

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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PROJECT

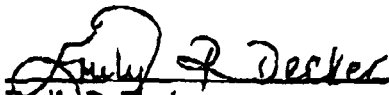
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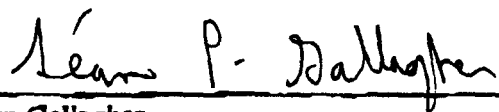
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

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.


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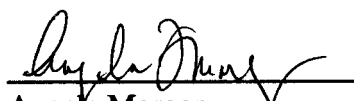

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QUALITY ASSURANCE STATEMENT

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.

<u>Phase</u>	<u>Date Inspected</u>	<u>Date Reported to Centre Management</u>	<u>Date Reported to Study Director and Sponsor Management</u>
1. Protocol Review	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

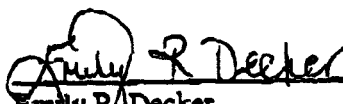
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CERTIFICATION OF AUTHENTICITY

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

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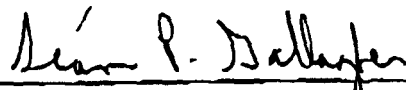
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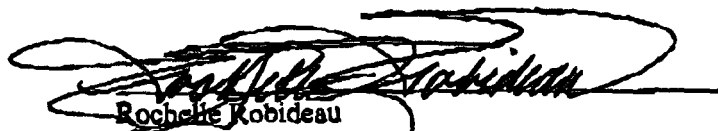
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STUDY IDENTIFICATION

"Extraction of Potassium Perfluorooctanesulfonate from 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	Study Initiation Date:	03/26/01
TIMETABLE:	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:

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Mitra Arjmand	Technician
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Lawrence Ord	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 $\pm$ standard deviations for PFOS in mallard membrane, albumen, and yolk samples were $91\% \pm 6\%$, $91\% \pm 16\%$, and $93\% \pm 13\%$, respectively. The average percent recoveries $\pm$ standard deviations for PFOS in quail membrane, albumen, and yolk samples were $102\% \pm 11\%$, $85\% \pm 5\%$, and $96\% \pm 7\%$, 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, Albumen 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, Albumen 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:

<u>Matrix</u>	<u>Centre ID</u>
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.

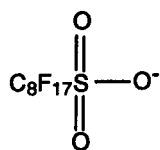
<u>Compound</u>	<u>Centre Control No..</u>	<u>TCR No.</u>	<u>Purity (%)</u>	<u>Expiration Date</u>
PFOS	00-023-042	TCR 00017-46	97.9	08/31/01

The molecular structure of PFOS is given below.

PFOS

Chemical Name = Perfluorooctanesulfonate

Molecular weight = 499 ($\text{C}_8\text{F}_{17}\text{SO}_3^-$)



Note: The neutral molecule and standard from which the PFOS (anion) is obtained, is potassium perfluorooctanesulfonate [$\text{C}_8\text{F}_{17}\text{SO}_3\text{K}$], molecular weight 538.

6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled “Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen 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 $\mu\text{g/mL}$ by dissolving 10 mg of the standard (corrected for purity and salt content) in methanol. From this solution, a 1.0 $\mu\text{g/mL}$ 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 C₈ (Jones Chromatography), 2.1 mm x 50 mm, 4μ

Column Temp.: 35° C

Injection Vol.: 10 μL

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

Mobile Phase (B): Methanol

Flow Rate: 0.3 mL/min.

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

Ions monitored:

<u>Analyte</u>	<u>Mode</u>	<u>Transition Monitored</u>	<u>Approximate Retention Time (min)</u>
PFOS	negative	499 → 99	4.40

Tune File Parameters

<u>Controls</u>	<u>Set</u>
IS-Iospray	-4200.0
DP-Declustering Potential	-51.0
FP-Focusing Potential	-230.0
EP-Entrance Potential	10.0
CE-Collision Energy	-70
CXP-Collision Cell Exit Potential	-5

DF-Deflector	300.0
CEM-Channel Electron Multiplier	2800.0
<u>Gas Flows</u>	<u>Set</u>
Nebulizer Gas	12
Curtain Gas	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:

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

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:

Recovery (%) =

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

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 quantifiable 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:

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

$$= 90\%$$

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 H₂O 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 $\pm$ standard deviations for PFOS in mallard membrane, albumen, and yolk samples were $91\% \pm 6\%$, $91\% \pm 16\%$, and $93\% \pm 13\%$, respectively. Individual recoveries for PFOS in the quail membrane, albumen, and yolk samples are detailed in **Table XXIX-XXXI**. The average percent recoveries $\pm$ standard deviations for PFOS in quail membrane, albumen, and yolk samples were $102\% \pm 11\%$, $85\% \pm 5\%$, and $96\% \pm 7\%$, 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.
 Centre Analytical Laboratories, Inc.

