AR226-1858

Exygen™ RESEARCH
Precise Research.
Proven Results.

Analytical Report

3M Environmental Technology and Safety Services

Analysis of Perfluorooctanesulfonate in Mallard and Quail Egg Yolk

Exygen Report No. 023-070

Testing Laboratory

Exygen Research
3058 Research Drive
State College, PA 16801

Requester

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001393

1 Introduction

Results are reported for the analysis of total perfluorooctanesulfonate (PFOS) in mallard and quail egg yolk samples. Also reported is the PFOS contained in each of the three fractions of egg yolk: very low density lipoprotein (VLDL), phosvitin, and lipovitellin. This study was conducted in accordance with the analytical phase protocol given in Attachment E.

2 Sample Receipt

The samples were submitted in plastic bags, labeled with magic marker and typed print. A copy of all sample log-in information is presented in Attachment A

Twelve mallard egg yolk samples and twelve quail egg yolk samples were received on 06/01/02. The samples were shipped frozen on dry ice via Federal Express (FedEx). The twelve mallard yolk samples were combined and homogenized into one sample. The same was done for the twelve quail yolk samples. The samples were stored frozen from time of receipt until analysis. Four mallard albumen and shell membrane samples and four quail albumen and shell membrane samples were also received on 06/01/02. These samples were not analyzed as part of this study.

3 Methods - Analytical and Preparatory

3.1 Sample Preparation

3.1.1 Total PFOS Content

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.

3.1.2 PFOS Content in Yolk Fractions

A 5.0 g sample was used for the fractionation. Ten milliliters of a solution containing 0.67 M $MgSO_4$, 1 mM phenylmethanesulfonylfluoride (PMSF), and 2 µM leupeptin were added to the sample. The sample was shaken for ~ 2 minutes and then the suspension was transferred to a centrifuge tube. The sample was centrifuged at 200,000 x *g* at 4°C for 24 hours. The sample was then separated into 2 segments: the very low density lipoprotein (VLDL) and the high density fraction (HDF).

The VLDL was dissolved in a solution containing 20 mM Tris-HCl, 150 mM NaCl, 0.2 mM EDTA, 1 mM PMSF, and 5 µM leupeptin. The HDF was resuspended in a solution containing 0.45 M $MgSO_4$, 1 mM PMSF, and 2 µM leupeptin. Both VLDL and HDF fractions were centrifuged at 200,000 x *g* at 4°C for 16 hours. The VLDL was then dissolved in a solution containing 20 mM Tris-HCl, 150 mM NaCl, 0.2 mM EDTA, 1 mM PMSF, and 5 µM leupeptin and stored at 4°C. The HDF was dissolved in a solution containing 0.45 M $MgSO_4$, 1 mM PMSF, and 2 µM

leupeptin and then two volumes of cold water was added dropwise and then stored overnight at 4°C.

The HDF supernatant (lipovitellin) was decanted and diluted with cold water and stored overnight at 4°C. The HDF precipitate (phosvitin) was dissolved in a solution containing 0.4 M $MgSO_4$, 1 mM PMSF, and 2 µM leupeptin and then diluted with cold water and stored overnight at 4°C. The lipovitellin and phosvitin were then recovered by centrifugation at 106,000 x *g* at 4°C for 1 hour and dissolved in a solution containing 1 M NaCl, 5 mM Tris-HCl, 1 mM PMSF, and 2 µM leupeptin.

All of the resulting precipitates (~1.0 g) from each fraction were 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

3.2 Sample Analysis by LC/MS/MS

In High Pressure Liquid Chromatography (HPLC), an aliquot of extract is injected and passed through a liquid-phase chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. Following HPLC separation, mass spectrometry provides a rapid and accurate means for analyzing a wide range of organic compounds. Molecules are ionized, fragmented, and detected. The ions characteristic of the compound is observed and quantitated against standards. Each sample was analyzed in duplicate.

An HP1000 system interfaced to a Micromass Quattro Ultima system was used to analyze the sample extracts. A gradient elution through a Jones Chromatography Genesis C-8 50 x 2.1 mm x 4µm column was used for separation.

The following gradient was performed:

Mobile Phase (A): 2mM Ammonium Acetate in Type I Water
Mobile Phase (B): Methanol

Time	%A	%B
0.0	60	40
0.4	60	40
1.0	10	90
7.0	10	90
7.5	0	100
9.0	0	100
9.5	60	40
14.0	60	40

The following parameters were used for operation of the mass spectrometer:

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	499->99

Gas Temperature	350°C
Drying Gas (N2)	7.0 L/min

4 Analysis

4.1 Calibration

A 6-point calibration curve was analyzed at the beginning, throughout, and at the end of the analytical sequence for PFOS. The calibration points were prepared for PFOS at 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ng/mL. The instrument response versus the concentration was plotted for each point. Using linear regression with 1/x weighting, the slope, y-intercept and coefficient of determination (r^2) were determined. A calibration curve is acceptable if $r^2 \geq 0.990$.

For the results reported here, calibration criteria were met. The calibration curves are included in the raw data in Attachment C.

4.2 Surrogates

Surrogate spikes are not a component of the analytical method.

4.3 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared by adding a known concentration of the analytes to control egg yolk samples. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 70-130% or the data is rejected. For the results reported here, the spikes were within the acceptable range.

4.4 Matrix Spikes

Matrix spikes were not included with this study.

4.5 Sample Related Comments

Duplicate injections were performed for all sample analyses.

5 Data Summary

Please see Attachment B for a detailed listing of the analytical results. Results are reported in parts per billion (ng/g) for PFOS.

6 Data/Sample Retention

Samples are disposed of one month after the report is issued unless otherwise specified. All electronic data is archived on retrievable media and hard copy reports are stored in data folders maintained by Exygen. Hardcopy data is stored for a minimum of five years. 3M Environmental will be notified 30 days prior to the disposal of hardcopy data.

7 Attachments

7.1 Attachment A: Chain of Custody

7.2 Attachment B: Analytical Results

7.3 Attachment C: Raw Analytical Data

7.4 Attachment D: Study Personnel

7.5 Attachment E: Analytical Phase Protocol

8 Signatures

Emily R. Decker, Principal Investigator — Date: 7/5/02

John M. Flaherty, Vice President — Date: 7/5/02

Exygen Research

Sample Login Report

Study Number: **023-070**
Protocol: **NA**

Exygen ID	Client Sample ID	Received	Matrix	Submitted By	Logged In By/Date	Initial Loc.	Condition of Samples	Comments
0201613	(Composite)	See Original Login	EGG YOLK	NA	RICK K 6-4-02	FREEZER 8	See Original Login	Composite of samples: 201551-201562
0201614	(Composite)	See Original Login	EGG YOLK	NA	RICK K 6-4-02	FREEZER 8	See Original Login	Composite of samples: 201563-201574

Verified By/Date: RK 6-4-02

Printed 6/4/2002

Exygen Research

Sample Login Report

Study Number: **023-070**
Protocol: **NA**

Exygen ID	Client Sample ID	Received	Matrix	Submitted By	Logged In By/Date	Initial Loc.	Condition of Samples	Comments
0201551	E01-1379-31882	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201552	E01-1379-31883	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201553	E01-1379-31884	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201554	E01-1379-31885	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201555	E01-1379-31895	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201556	E01-1379-31896	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201557	E01-1379-31897	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201558	E01-1379-31898	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201559	E01-1379-31939	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201560	E01-1379-31943	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201561	E01-1379-31947	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201562	E01-1379-31951	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201563	E01-1378-31638	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201564	E01-1378-31639	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201565	E01-1378-31640	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201566	E01-1378-31641	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201567	E01-1378-31654	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201568	E01-1378-31655	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.

Verified By/Date: 6-3-02

Printed 6/3/2002

Exygen Research

Sample Login Report

Study Number: **023-070**
Protocol: **NA**

Exygen ID	Client Sample ID	Received	Matrix	Submitted By	Logged In By/Date	Initial Loc.	Condition of Samples	Comments
0201569	E01-1378-31656	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201570	E01-1378-31657	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201571	E01-1378-31694	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201572	E01-1378-31698	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201573	E01-1378-31702	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201574	E01-1378-31706	6-1-02 21:15	EGG YOLK	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201575	E01-1379-31940	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201576	E01-1379-31944	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201577	E01-1379-31948	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201578	E01-1379-31952	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201579	E01-1378-31695	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201580	E01-1378-31699	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201581	E01-1378-31703	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201582	E01-1378-31707	6-1-02 21:15	EGG ALBUMEN	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201583	E01-1379-31942	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201584	E01-1379-31946	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201585	E01-1379-31950	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201586	E01-1379-31954	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.

Verified By/Date: 6-3-02

Exygen Research

Sample Login Report

Study Number: **023-070**
Protocol: **NA**

Exygen ID	Client Sample ID	Received	Matrix	Submitted By	Logged In By/Date	Initial Loc.	Condition of Samples	Comments
0201587	E01-1378-31697	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201588	E01-1378-31701	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201589	E01-1378-31705	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.
0201590	E01-1378-31709	6-1-02 21:15	EGG SHELL MEMBRANE	NA	LAWRENCE O 6-3-02	FREEZER 8	F-DRY ICE-A	Stored in FREEZER 8 until Log-In.

