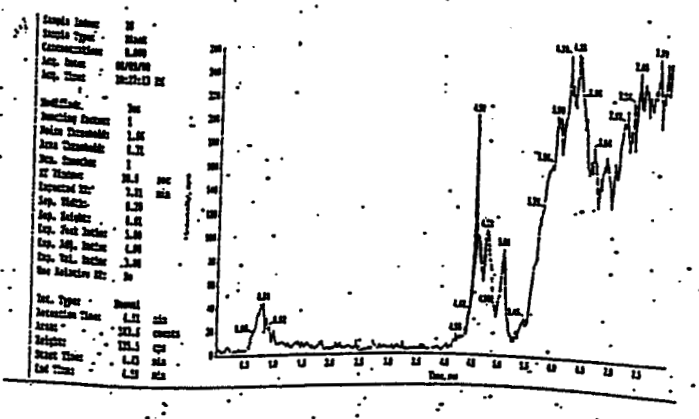

Figure 4: Representative Chromatogram of a Mallard Egg Yolk Control

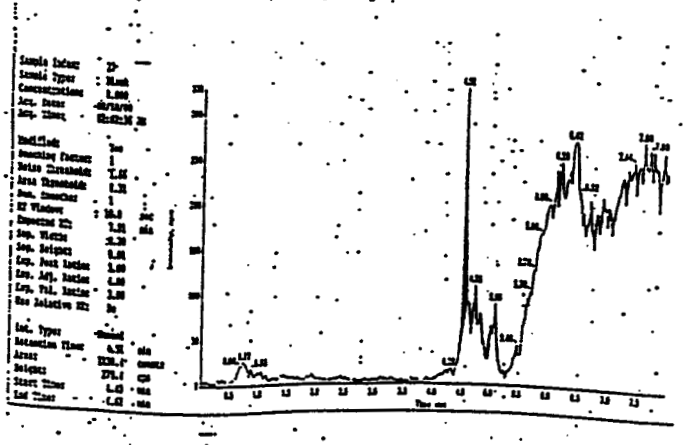

Figure 5: Representative Chromatogram of a Quail Egg Albumen Control

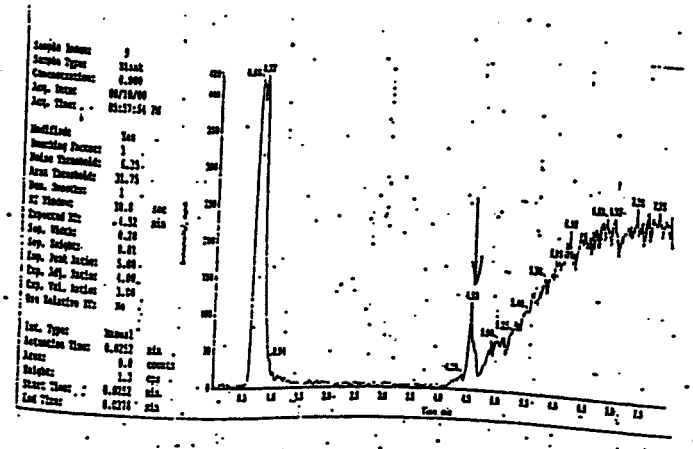

Figure 6: Representative Chromatogram of a Mallard Egg Albumen Control

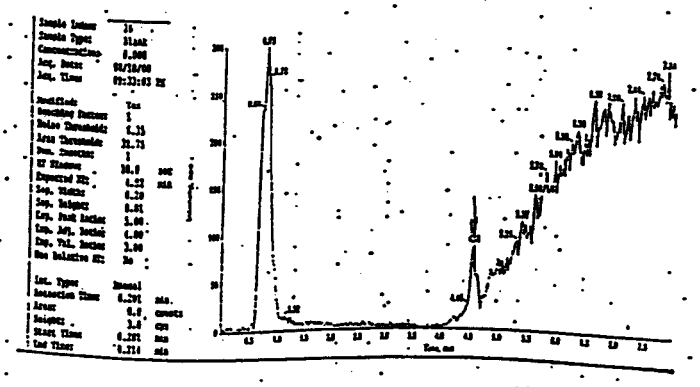

Figure 7: Representative Chromatogram of a Quail Egg Membrane Control Fortified at 10 ng/g (ppb)