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

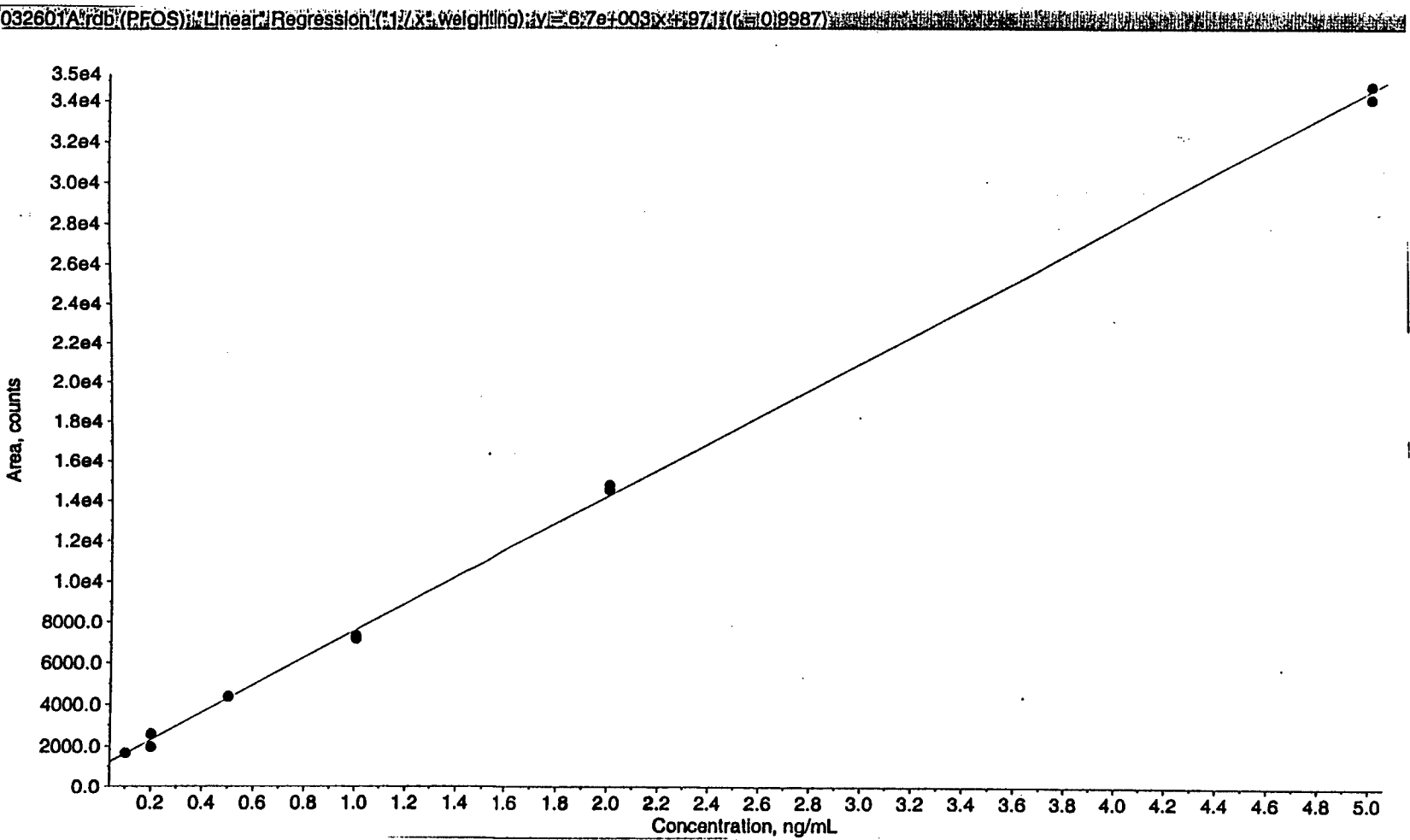


Figure 2. Chromatogram