Verified By/Date: 6-3-02

3M ENVIRONMENTAL LABORATORY
CONTRACT LABORATORY WORK ORDER BY SAMPLE

Project: E01-1379

Contract Lab(s): EXYGEN

Requester: Robideau, Rochelle R (0002-03E-09)
Department: 502180 Site Source:
Project Number:
Date Received: 10/4/2001
Project Description: N. Bobwhite Reproduction (Eggs)

Completion Date:
Project Lead: Rochelle R. Robideau
Phone Number: 651-778-7065
Email Address: rrrobideau@mmm.com

Ship Date: 5/31/02

Comments:

NOTE: MISC SERV = Protein separation to be conducted by Exygen Research.

3M Sample	Sampled Date	Sample Description	
E01-1379-31882	6/13/2001	10 ppm a.i. 230 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31883	6/13/2001	10 ppm a.i. 232 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31884	6/13/2001	10 ppm a.i. 234 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31885	6/13/2001	10 ppm a.i. 236 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31895	6/13/2001	10 ppm a.i. 229 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31896	6/13/2001	10 ppm a.i. 231 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31897	6/13/2001	10 ppm a.i. 233 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31898	6/13/2001	10 ppm a.i. 235 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31939	6/21/2001	10 ppm a.i. 226 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002

3M ENVIRONMENTAL LABORATORY
CONTRACT LABORATORY WORK ORDER BY SAMPLE

Project: E01-1379 (cont.) Contract Lab(s): EXYGEN

3M Sample	Sampled Date	Sample Description	
E01-1379-31940	6/21/2001	10 ppm a.i. 226 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31942	6/21/2001	10 ppm a.i. 226 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31943	6/21/2001	10 ppm a.i. 230 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31944	6/21/2001	10 ppm a.i. 230 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31946	6/21/2001	10 ppm a.i. 230 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1379-31947	6/21/2001	10 ppm a.i. 232 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1379-31948	6/21/2001	10 ppm a.i. 232 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1379-31950	6/21/2001	10 ppm a.i. 232 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1379-31951	6/21/2001	10 ppm a.i. 234 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1379-31952	6/21/2001	10 ppm a.i. 234 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1379-31954	6/21/2001	10 ppm a.i. 234 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002

3M Environmental Laboratory

Form 38778 - PWO

Shipping Address:
3M Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Telephone:
Sample Receiving: (651) 778-4948
Alternate: (651) 778-6763
FAX: (651) 778-6176

Chain of Custody /Request for Laboratory Analytical

5808

Project ID/Project Name	N. Bobwhite Reproduction (Eggs)
Template #	Final Report Due Date
Project Lead	Rochelle R. Robideau — Internal Due Date
Dept. # (main)	502180 — Class/Job/Project #

3M Env. Lab Project # For Internal Use Only

E01-1379

Report Results to:

Contact Name	Robideau, Rochelle R
Company	3M
Mailing Address	0002-03E-09
City, State, Zip	
Telephone #	1 651 778-7065 FAX # 1 (651) 778-6176

Date Available	6/1
Date Due	6/30 / CONTACT ROCHELLE
Contract Lab	EXYGEN

Special Instructions and/or Specific Regulatory Requirements:
(method, limit of detection, reporting units, etc.)

Preservatives: EXYGEN

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/ Media	HNO_3	H_2SO_4	VOCs	None	Other FROZEN	Total Number of Containers	Analysis Requested: Complete below. Attach any associated information. PROTEIN SDS.
						Enter the number of containers of each						(Enter an 'X' in the box below to indicate request)
1.	10 ppm a.i. 230 H Yolk	31882	6/13/01							1	1	X
2.	10 ppm a.i. 232 H Yolk	31883	6/13/01							1	1	X
3.	10 ppm a.i. 234 H Yolk	31884	6/13/01							1	1	X
4.	10 ppm a.i. 236 H Yolk	31885	6/13/01							1	1	X
5.	10 ppm a.i. 229 I Yolk	31895	6/13/01							1	1	X
6.	10 ppm a.i. 231 I Yolk	31896	6/13/01							1	1	X
7.	10 ppm a.i. 233 I Yolk	31897	6/13/01							1	1	X
8.	10 ppm a.i. 235 I Yolk	31898	6/13/01							1	1	X
9.	10 ppm a.i. 226 J Yolk	31939	6/21/01							1	1	X
10.	10 ppm a.i. 226 J Albumen	31940	6/21/01							1	1	X
11.	10 ppm a.i. 226 J Shell Membrane	31942	6/21/01							1	1	X
12.	10 ppm a.i. 230 J Yolk	31943	6/21/01							1	1	X

Collected by (print):

Collector's signature:

Chain of Custody

Item #	Relinquished by/Affiliation	Time	Date	Shipped Via:	Received by/Affiliation	Time	Date
* 1-12	[signature] /3M	14:30	5/31/02	FED-EX	[signature] 6-3-02	2115	6-1-02

Sample Condition Upon Receipt: O Acceptable O Other:

Temperature: 'C O Received on Ice

Other Associated CoCs: ORIGINAL WILDLIFE INTERNATIONAL COC'S

Copies to: RRR

Comments: * TO EXYGEN RESEARCH

Page 1 Of 2

Original - Accompanying Samples

Last Page - Originator

See Reverse Side for Instructions

3M Environmental Laboratory

Form 38778 - PWO

Shipping Address:
3M Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Telephone:
Sample Receiving: (651) 778-4948
Alternate: (651) 778-6753
FAX: (651) 778-6176

Chain of Custody /Request for Laboratory Analytical

5809

3M Env. Lab Project # For Internal Use Only: E01-1379

Project ID/Project Name	~~N. Bobwhite Reproduction (Eggs)~~		
Template #		Final Report Due Date	
Project Lead	~~Rochelle R. Robideau~~	Internal Due Date	
Dept. # (main)	~~502180~~	Class/Job/Project #	

Report Results to:

Contact Name	Robideau, Rochelle R	Date Available	6/1
Company	3M	Date Due	6/30 / CONTACT ROCHELLE
Mailing Address	0002-03E-09	Contract Lab	EXYGEN
City, State, Zip			
Telephone #	1 651 778-7065	FAX #	1 (651) 778-6176

Special Instructions and/or Specific Regulatory Requirements: (method, limit of detection, reporting units, etc.)

Preservatives: (Enter the number of containers of each)

Analysis Requested: Complete below. Attach any associated information. (Enter an 'X' in the box below to indicate request)

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/ Media	HNO₃	H₂SO₄	VOCs	None	Other	Total Number of Containers	PROTEIN SEP	PFOS
1.	10 ppm a.i. 230 J Albumen	31944	6/21/01									X	
2.	10 ppm a.i. 230 J Shell Membrane	31946	6/21/01									↓	
3.	10 ppm a.i. 232 J Yolk	31947	6/21/01										X
4.	10 ppm a.i. 232 J Albumen	31948	6/21/01										↓
5.	10 ppm a.i. 232 J Shell Membrane	31950	6/21/01										↓
6.	10 ppm a.i. 234 J Yolk	31951	6/21/01										↓
7.	10 ppm a.i. 234 J Albumen	31952	6/21/01										↓
8.	10 ppm a.i. 234 J Shell Membrane	31954	6/21/01										↓
9.													
10.													

Chain of Custody

Collected by (print):

Collector's signature:

Item #	Relinquished by/Affiliation	Time	Date	Shipped Via:	Received by/Affiliation	Time	Date
*1-8	[signature] / 3M	14:30	5/31/02	FED-EX	[signature] (EC) CUS 6-3-02	2115	6-1-02

Sample Condition Upon Receipt: O Acceptable O Other:

Temperature: 'C O Received on Ice ✓

Other Associated CoCs: ORIGINAL WILDLIFE INTERNATIONAL COC'S

Copies to: RRR

Comments: * TO EXYGEN RESEARCH

Original - Accompanying Samples Last Page - Originator See Reverse Side for Instructions

001330

3M ENVIRONMENTAL LABORATORY
CONTRACT LABORATORY WORK ORDER BY SAMPLE

Project: E01-1378

Contract Lab(s): EXYGEN

Requester: Robideau, Rochelle R (0002-03E-09)
Department: 502180 Site Source:
Project Number:
Date Received: 10/4/2001
Project Description: Mallard Reporduction (Eggs)

Completion Date:
Project Lead: Rochelle R. Robideau
Phone Number: 651-778-7065
Email Address: rrrobideau@mmm.com

Ship Date: 5/31/02

Comments:

NOTE: MISC SERV = Protein separation conducted by Exygen Research.