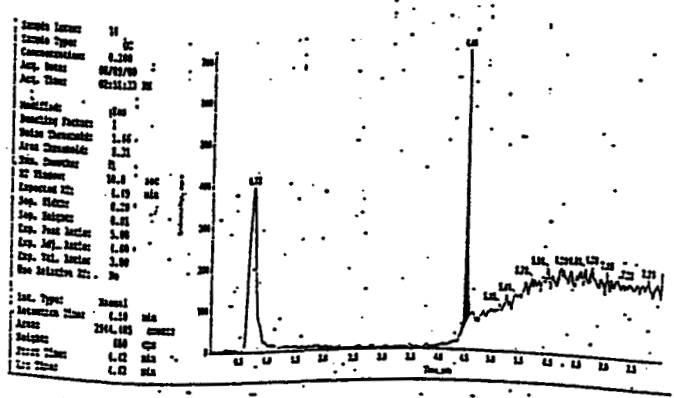

Figure 8: Representative Chromatogram of a Mallard Egg Membrane Control Fortified at 10.0 ng/g (ppb)

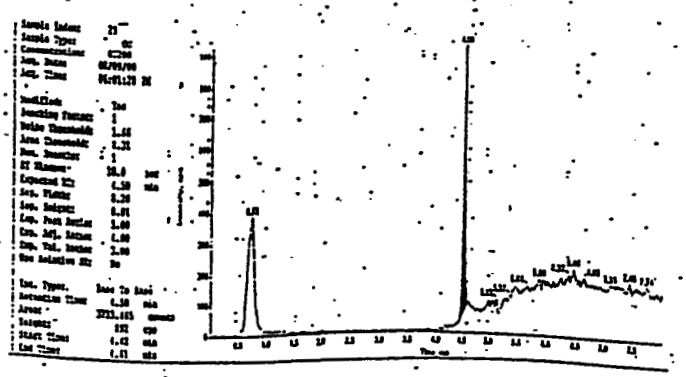

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Figure 9: Representative Chromatogram of a Quail Egg Yolk Control Fortified at 10.0 ng/g (ppb)

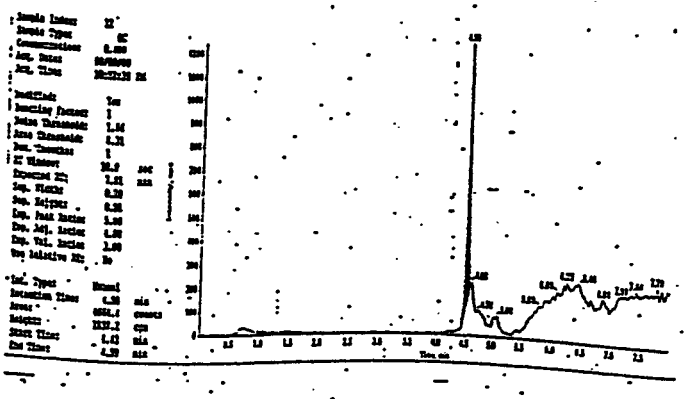


Figure 10: Representative Chromatogram of a Mallard Egg Yolk Control Fortified at 10.0 ng/g (ppb)

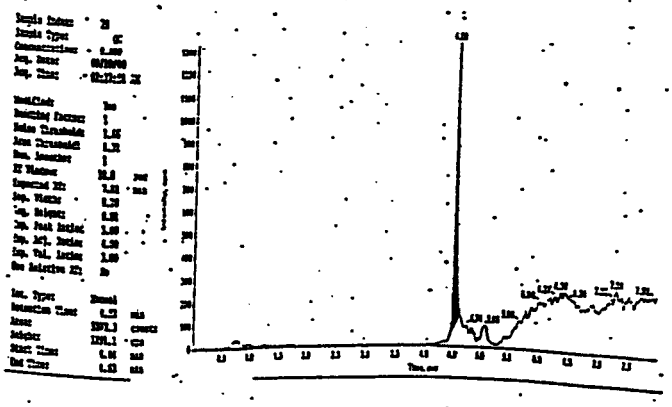


Figure 11: Representative Chromatogram of a Quail Egg Albumen Control Fortified at 10.0 ng/g (ppb)