Representing a 0.1 ng/mL standard for PFOS

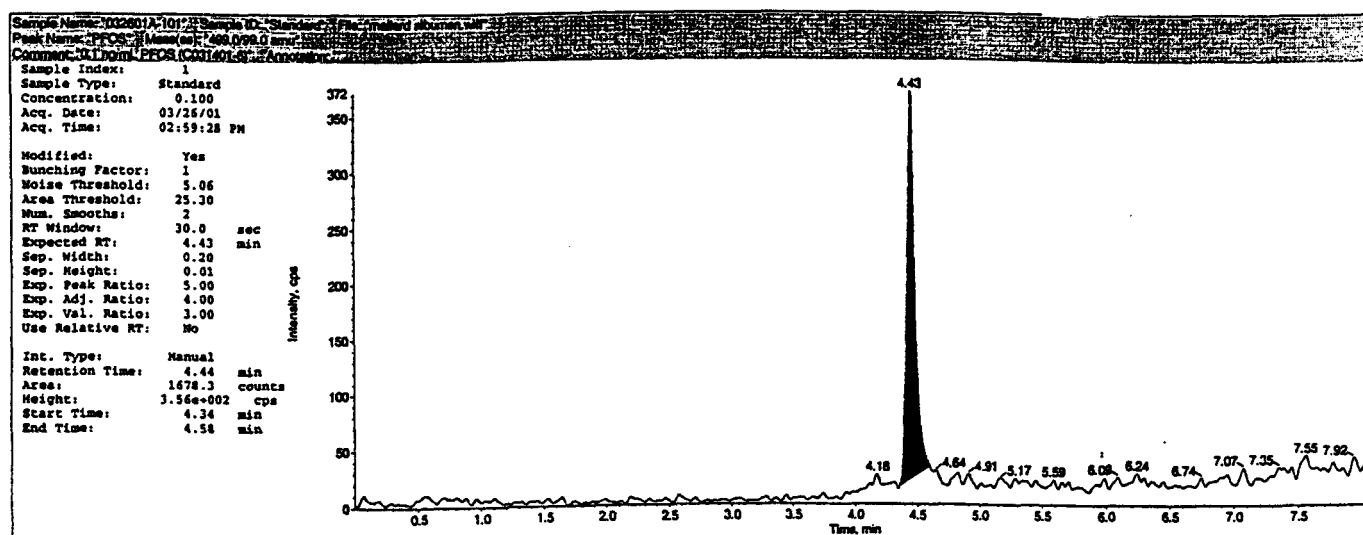
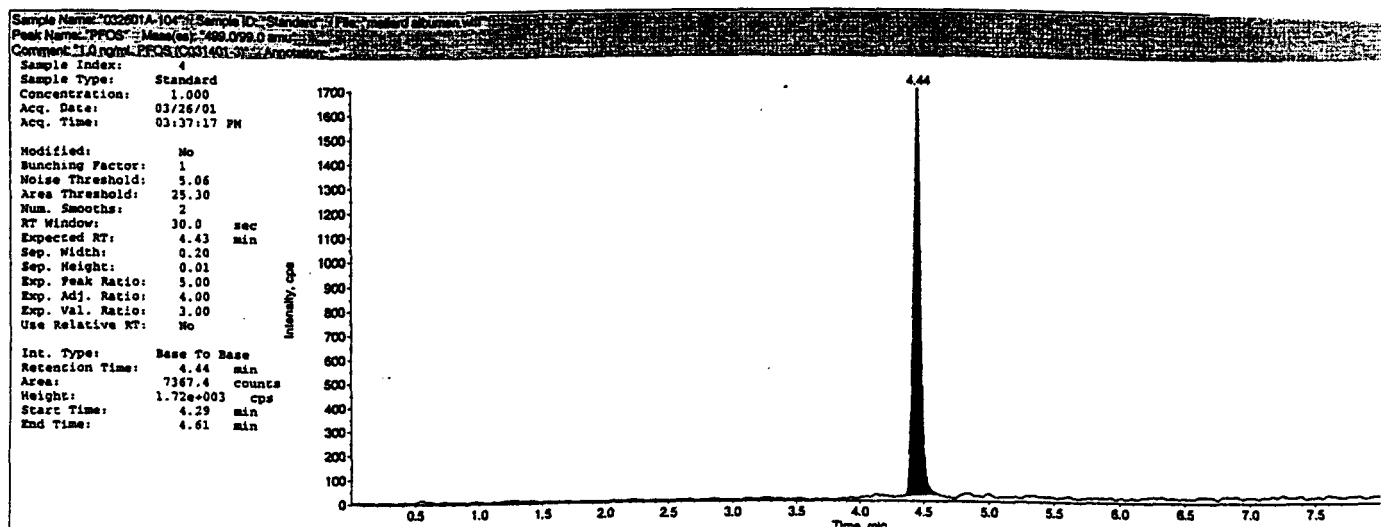