3M Sample	Sampled Date	Sample Description	
E01-1378-31638	6/13/2001	10 ppm a.i. 230 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31639	6/13/2001	10 ppm a.i. 232 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31640	6/13/2001	10 ppm a.i. 234 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31641	6/13/2001	10 ppm a.i. 236 H Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31654	6/13/2001	10 ppm a.i. 229 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31655	6/13/2001	10 ppm a.i. 231 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31656	6/13/2001	10 ppm a.i. 233 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31657	6/13/2001	10 ppm a.i. 235 I Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31694	6/21/2001	10 ppm a.i. 230 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002

3M ENVIRONMENTAL LABORATORY
CONTRACT LABORATORY WORK ORDER BY SAMPLE

Project: E01-1378 (cont.) Contract Lab(s): EXYGEN

3M Sample	Sampled Date	Sample Description	
E01-1378-31695	6/21/2001	10 ppm a.i. 230 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31697	6/21/2001	10 ppm a.i. 230 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31698	6/21/2001	10 ppm a.i. 232 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31699	6/21/2001	10 ppm a.i. 232 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31701	6/21/2001	10 ppm a.i. 232 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
MISC_SERV	Misc. Services	Misc. Services	6/30/2002
E01-1378-31702	6/21/2001	10 ppm a.i. 234 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1378-31703	6/21/2001	10 ppm a.i. 234 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1378-31705	6/21/2001	10 ppm a.i. 234 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1378-31706	6/21/2001	10 ppm a.i. 236 J Yolk	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1378-31707	6/21/2001	10 ppm a.i. 236 J Albumen	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002
E01-1378-31709	6/21/2001	10 ppm a.i. 236 J Shell Membrane	
Analysis Code	Analytical Method	Components	Analysis Due Date
PFOS	PFOS by ESMS	PFOS	6/30/2002

3M Environmental Laboratory

Form 38778 - PWO

Shipping Address:
3M Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Telephone:
Sample Receiving: (651) 778-4948
Alternate: (651) 778-6753
FAX: (651) 778-6176

Chain of Custody /Request for Laboratory Analytical 5810

Project ID/Project Name	~~Mallard Reporduction (Eggs)~~
Template #	Final Report Due Date
Project Lead	Rochelle R. Robideau — Internal Due Date
Dept. # (main)	502180 — Class/Job/Project #

3M Env. Lab Project # For Internal Use Only

E01-1378

Report Results to:

Contact Name	Robideau, Rochelle R
Company	3M
Mailing Address	0002-03E-09
City, State, Zip	
Telephone #	1 651 778-7065
FAX #	1 (651) 778-6176

Date Available	6/1
Date Due	6/30 / CONTACT ROCHELLE
Contract Lab	EN4GEN

Special Instructions and/or Specific Regulatory Requirements:
(method, limit of detection, reporting units, etc.)

Preservatives: HNO3 | H2SO4 | VOCs | None | FROZEN Other

Total Number of Containers

Analysis Requested:
Complete below. Attach any associated information.

Pesticides

Enter the number of containers of each | (Enter an 'X' in the box below to indicate request)

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/ Media	HNO3	H2SO4	VOCs	None	FROZEN Other	Total Number of Containers	Pesticides
1.	10 ppm a.i. 230 H Yolk	31638	6/13/01							1	1	X
2.	10 ppm a.i. 232 H Yolk	31639	6/13/01									
3.	10 ppm a.i. 234 H Yolk	31640	6/13/01									
4.	10 ppm a.i. 236 H Yolk	31641	6/13/01									
5.	10 ppm a.i. 229 I Yolk	31654	6/13/01									
6.	10 ppm a.i. 231 I Yolk	31655	6/13/01									
7.	10 ppm a.i. 233 I Yolk	31656	6/13/01									
8.	10 ppm a.i. 235 I Yolk	31657	6/13/01									
9.	10 ppm a.i. 230 J Yolk	31694	6/21/01									
10.	10 ppm a.i. 230 J Albumen	31695	6/21/01									
11.	10 ppm a.i. 230 J Shell Membrane	31697	6/21/01									
12.	10 ppm a.i. 232 J Yolk	31698	6/21/01									

Collected by (print):

Collector's signature:

Chain of Custody

Item #	Relinquished by/Affiliation	Time	Date	Shipped Via:	Received by/Affiliation	Time	Date
*1-12	Paul Smith / 3M	1413o	5/31/02	FED-EX	[illegible] @LNO 6-3-02	2115	6-1-02

Sample Condition Upon Receipt: O Acceptable O Other:

Temperature: 'C O Received on Ice ✓

Other Associated CoCs: ORIGINAL COCS WILDLIFE INTERNATIONAL

Copies to: RRR

Comments:

Page 1 Of 1

Original - Accompanying Samples

3M Environmental Laboratory

Form 38778 - PWO

Shipping Address:
3M Bldg. 2-3E-09
935 Bush Avenue
St. Paul, MN 55106

Telephone:
Sample Receiving: (651) 778-4981
Alternate: (651) 778-6753
FAX: (651) 778-6176

Chain of Custody /Request for Laboratory Analytical

5811

Project ID/Project Name	~~Mallard Reproduction (Eggs)~~
Template #	Final Report Due Date
Project Lead	~~Rochelle R. Robideau~~ Internal Due Date
Dept. # (main)	502180 Class/Job/Project #

3M Env. Lab Project # For Internal Use Only

E01-1378

Report Results to:

Contact Name	Robideau, Rochelle R
Company	3M
Mailing Address	0002-03E-09
City, State, Zip	
Telephone #	1 651 778-7065 FAX # 1 (651) 778-6176

Date Available	6/1
Date Due	6/30 CONTACT ROCHELLE
Contract Lab	57460N

Special Instructions and/or Specific Regulatory Requirements:
(method, limit of detection, reporting units, etc.)

Preservatives: HNO3 | H2SO4 | VOCs | None | Other Frozen

Total Number of Containers

Analysis Requested:
Complete below. Attach any associated information.

Frozen tissue pkg

(Enter an 'X' in the box below to indicate request)

Item #	Client Sample Identification	3M LIMS#	Date Sampled	Time Sampled	Matrix/ Media	Enter the number of containers of each															
1.	10 ppm a.i. 232 J Albumen	31699	6/21/01							1	1	X									
2.	10 ppm a.i. 232 J Shell Membrane	31701	6/21/01									↓									
3.	10 ppm a.i. 234 J Yolk	31702	6/21/01										X								
4.	10 ppm a.i. 234 J Albumen	31703	6/21/01																		
5.	10 ppm a.i. 234 J Shell Membrane	31705	6/21/01																		
6.	10 ppm a.i. 236 J Yolk	31706	6/21/01																		
7.	10 ppm a.i. 236 J Albumen	31707	6/21/01																		
8.	10 ppm a.i. 236 J Shell Membrane	31709	6/21/01																		
9.																					
10.																					

Chain of Custody

Collected by (print):

Collector's signature:

Item #	Relinquished by/Affiliation	Time	Date	Shipped Via:	Received by/Affiliation	Time	Date
#1-8	[signature] (3M	14:30	5/31/01	Fed-Ex	[signature] CCLNO 6-3-02	2115	6-1-02

Sample Condition Upon Receipt: O Acceptable O Other:

Temperature: 'C O Received on Ice

Other Associated CoCs: ORIGINAL WILDLIFE INTERNATIONAL COC'S

Copies to: RRR ✓

Comments:

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Sample "Condition Upon Receipt" Form