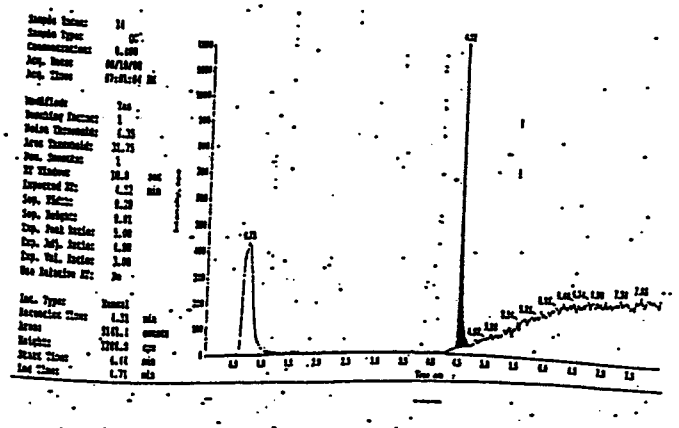
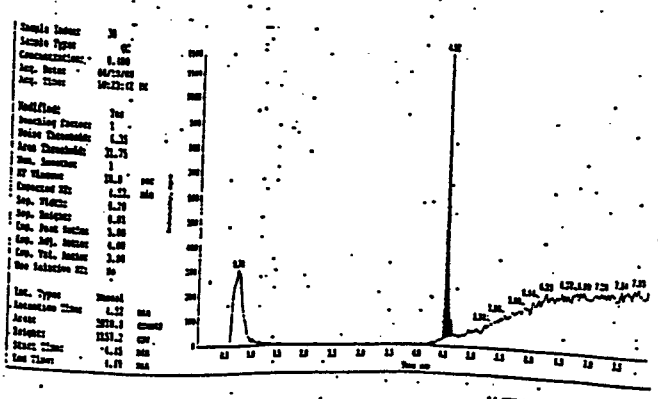


Figure 12: Representative Chromatogram of a Mallard Egg Albumen Control Fortified at 10.0 ng/g (ppb)



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**PROTOCOL AMENDMENT**
Amendment Number: 1
Effective Date: 3/23/01

Centre Study Number: 023-063 Centre Protocol Number: 00P-023-063

DESCRIPTION OF AMENDED SECTION

1. Title
2. § 8 Analytical Method 4.4.4.f.

AMENDED TO

1. Change the analytical phase title to read:
   Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk for Analysis Using HPLC-Electrospray/Mass Spectrometry (This will also change on Page 2 and Page 3)
2. Change the first sentence to read:
   Fortification recoveries within 70-120% are acceptable for fortifications at the LOQ level.

RATIONALE

1. Per Sponsor request to maintain consistency with method.
2. Reflect the requirements that were followed during the method validation.

IMPACT ON THE STUDY

1-2. No negative impact on the study.

Sean P Dalleyfer
Study Director Signature

6/26/01
Date

Sponsor Representative Signature

6/29/01
Date

Centre QAU Review ARM 6/27/01

February 12, 1998/1

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**PROTOCOL AMENDMENT**
Amendment Number: 2
Effective Date: 3/26/01

Centre Study Number: 023-063 Centre Protocol Number: 00P-023-063

DESCRIPTION OF AMENDED SECTION

1. § Section 9: Analytical Method
2. § Section 9: Analytical Method
3. § Section 10: Experimental Design

AMENDED TO

1. Amend Page 21 Section 4.3.2 a. to read:
   Prior to weighing and extracting the egg membrane samples, the membranes will be rinsed with ASTM Type I $H_2O$ and patted dry.
2. On Page 24 Section 4.4.4.d, remove:
   One ASTM Type I water blank
3. Amend Page 6 last paragraph, second sentence to read:
   For egg yolk and albumen, ~ 0.5 g samples and egg membrane ~ 0.1 g samples will be weighed and dried in an oven at ~ 105°C for ~ 12-14 hours.

RATIONALE

1. At the instruction of the study director to ensure that no contamination of egg yolk or albumen is present in the sample.
2. This sample is only to check for PFOS residue present in the ASTM Type I $H_2O$ used.
3. To account for sample availability.

IMPACT ON THE STUDY

1-3. No negative impact on the study.

Study Director Signature 6/26/01 Date

Sponsor Representative Signature 6/29/01 Date

Centre QAU Review 6/27/01

February 12, 1998/1

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# PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 03/27/01, (2) 04/02/01

Centre Study Number: 023-063 Centre Protocol Number: 00P-023-063

DESCRIPTION OF DEVIATION

1. § 8 Analytical Method 4.4.4.f: Quantified Centre sample 0103196 and its duplicate injection when the concentrations were greater than that of the highest standard in Set 032601C.

2. § Amendment #1: Accepted recoveries of 58% and 61% for Centre sample 0003800 Spk A and its duplicate injection in Set 033001C.

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

1-2. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 4/3/01

IMPACT ON THE STUDY

1. No negative impact because the areas were < 5% greater than the highest standard.

2. No negative impact because Spk B gave acceptable recoveries and sample results reported in the set were only for confirmation.

Emily R Decker — Principal Investigator Signature — Date 9/6/01

Leon P. Dalbey — Study Director Signature — Date 6/26/01

Sponsor Management Signature (REPRESENTATIVE) — Date 6/29/01

CEO 9/6/01

CAL QAU Review AKR 6/27/01

February 12, 1998/2