Figure 3. Chromatogram Representing a 1.0 ng/mL standard for PFOS



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

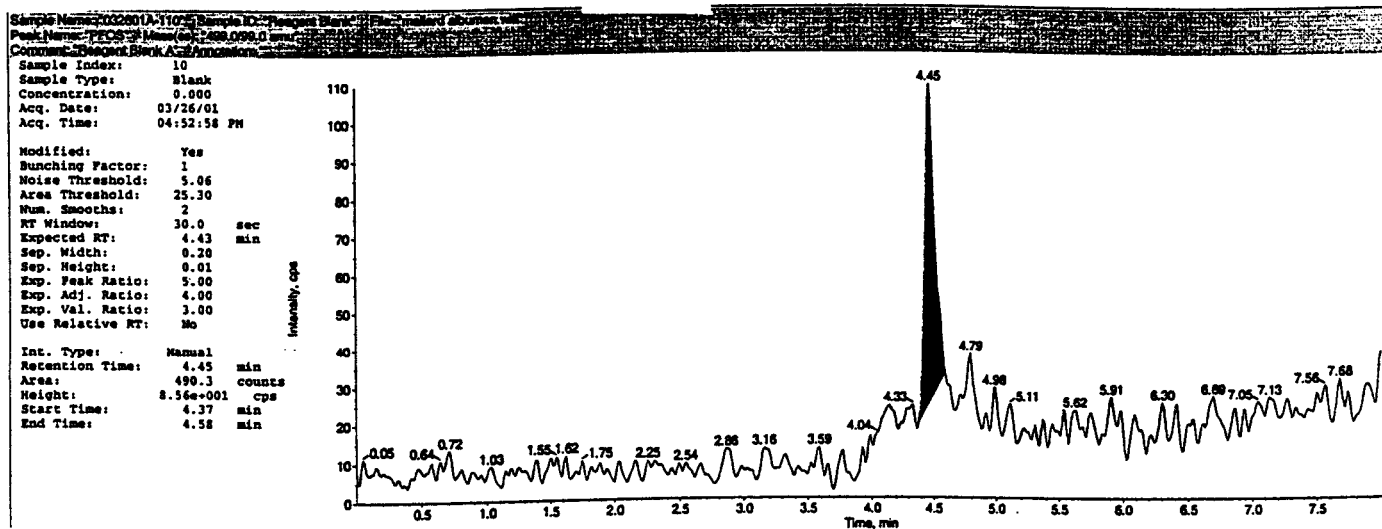


Figure 5. Chromatogram Representing Control Mallard Membrane for PFOS (Centre ID: 0003802 Blank A, Set: 032601C)