Protocol # NA

Exygen Study # 023-070 R.K 6-3-02

Date & Time Received 6-1-02 / 2115

Condition of Samples F- Dry Ice - A

Temporary Storage Location F-8

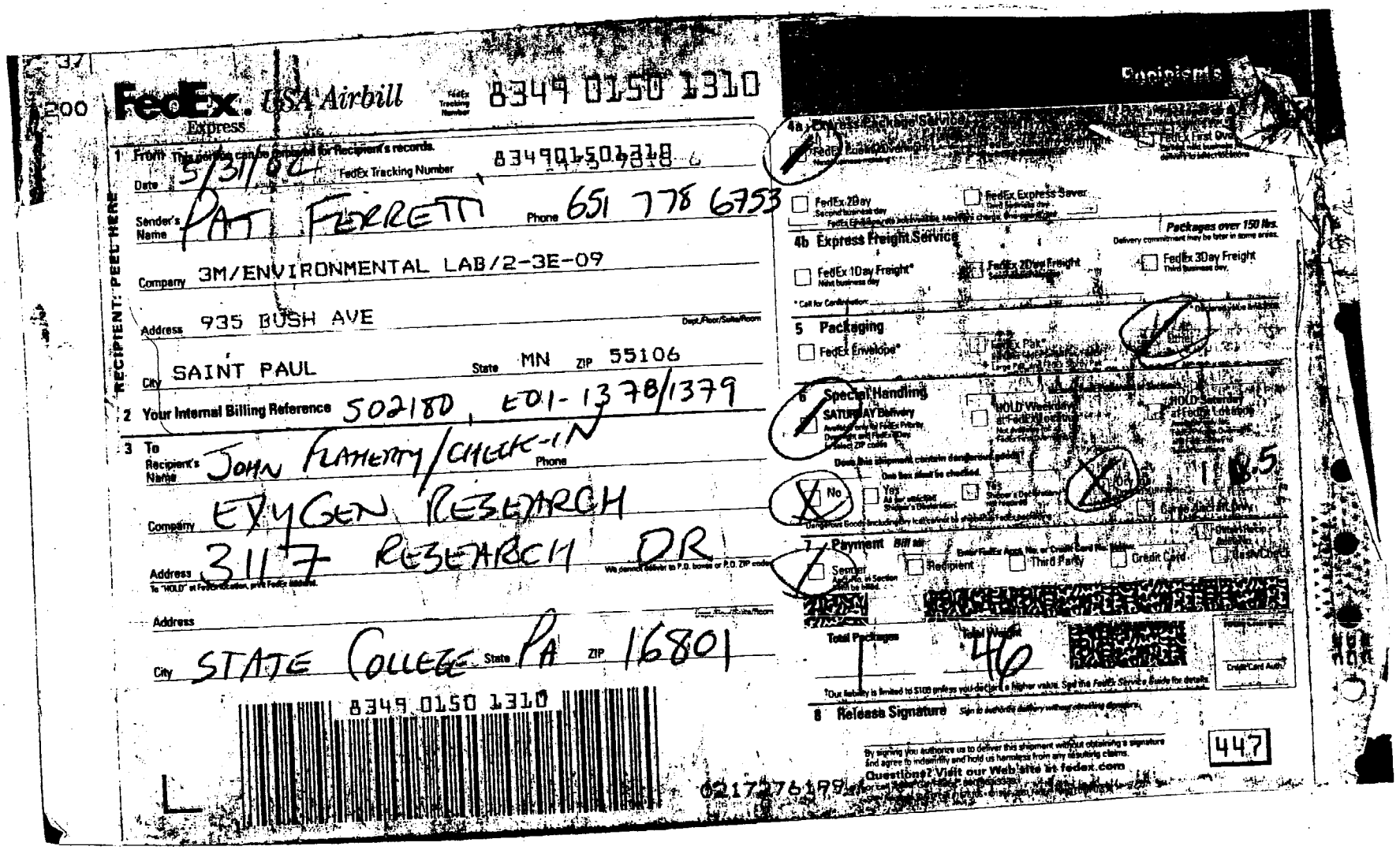
Initials & Date RK 6-1-02

Waybill # 8349 0150 1310 (FedEX)

Comments: Saturday delivery, did not have study number. RK 6-1-02

May 24, 2002/3

3058 Research Drive
State College, PA 16801, USA
T: 800.281.3219
F: 814.272.1019
exygen.com



FedEx. USA Airbill
Express
8349 0150 1310
Recipient's
RECIPIENT: PEEL HERE
1 From This section can be printed for Recipient's records.
Date 5/31/04
FedEx Tracking Number 8349 0150 1310 6
Sender's Name PAT FERRETTI Phone 651 778 6753
Company 3M/ENVIRONMENTAL LAB/2-3E-09
Address 935 BUSH AVE
City SAINT PAUL State MN ZIP 55106
2 Your Internal Billing Reference 502180, E01-1378/1379
3 To
Recipient's Name JOHN FLAHERTY/CHECK-IN Phone
Company EXYGEN RESEARCH
Address 3117 RESEARCH DR
Address
City STATE COLLEGE State PA ZIP 16801
8349 0150 1310
4a Express Package Service
FedEx Priority Overnight
FedEx First Overnight
FedEx 2Day
FedEx Express Saver
4b Express Freight Service
Packages over 150 lbs.
FedEx 1Day Freight
FedEx 2Day Freight
FedEx 3Day Freight
5 Packaging
FedEx Envelope
FedEx Pak
FedEx Box
6 Special Handling
SATURDAY Delivery
HOLD Weekday at FedEx Location
HOLD Saturday at FedEx Location
No
Yes
OR
7 Payment Bill to:
Sender
Recipient
Third Party
Credit Card
Cash/Check
Total Packages
Total Weight
Credit Card Auth.
8 Release Signature
By signing you authorize us to deliver this shipment without obtaining a signature and agree to indemnify and hold us harmless from any resulting claims.
Questions? Visit our Web site at fedex.com
447
0217276199

Exygen Research

Sample Login Report

Study Number: **023-070**
Protocol: **NA**

Exygen ID	Client Sample ID	Received	Matrix	Submitted By	Logged In By/Date	Initial Loc.	Condition of Sample	Comments
0201615	NA	6-5-02 12:00	EGG	NA	LAWRENCE O 6-5-02	FREEZER 8	REFRIGERATED	Purchased at Weis Markets #33, State College, PA

Verified By/Date: 6-5-02

For Emily

LNO
6-5-02

THANK YOU FOR SHOPPING

WEIS MARKETS # 33 STATE COLLEGE, PA.

Item			Price
*WQ EGGS L			.79 F
**** TAX	.00	BAL	.79
Cash			1.00
CHANGE			.21

6/05/02 12:29 PM 0033 02 0150 145

ASK US HOW OUR WEIS SHOPPERS CLUB
CAN SAVE YOU $$'S EVERYDAY ...
WEIS CLUB MEMBERS
HAVE THE POWER TO SAVE MORE

"THIS IS AN EXACT COPY OF
THE ORIGINAL DOCUMENT."

BY LNO DATE 6-5-02

Exygen™ RESEARCH
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Analytical Report

Summary of PFOS Residue Found (ppb) in Combined Mallard Egg Yolk Samples

Sponsor ID	Analyte Found (ppb)	Fort. Level (ppb)	Recovery (%)
na (Reagent Control)	NQ	-	-
na (Reagent Control)^	NQ	-	-
na (Reagent Control)	NQ	-	-
na (Reagent Control)^	NQ	-	-
na (Matrix Control)	NQ	-	-
na (Matrix Control)^	NQ	-	-
na (Matrix Control)	NQ	-	-
na (Matrix Control)^	NQ	-	-
na (LCS)	108	100	108
na (LCS)	111	100	111
na (LCS)	103	100	103
na (LCS)	100	100	100
**	53600	-	-
**^	52300	-	-
** Dup	52600	-	-
** Dup^	52500	-	-

NQ = Result is above the detection limit of ~ 0.5 ppb but below the quantitation limit of 10 ppb.

^ Duplicate Injection

** composite of samples E01-1378-31638, 31639, 31640, 31641, 31654, 31655, 31656, 31657, 31694, 31698, 31702, 31706

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State College, PA 16801, USA
T: 814.272.1039
F: 814.231.1580
exygen.com

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Analytical Report

Summary of PFOS Residue Found (ppb) in Combined Quail Egg Yolk Samples

Sponsor ID	Analyte Found (ppb)	Fort. Level (ppb)	Recovery (%)
na (Reagent Control)	NQ	-	-
na (Reagent Control)^	NQ	-	-
na (Reagent Control)	NQ	-	-
na (Reagent Control)^	NQ	-	-
na (Matrix Control)	NQ	-	-
na (Matrix Control)^	NQ	-	-
na (Matrix Control)	NQ	-	-
na (Matrix Control)^	NQ	-	-
na (LCS)	104	100	104
na (LCS)	102	100	102
na (LCS)	106	100	106
na (LCS)	109	100	109
**	75000	-	-
**^	74300	-	-
** Dup	48700	-	-
** Dup^	49900	-	-

NQ = Result is above the detection limit of ~ 0.5 ppb but below the quantitation limit of 10 ppb.

^ Duplicate Injection

** composite of samples E01-1379-31882, 31883, 31884, 31885, 31895, 31896, 31897, 31898, 31939, 31943, 31947, 31951

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Analytical Report

Summary of PFOS Residue Found (ppb) in Mallard*/Quail** Egg Yolk Fractions

Sponsor ID	Analyte Found (ppb)	Fort. Level (ppb)	Recovery (%)
na (Reagent Control)	ND	-	-
na (Reagent Control)^	ND	-	-
na (Reagent Control)	ND	-	-
na (Reagent Control)^	ND	-	-
na (Matrix Control)-VLDL	13.5	-	-
na (Matrix Control)-VLDL^	14.3	-	-
na (Matrix Control)-Phosvitin	NQ	-	-
na (Matrix Control)-Phosvitin^	NQ	-	-
na (Matrix Control)-Lipovitellin	NQ	-	-
na (Matrix Control)-Lipovitellin^	NQ	-	-
*-Mallard VLDL	41800	-	-
*-Mallard VLDL^	42500	-	-
*-Mallard Phosvitin	3600	-	-
*-Mallard Phosvitin^	3580	-	-
*-Mallard Lipovitellin	8910	-	-
*-Mallard Lipovitellin^	8830	-	-
**-Quail VLDL	39700	-	-
**-Quail VLDL^	39900	-	-
**-Quail Phosvitin	815	-	-
**-Quail Phosvitin^	838	-	-
**-Quail Lipovitellin	1300	-	-
**-Quail Lipovitellin^	1340	-	-

ND = Result is below the detection limit of ~ 0.5 ppb.
NQ = Result is above the detection limit of ~ 0.5 ppb but below the quantitation limit of 10 ppb.
^ Duplicate Injection
* composite of samples E01-1378-31638, 31639, 31640, 31641, 31654, 31655, 31656, 31657, 31694, 31698, 31702, 31706
** composite of samples E01-1379-31882, 31883, 31884, 31885, 31895, 31896, 31897, 31898, 31939, 31943, 31947, 31951

3058 Research Drive
State College, PA 16801, USA
T: 814.272.1039
F: 814.231.1580
exygen.com

Exygen RESEARCH
3058 Research Drive
State College, PA 16801
T: (814) 272-1039
F: (814) 231-1580

STUDY PERSONNEL LOG

PROTOCOL NA **EXYGEN STUDY** 023-070

PRINTED NAME	SIGNATURE	INITIALS
EMILY R. DECKER	Emily R Decker	ERD
Rickey Keller	Rickey Keller	RK
Lawrence N. Ord	Lawrence N. Ord	LNO
	ERD 3/5/02	

ANALYTICAL PHASE PROTOCOL

ANALYTICAL PHASE TITLE

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM MALLARD AND QUAIL EGG YOLK 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

Exygen Research (Exygen)
3058 Research Drive
State College, PA 16801
Phone 814-272-1039

Title: EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM MALLARD AND QUAIL EGG YOLK FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

TABLE OF CONTENTS

Title:

EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE FROM MALLARD AND QUAIL EGG YOLK FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

1. PURPOSE

The purpose of this study is to analyze mallard and quail egg yolk samples and their fractions for residues of perfluorooctanesulfonate (PFOS) using methods entitled, "Lipovitellin, Phosvitin, and Very Low Density Lipoprotein (VLDL) Isolation from Chicken and Japanese Quail Egg Yolk" and "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen and Yolk by LC/MS/MS." Both methods can be found in the Appendix.