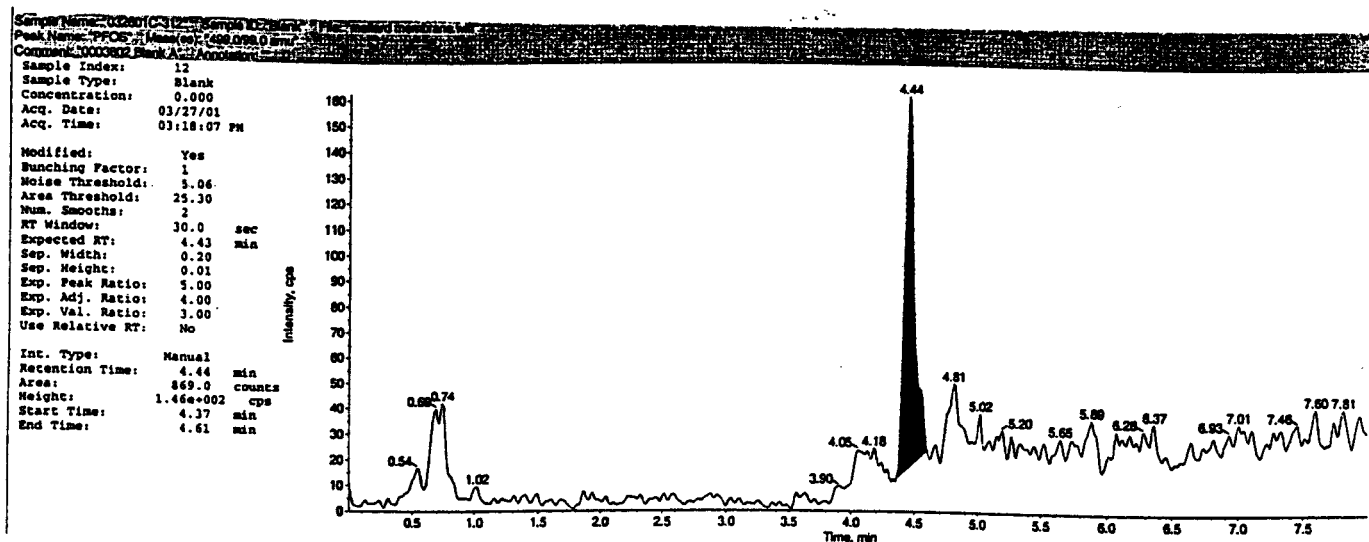
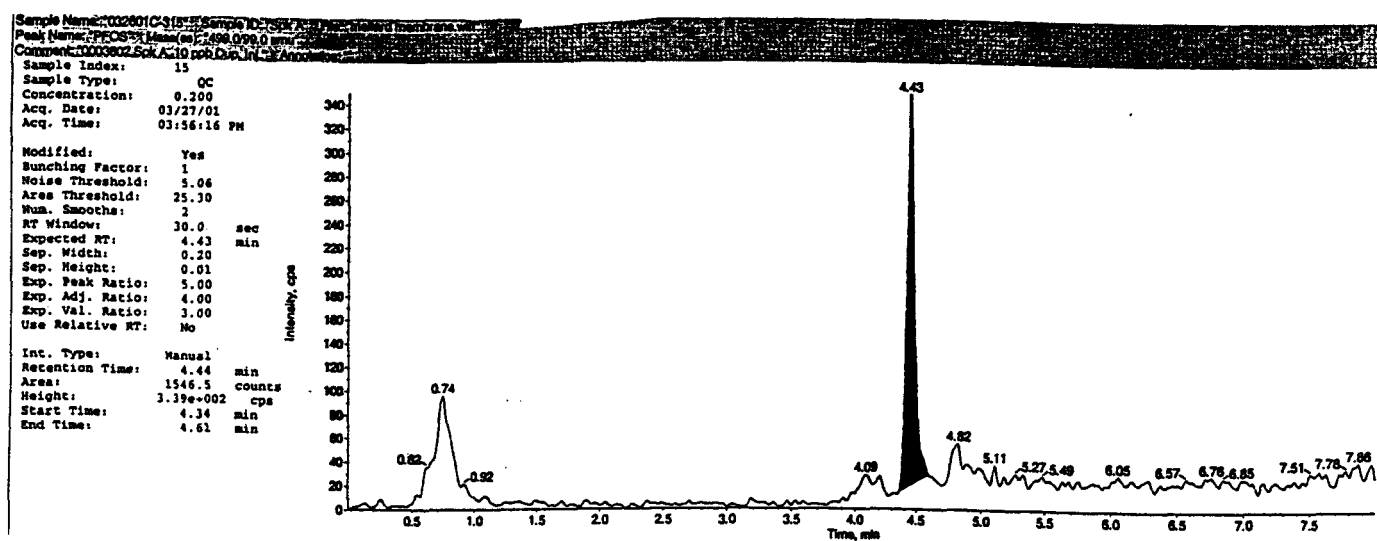
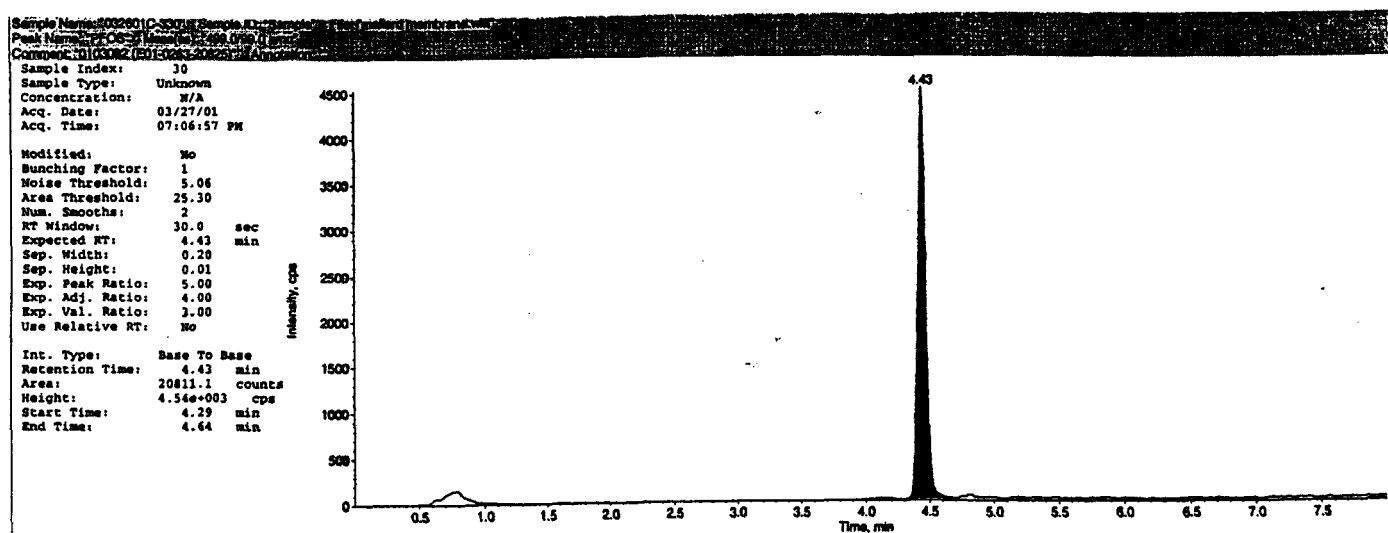


Figure 6. Chromatogram Representing Control Mallard Membrane Fortified with 10 ppb of PFOS, (Centre ID: 0003802 Spk A Dup. Inj., Set: 032601C)



**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

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

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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.

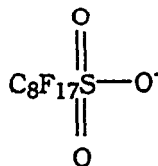
PFOS

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-heptafluoro-, 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.

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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 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 $< -10^{\circ}\text{C}$, except when removed for extraction and analysis as described in the method. The samples will be kept isolated from the test substance during storage.

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

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. There 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., $\mu\text{g/ml}$, 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

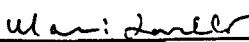
- 1 U.S. Environmental Protection Agency. Toxic Substances Control Act (TSCA). Good Laboratory Practice Standards. Final Rule, 40 CFR Part 792.

Centre Protocol No. 00P-023-063

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

3/28/01
Date

Principal Investigator, Centre



Emily Stauffer
Scientist

3/28/01
Date

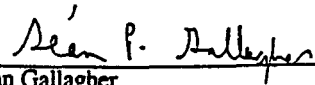
Facility Management, Centre



John Flaherty
Operations Manager

3/28/01
Date

Study Director, Wildlife International, Ltd.



Sean Gallagher
Senior Biologist

3/26/01
Date

Sponsor, 3M



Rochelle Robideau
Environmental Technologist

3/22/01
Date

Centre Protocol No. 00P-023-063

APPENDIX: ANALYTICAL METHOD

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

Centre Protocol No. OOP-023-063

TITLE

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

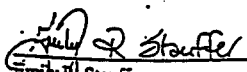
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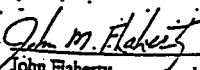
Centre Protocol No. 00P-023-063

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
Principal Investigator
Centre Analytical Laboratories, Inc.
3/16/01
Date


John Flaherty
Laboratory Manager
Centre Analytical Laboratories, Inc.
3/16/01
Date


Richard A. Grazzini, Ph.D.
President
Centre Analytical Laboratories, Inc.
3-16-01
Date


Roselle Robideau
Sponsor Representative
3M Environmental Laboratory
3/16/01
Date

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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.

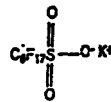
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2. EXPERIMENTAL COMPOUNDS

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-heptafluoro-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 533).

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

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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

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

b. 0.1 $\mu\text{g/mL}$ Fortification Solution - Pipette 10.0 mL of the 1.0 $\mu\text{g/mL}$ fortification solution into a 100-mL volumetric flask and bring up to volume with methanol.

c. 0.01 $\mu\text{g/mL}$ Fortification Solution - Pipette 10.0 mL of the 0.1 $\mu\text{g/mL}$ 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 $\mu\text{g/mL}$ and 0.01 $\mu\text{g/mL}$ fortification solutions.

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

Initial Conc. ($\mu\text{g/mL}$)	Volume (mL)	Diluted to (mL)	Final Conc. ($\mu\text{g/mL}$)
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

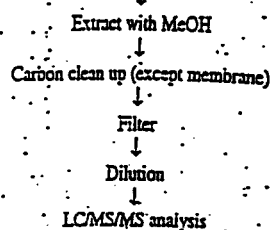
Store all calibration standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

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 (0.1 g for membrane), Fortify if needed.



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.

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Centre Method No: 00M-023-015

4.3.1 Egg Membrane

- Allow sample to thaw.
- Weigh 0.1 g (± 0.005 g) 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.
- 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.
- Make a 5X dilution by transferring 0.1 mL of filtrate to HPLC vials and adding 0.4 mL of methanol using disposable micropipettes (100-200 μ L).
- 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

- Allow sample to thaw.
- Weigh 1.0 g (± 0.05 g) 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.
- Add 25 mL of MeOH, replace lid tightly, and shake on a wrist-action shaker for 15 minutes. Centrifuge tubes at $\sim 2,000$ rpm for ~ 10 minutes.
- 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.
- 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.

Centre Protocol No. 00P-023-063

Centre Method No: 00M-023-015

4.4. ANALYSIS BY LC/MS/MS

4.4.1: LC/MS/MS System and Operating Conditions (Turbolonspray)

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

HPLC Column: Genesis C₈ (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 C₁₈ 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.

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

<u>Controls</u>	<u>Set</u>
IS-Ispray	-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-Quad 1 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

<u>Gas Flows</u>	<u>Set</u>
Nebulizer Gas	.12
Curtain Gas	.13
Collision Gas	4
TIS Temperature	350°C

4.4.3. Calibration Procedures

- Inject the same volume (between 10 to 20 µL) of each calibration standard (prepared in MeOH) into the LC/MS/MS.
- Use linear, 1/x weighted standard curves for quantification. Linear standard curves are generated for each set by linear regression using the

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appropriate software system. Any calibration standards falling outside $\pm 30\%$, 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 ($r^2 \geq 0.985$). If calibration results fall outside these limits, then appropriate steps should be taken to adjust instrument operation, and the relevant set of samples must be reanalyzed.

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.

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- 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.
- k. 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.

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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:

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

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:

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

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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.

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TABLES

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

Sample	Fort. Level (pob)	% 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 (pob)	% 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	62
0003795 Spk B	10	62
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 Spk I	250	88
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 Spk I	250	88
AVERAGE:		88
STANDARD DEVIATION:		5
RELATIVE STANDARD DEVIATION:		6

NA = Not Applicable

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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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FIGURES

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[illegible][illegible]

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Figure 3: Representative Chromatogram of a Quail Egg Yolk Control