2. REFERENCE MATERIAL

The following analytical standard will be used:

Test Material	Lot Number	Purity (%)
PFOS	215	TBD

Chemical name and structure of the compound is presented below.

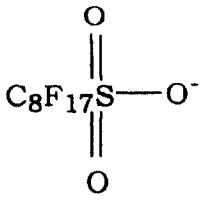
PFOS

Chemical Name: Perfluorooctanesulfonate

IUPAC Name: 1-Octanesulfonic acid,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt

CAS Number: 2795-39-3

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



Note: The neutral molecule and standard from which the PFOS (anion) is obtained 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 Exygen. Forms documenting chain-of-custody and shipping records for tracking of the test substances will be included as part of the raw data package.

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

HAZARD INFORMATION

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

3. SPONSOR

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

Sponsor Representative:
Bill Reagen

4. TESTING FACILITY / PERFORMING LABORATORY

Exygen Research
3058 Research Drive
State College, PA 16801

Project Manager:
Emily R. Decker, Exygen

5. PROPOSED EXPERIMENTAL TIME-FRAME

Analytical Start Date	June 3, 2002
Analytical Termination Date	June 21, 2002
Report Issued	July 31, 2002

6. SAMPLE PROCESSING, STORAGE AND IDENTIFICATION

All mallard egg yolk samples received will be combined into one composite sample using a blender. The same process will also be used for all of the quail egg yolk samples.

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

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

7. ANALYTICAL METHOD

The composite mallard/quail egg yolk samples will be extracted and analyzed first according to the analytical method titled "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen and Yolk by LC/MS/MS" to determine the total amount of PFOS in each sample. Then the mallard/quail composite yolk samples will be separated into three fractions using the method "Lipovitellin, Phosvitin, and Very Low Density Lipoprotein (VLDL) Isolation from Chicken and Japanese Quail Egg Yolk." Each fraction will then be extracted and analyzed according to the method "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen and Yolk by LC/MS/MS".

8. EXPERIMENTAL DESIGN

Samples obtained by 3M Environmental will be shipped to Exygen Research for analysis. Samples will be extracted and analyzed at Exygen according to method, "Determination of Perfluorooctanesulfonate in Egg Membrane, Albumen and Yolk by LC/MS/MS."

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.

The average recovery and relative standard deviation of the fortified samples will be calculated. If necessary, apply a standard test for outliers. If an outlier exists, then it may be excluded from the statistical analysis. Also, the average residue found and standard deviation for each matrix will be calculated.

9. RECORDS

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

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

All chromatograms will contain the following:

a. Sample identification, date, arrow or other indication of the area of interest, and injection number corresponding to the run.

b. Additionally, fortifications will include the fortification level of the analyte.
c. Analytical standard chromatograms will additionally include the concentration (e.g., µg/ml, ng/mL, ppb, ppt, etc.).

Each data set will contain information on temperatures, flow rates, column parameters, gases, instrument parameters, and instrument type, etc.

10. DATA AND REPORT

1. All raw data and the original signed protocol will be maintained in the study file. This data includes the laboratory notebooks, analytical standard solution preparation, sample chain of custody sheets, sample work sheets, chromatograms, calibration curves, and any other appropriate data generated.

2. A report will be issued by Exygen at the completion of the study according to the Sponsor's specifications. 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. Tables containing all applicable data
   6. All chromatographic and instrumental conditions
   7. A complete listing of Exygen study personnel

11. COSTS

The total cost for performance of the study will be $30,000.00.

12. PROTOCOL APPROVAL

Project Manager, Exygen

Emily R Decker

Emily Decker
Scientist

5/31/02
Date

Sponsor, 3M

Bill Reagen

05/30/02
Date

APPENDIX: ANALYTICAL METHODS

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

B. "Lipovitellin, Phosvitin, and Very Low Density Lipoprotein (VLDL) Isolation from Chicken and Japanese Quail Egg Yolk"

TITLE

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

AUTHORS

Emily Stauffer and John Flaherty

DATE ISSUED

January 2, 2001

SPONSOR

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CENTRE STUDY NUMBER

023-015

CENTRE METHOD NUMBER

00M-023-015

TOTAL NUMBER OF PAGES

28

MANAGEMENT APPROVAL

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

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

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6-MAR-01
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Rochelle Robideau — Date
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3M Environmental Laboratory

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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.

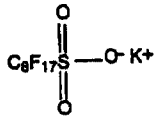
Perfluorooctanesulfonate is extracted from each matrix with methanol (MeOH). For egg membrane samples, the methanol extract is passed through a membrane filter. For egg albumen and yolk samples, a 0.5g of carbon is added to an aliquot of the methanol extract. Quantification of PFOS is accomplished by 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.

2. EXPERIMENTAL COMPOUNDS

The molecular structure of PFOS is given below:

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



Chemical Name : Perfluorooctanesulfonate
IUPAC name : 1-Octanesulfonic acid,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-potassium salt
CAS # : 2795-39-3

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

3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

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

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

3.2. STANDARDS

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

3.3. EQUIPMENT AND SUPPLIES

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

Note:

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

3.4. SOLUTIONS

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

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

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

3.5. Preparation of Stock, Fortification, and Calibration Solutions

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

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

3.5.1. Stock Solution

Prepare stock solution of PFOS at 100 µg/mL by weighing out 10.0 mg of analytical standard (corrected for percent salt). Adjust final volume to 100 mL with methanol in a 100-mL volumetric flask. Store this stock solution (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

3.5.2. Fortification Solutions

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

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

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

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

3.5.3. Calibration Standards

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

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

Initial Conc. (µg/mL)	Volume (mL)	Diluted to (mL)	Final Conc. (µg/mL)
0.1	5	100	0.005
0.1	2	100	0.002
0.1	1	100	0.001
0.01	5	100	0.0005
0.01	1	100	0.0001
0.01	0.5	100	0.00005

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

4. METHOD

4.1. FLOW DIAGRAM

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

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

4.2. SAMPLE PROCESSING

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

4.3. SAMPLE EXTRACTION

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

4.3.1 Egg Membrane

a. Allow sample to thaw.

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

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

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

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

4.3.2. Egg Yolk and Albumen

a. Allow sample to thaw.

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

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

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

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

4.4. ANALYSIS BY LC/MS/MS

4.4.1. LC/MS/MS System and Operating Conditions (TurboIonspray)

Mass Spec: PE SCIEX API 3000,Biomolecular Mass Analyzer

Interface: SCIEX TurboIon Spray Liquid Introduction Interface
Harvard infusion pump

Computer: Power Macintosh G3

Software: PE SciexAnalyst 1.1
Windows NT

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

HPLC Column: Genesis C_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in ASTM type I water
Mobile Phase (B): Methanol
Flow Rate: 0.3 mL/min.

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

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

Ions monitored:

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

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

4.4.2. Example Tune File Parameters

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

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

Controls	Set
IS-Iospray	-4000.0
OR-Orifice	-61.0
RNG-Focus Ring	-270.0
Q0-Quad 0 Rod Offset	10.0
IQ1- Inter quad 1 lens	9.3
ST-Stubbies	15.0
RO1-Quad1 Rod Offset	9.3
IQ2-Inter quad 2 lens	20.0
RO2-Quad 2 rod offset	84.0
ST3-Stubbies	100.0
RO3-Quad 3 rod offset	86.0
DF-CEM Deflection Plate	300.0
CEM-Channel Electron Multiplier	2400.0

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

4.4.3. Calibration Procedures

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

b. Use linear, 1/x weighted standard curves for quantification. Linear standard curves are generated for each set by linear regression using the

appropriate software system. Any calibration standards falling outside ± 30%, based on its calculated concentration, must be excluded from the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 30% of the total number of standards injected.

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

4.4.4. Sample Analysis

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

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

c. Inject an entire set of standards (six) at the beginning of the run and inject standards interspersed about every 5-10 samples. All sample injections must be bracketed by standard injections (see Section 4.5).

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

e. All samples must be analyzed with duplicate injections.

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

f. The concentration of each sample, fortification, control, etc. is determined from the standard curve based on the peak area of the analyte in all standards injected during a set. The standard responses must bracket responses of the residue found in the sample set. If necessary, dilute the samples and re-analyze to give a response within the standard curve range.