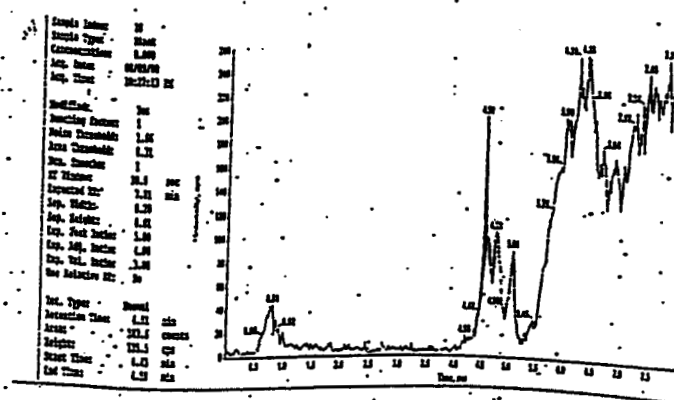
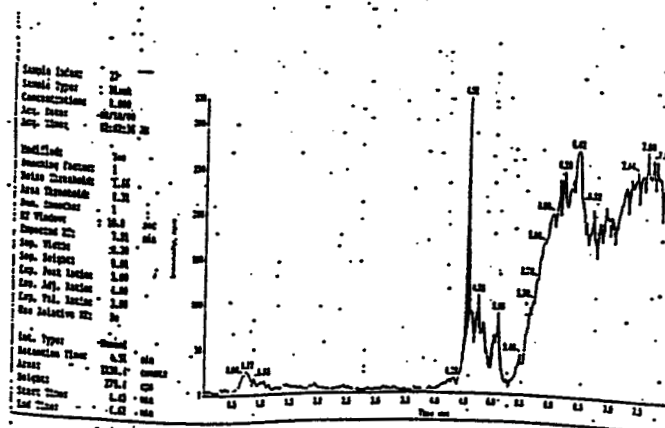


Figure 4: Representative Chromatogram of a Mallard Egg Yolk Control



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Figure 5: Representative Chromatogram of a Quail Egg Albumen Control

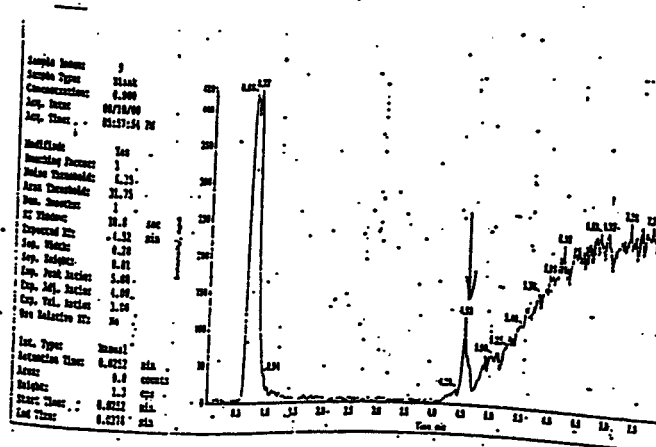
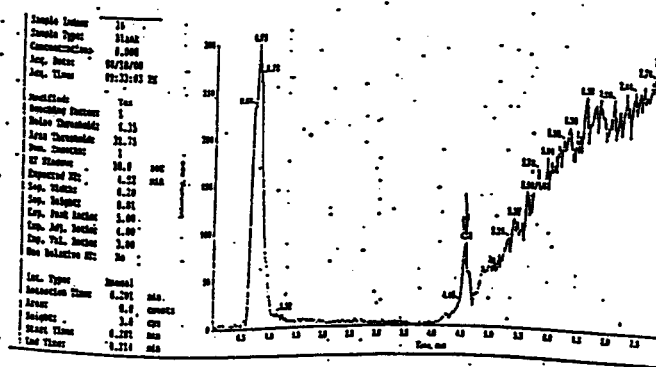


Figure 6: Representative Chromatogram of a Mallard Egg Albumen Control



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

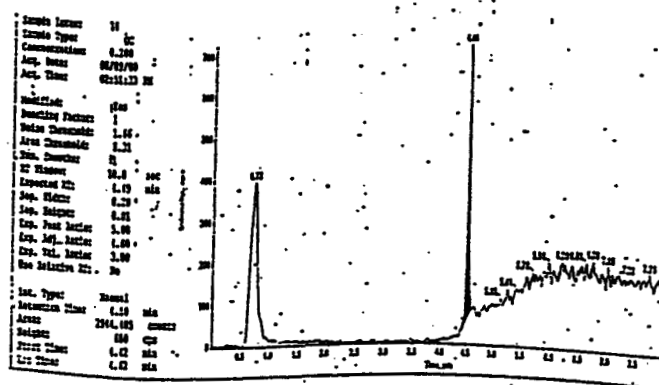
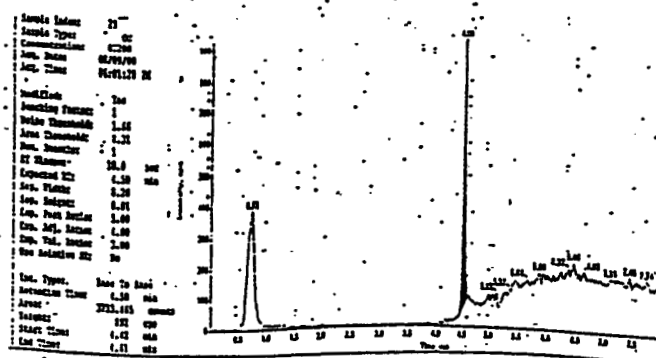


Figure 8: Representative Chromatogram of a Mallard Egg Membrane Control Fortified at 10.0 ng/g (ppb)



[illegible][illegible]

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

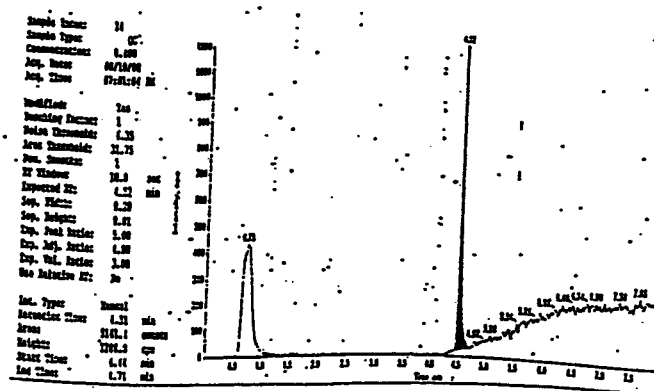
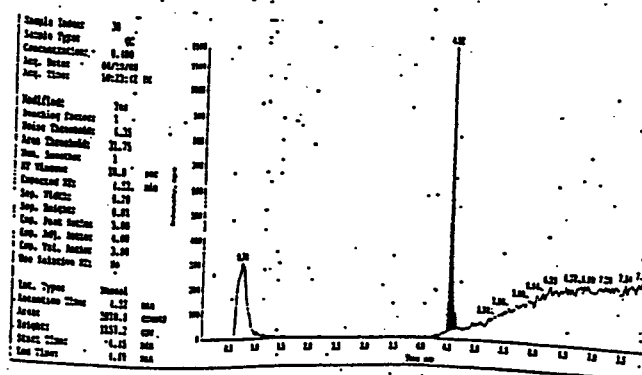


Figure 12: Representative Chromatogram of a Mallard Egg Albumen Control Fortified at 10.0 ng/g (ppb)





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PROTOCOL AMENDMENT

Amendment Number: 1Effective Date: 3/23/01Centre Study Number: 023-063Centre Protocol Number: 00P-023-063

DESCRIPTION OF AMENDED SECTION

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

AMENDED TO

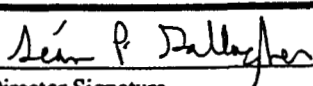
1. Change the analytical phase title to read:
Extraction of Potassium Perfluorooctanesulfonate from Egg Membrane, Albumen, and Yolk for Analysis Using HPLC-Electrospray/Mass Spectrometry (This will also change on Page 2 and Page 3)
2. Change the first sentence to read:
Fortification recoveries within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

RATIONALE

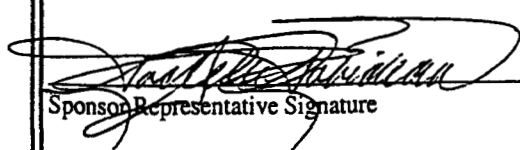
1. Per Sponsor request to maintain consistency with method.
2. Reflect the requirements that were followed during the method validation.

IMPACT ON THE STUDY

- 1-2. No negative impact on the study.


Study Director Signature

6/26/01
Date


Sponsor Representative Signature

4/29/01
Date
Centre QAU Review ARM 6/27/01

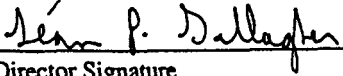
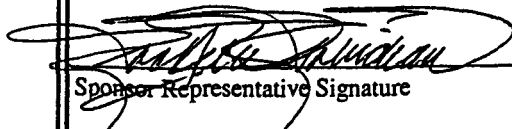
February 12, 1998/1



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PROTOCOL AMENDMENT Amendment Number: <u>2</u> Effective Date: <u>3/26/01</u>		Page 1 of 1
Centre Study Number: <u>023-063</u> Centre Protocol Number: <u>00P-023-063</u>		
<u>DESCRIPTION OF AMENDED SECTION</u>		
1. § Section 9: Analytical Method 2. § Section 9: Analytical Method 3. § Section 10: Experimental Design		
<u>AMENDED TO</u>		
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 ₂ O 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.		
<u>RATIONALE</u>		
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 ₂ O used. 3. To account for sample availability.		
<u>IMPACT ON THE STUDY</u>		
1-3. No negative impact on the study.		
 Study Director Signature	<u>6/26/01</u> Date	
 Sponsor Representative Signature	<u>6/29/01</u> Date	
Centre QAU Review <u>QAU 6/27/01</u>		February 12, 1998/1



**Centre Analytical
Laboratories, Inc.**

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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. Stuffer 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 9/6/01
Principal Investigator Signature Date

Sean P. Gallagher 6/26/01
Study Director Signature Date

[Signature] 6/29/01
Sponsor/Management Signature Date

REPRESENTATIVE
CLO 9/6/01

CAL QAU Review AKM 6/27/01

February 12, 1998/2