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

h. Fortification recoveries within 60 to 130% are acceptable for fortifications at the LOQ level. Recoveries between 70 to 120% are acceptable for fortifications at levels greater than the LOQ. Failure to meet these criteria requires an investigation of cause and a full reanalysis of the affected samples.

i. Samples in which no peaks are detected at the corresponding analyte retention times will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention times but are less than the lowest standard will be reported as NQ (not quantifiable).

j. Background levels of analyte found in control/blank samples that correspond to values below the LOQ, but are still quantifiable, will be used to correct fortification recoveries.

j. If samples are not loaded on the instrument to be analyzed the day they are extracted, samples must be stored refrigerated at approximately 2°C to 6°C until analysis and analyzed preferably within a week.

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

4.5. PERFORMANCE CRITERIA

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

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

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

4.6. Time Required for Analysis

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

5. CALCULATIONS

5.1 Analyte Found:

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

Equation 1:

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

5.2 Component Residue Concentration

Determine the component residue concentration using Equation 2

Equation 2:

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

Where DF = dilution factor and FV = final volume

Note: ng/g = ppb

5.3 Percent Recovery

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

Equation 3:

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

6. SAFETY

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

TABLES

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

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

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

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

NA = Not Applicable

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

Sample	Fort. Level (ng/g)	% Recovery
0003795 Matrix Blank A	0	NA
0003795 Matrix Blank B	0	NA
0003795 Matrix Blank C	0	NA
0003795 Spk A	10	69
0003795 Spk B	10	68
0003795 Spk C	10	70
0003795 Spk D	50	72
0003795 Spk E	50	80
0003795 Spk F	50	83
0003795 Spk G	250	85
0003795 Spk H	250	89
0003795 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

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

FIGURES

Figure 1: Representative Chromatogram of a Quail Egg Membrane Control

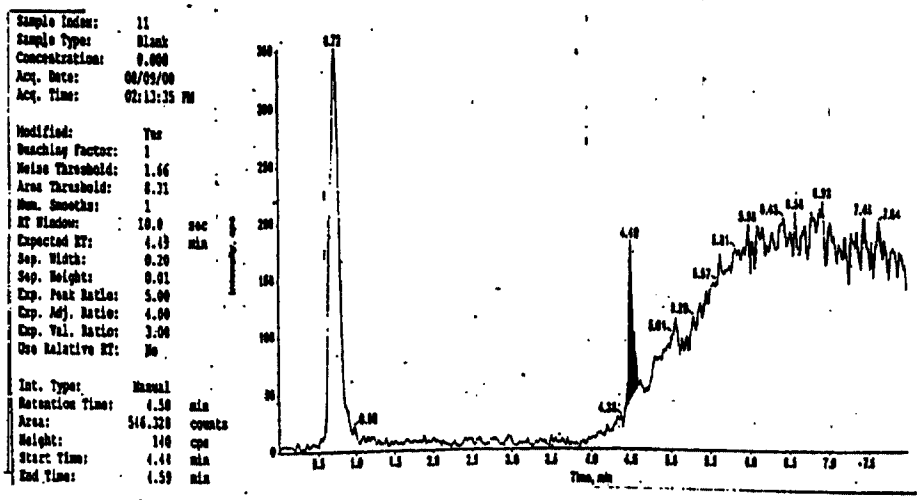


Figure 2: Representative Chromatogram of a Mallard Egg Membrane Control

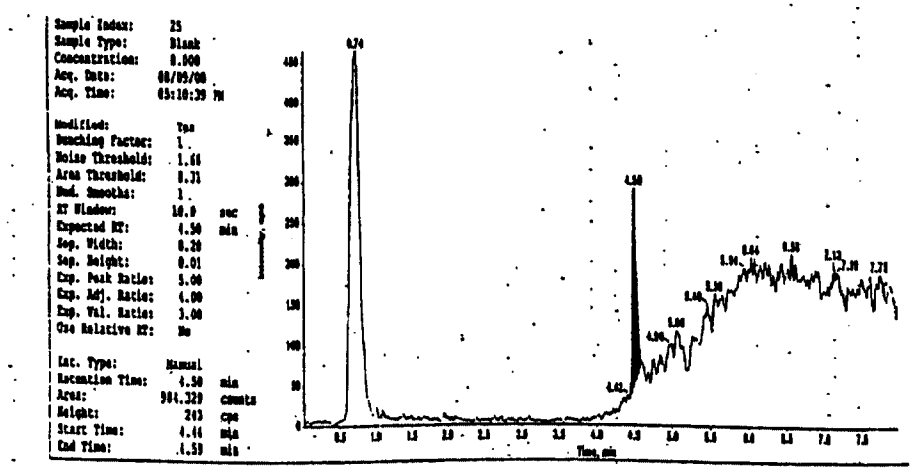


Figure 3: Representative Chromatogram of a Quail Egg Yolk Control

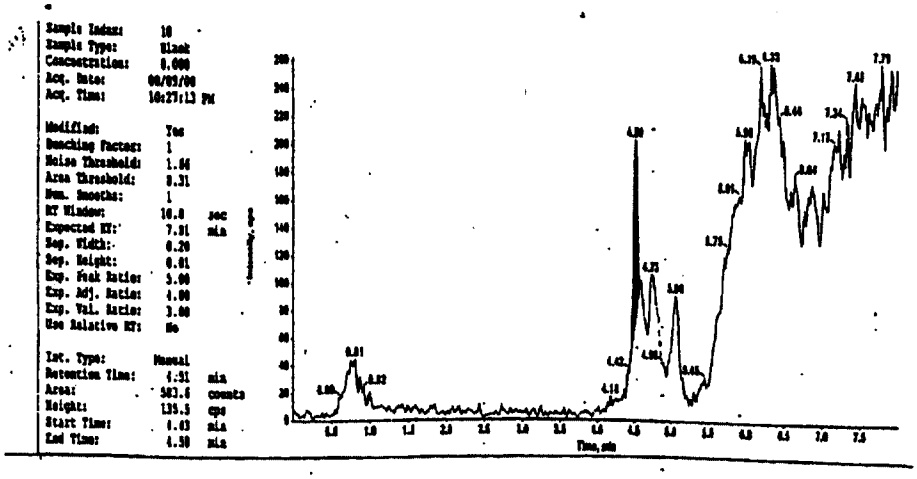


Figure 4: Representative Chromatogram of a Mallard Egg Yolk Control

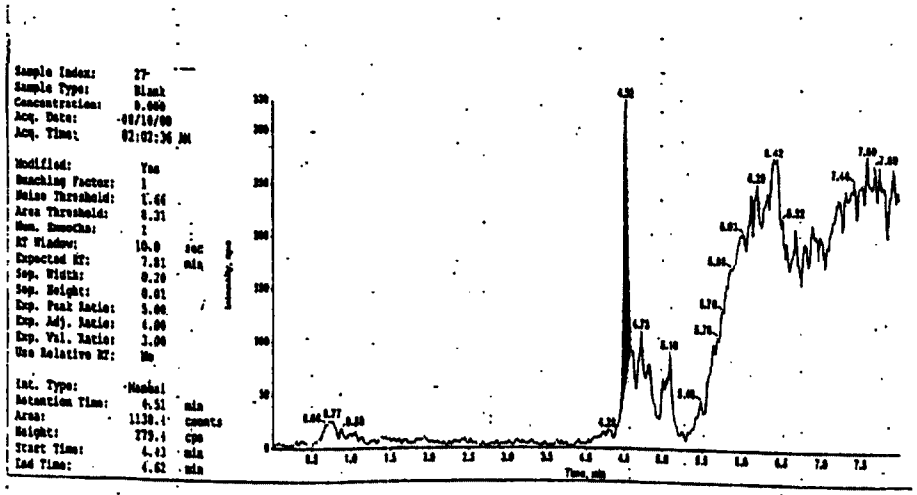


Figure 5: Representative Chromatogram of a Quail Egg Albumen Control

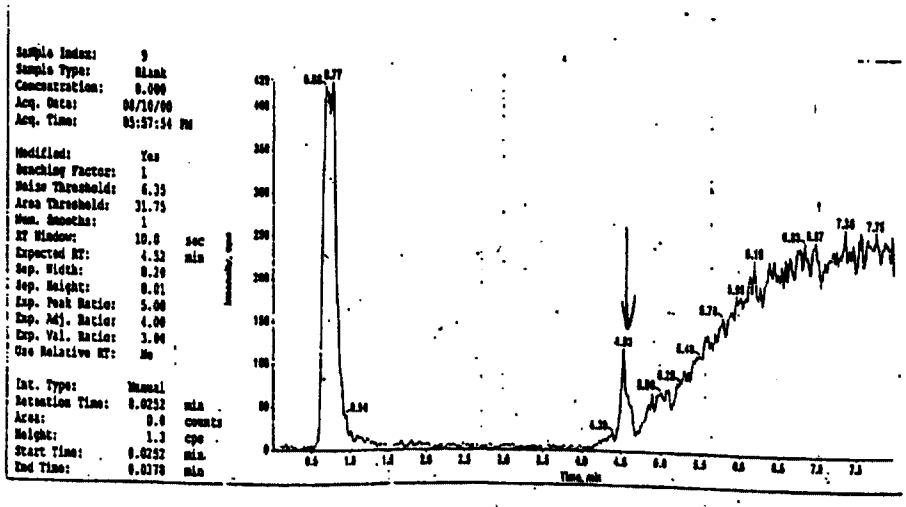


Figure 6: Representative Chromatogram of a Mallard Egg Albumen Control

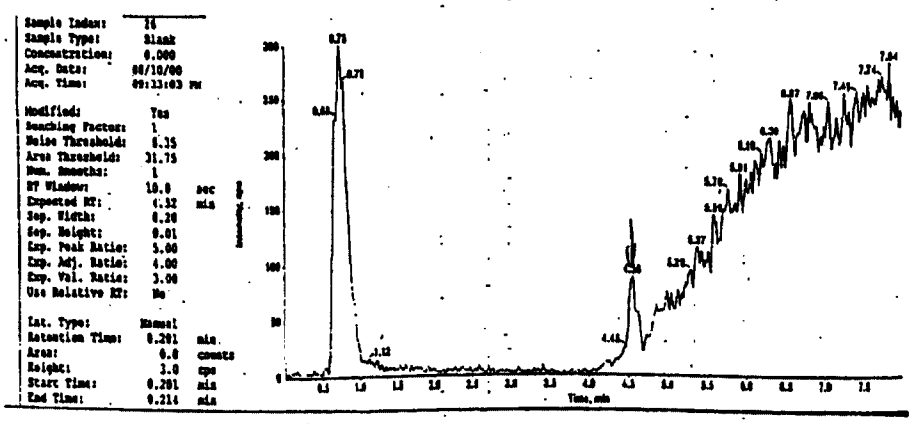


Figure 7: Representative Chromatogram of a Quail Egg Membrane Control Fortified at 10 ng/g (ppb)

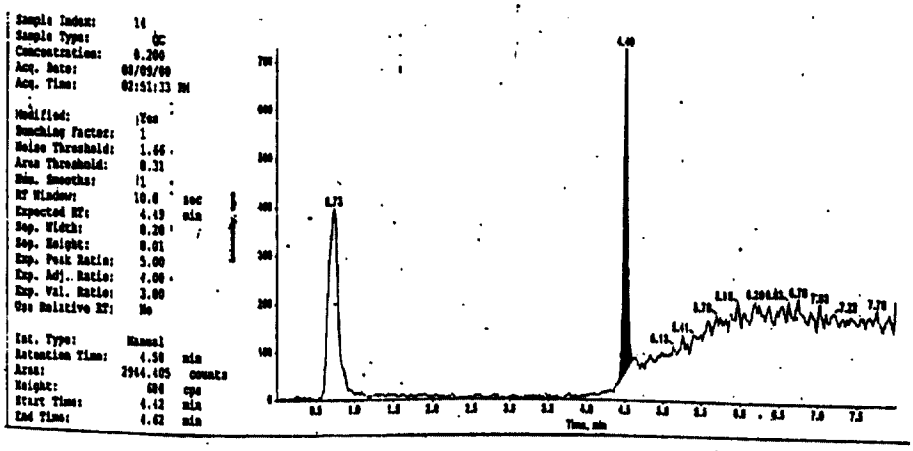


Figure 8: Representative Chromatogram of a Mallard Egg Membrane Control Fortified at 10.0 ng/g (ppb)

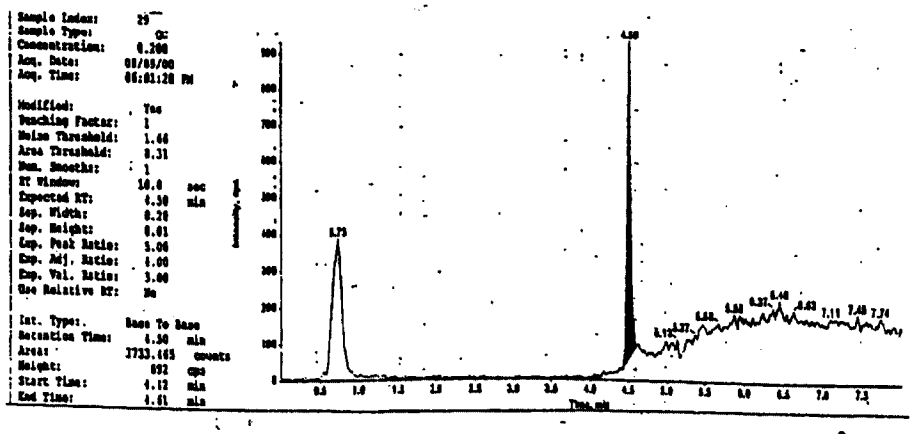


Figure 9: Representative Chromatogram of a Quail Egg Yolk Control Fortified at 10.0 ng/g (ppb)

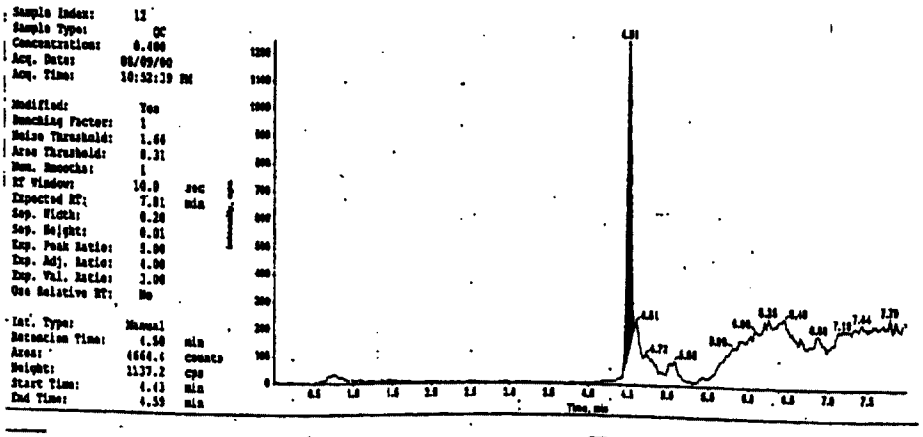


Figure 10: Representative Chromatogram of a Mallard Egg Yolk Control Fortified at 10.0 ng/g (ppb)

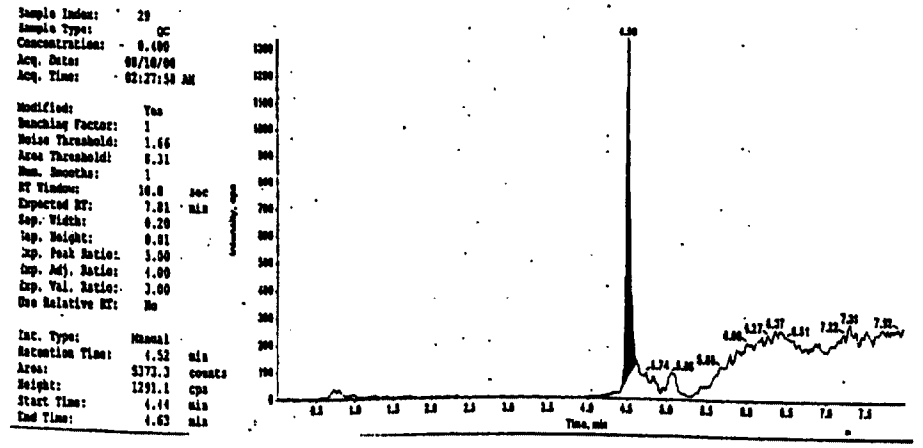


Figure 11: Representative Chromatogram of a Quail Egg Albumen Control Fortified at 10.0 ng/g (ppb)

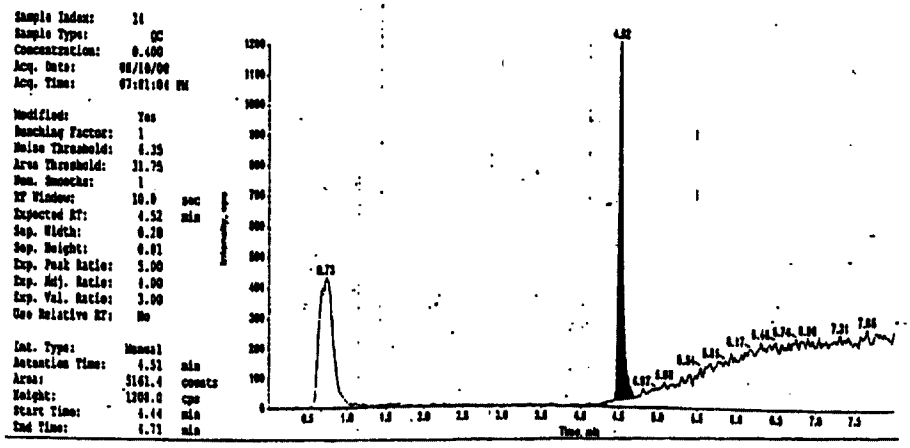
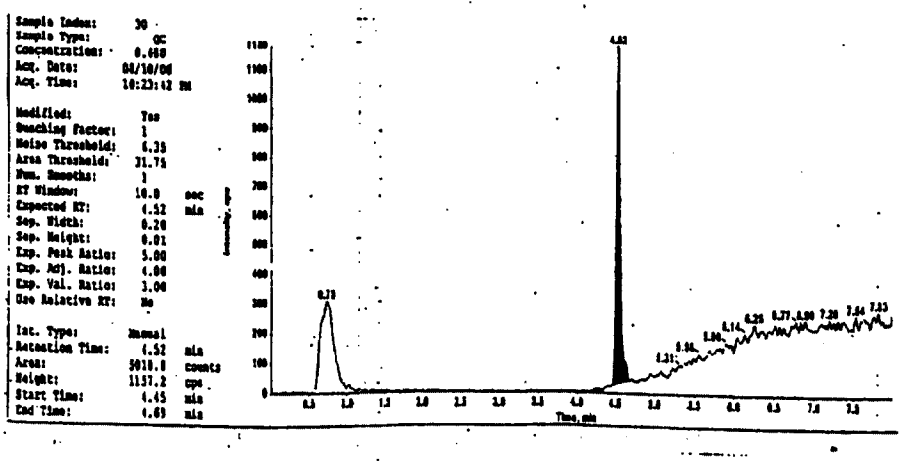


Figure 12: Representative Chromatogram of a Mallard Egg Albumen Control Fortified at 10.0 ng/g (ppb)



PLUS VITELLOGENIN

Lipovitellin, Phosvitin, and Very Low Density Lipoprotein (VLDL) Isolation from Chicken and Japanese Quail Egg Yolk (Bernardi and Cook, ~~*BBA* 44:86-~~ 96 [1960] and Stifani *et al.*, *JBC* 265:882-888 [1990])

(May, 1994)

1) Break open egg, separate yolk, and roll yolk on a moist paper towel to remove adhering albumen.

2) Puncture yolk membrane and let yolk drain into a graduated cylinder.

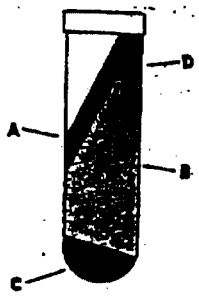
3) Dilute 1 volume of egg yolk with 2 volumes of a solution containing 0.67 M $MgSO_4$, 1 mM phenylmethanesulfonylfluoride (PMSF), and 2 µM leupeptin.

4) Cover cylinder tightly with Parafilm®M and mix well by gently inverting several times.

5) Using a 20 gauge needle and 12 cc syringe, transfer the suspension to Beckman polyallomer Quick-Seal® tubes (tube size will depend on amount of yolk prepared and rotors available).

6) Seal tubes, place into appropriate rotor, and centrifuge at 200,000 x *g* at 4 °C for 24 hours.

7) Following centrifugation, 4 layers will be evident in the tubes (see diagram at right): A, a firm layer of yellow gel (VLDL); B, a clear colorless solution; C, a viscous yellow solution grading to a firm pellet at the bottom; D, a fluffy yellow suspension (VLDL). **Layers B and C constitute the high density fraction (HDF) and layer A and the suspended material in D constitute the (very) low density fraction.**



8) Using a 20 gauge needle and 12 cc syringe, make two punctures in the top of the tube and withdraw layer D as well as several milliliters below the level of layer A until layer B is reached.

9) With a scalpel, cut the tube above the level of the remaining liquid. The yellow upper pellet of the tube (VLDL) is then redissolved in a solution containing 20 mM Tris-HCl (pH 8), 150 mM NaCl, 0.2 mM EDTA, 1 mM PMSF, and 5 µM leupeptin. The lower pellet (high density fraction, HDF; Layer C) should be *gently* washed with, and then resuspended in, a solution containing 0.45 M $MgSO_4$, 1 mM PMSF,

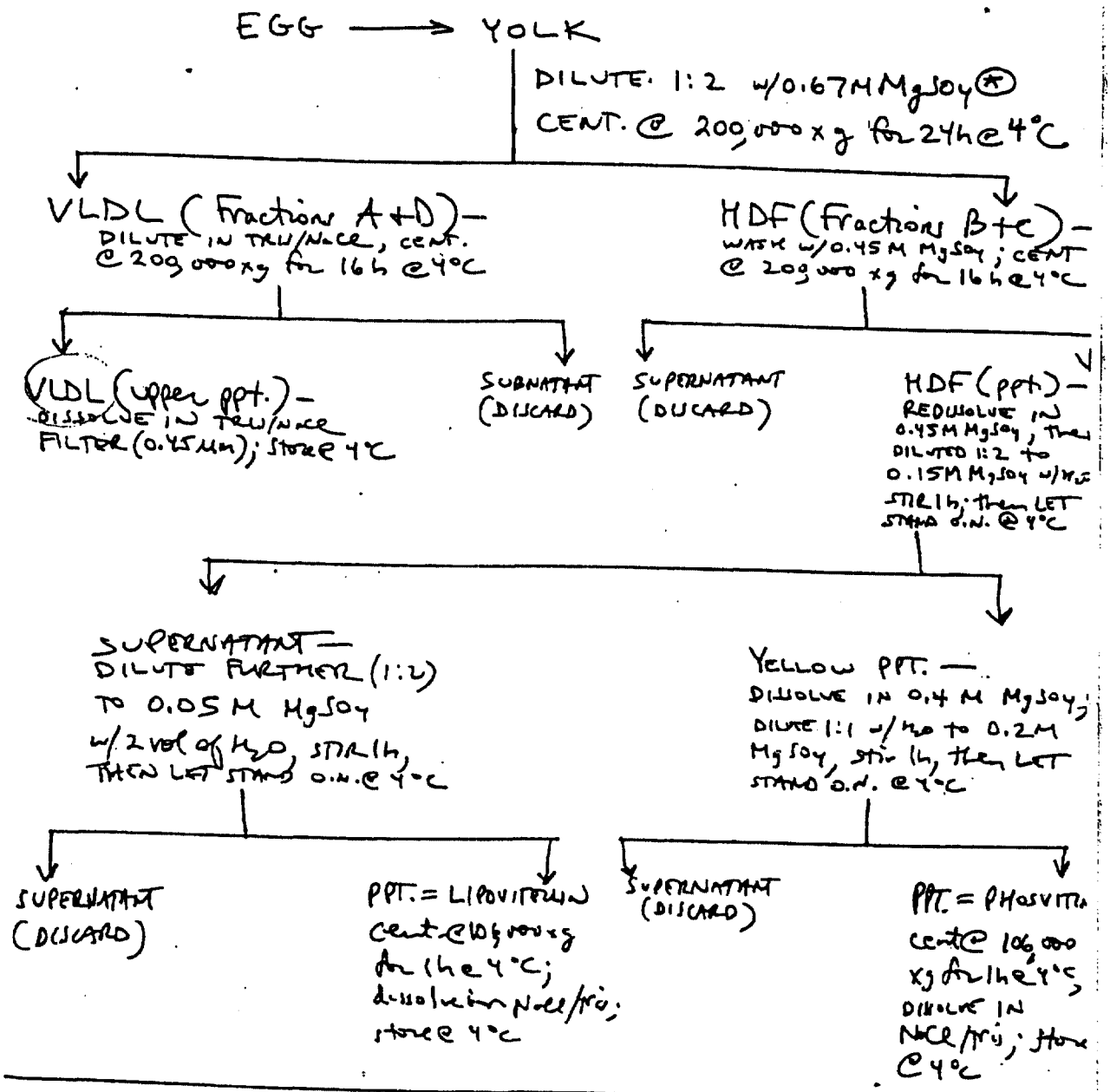
and 2 µM leupeptin. Both VLDL and HDF solutions are then centrifuged at 200,000 x *g* at 4 °C for 16 hours.

10) Following centrifugation of the VLDL solution, the upper pellet (VLDL) is re-dissolved in a minimal amount of 20 mM Tris-HCl (pH 8), 150 mM NaCl, 0.2 mM EDTA, 1 mM PMSF, and 5 µM leupeptin, filtered (0.45 µM), sodium azide added to a final concentration of 1 mM (i.e., dilute stock solution 1000 x), aliquoted, and stored at 4 °C. All of the VLDL preparation is now completed. The lower pellet (Layer C) of the HDF tube is first re-dissolved in a solution of 0.45 M $MgSO_4$, 1 mM PMSF, and 2 µM leupeptin. Then, two volumes of cold "e-pure" water are added to the HDF solution dropwise, with stirring, at 4 °C (thus, $MgSO_4$ concentration is now 0.15 M). The solution is then left standing (without stirring) at 4 °C overnight.

11) On the following morning, a yellow gelatinous precipitate should be adhered to the bottom of the flask. Decant (and save) the supernate (see step 12), then dissolve the precipitate in a solution of 0.4 M $MgSO_4$, 1 mM PMSF, and 2 µM leupeptin. Add one volume of cold "e-pure" water dropwise (thus solution is diluted to 0.2 M $MgSO_4$) and let stand overnight (without stirring) at 4 °C. On the following morning, the precipitate (phosvitin) is recovered by centrifugation at 106,000 x *g* at 4 °C for 1 hour and the resulting pellet is dissolved in buffer containing 1 M NaCl, 5 mM Tris-HCl (pH 7.8), 1 mM PMSF, 2 µM leupeptin, and sodium azide added to a final concentration of 1 mM (i.e., dilute stock solution 1000 x). Aliquot and store at 4 °C.

12) The "supernate" (0.15 M $MgSO_4$; see step 11) should be decanted, diluted with two volumes of cold "e-pure" water (final concentration of 0.05 M $MgSO_4$) dropwise with stirring at 4 °C, and allowed to stand overnight (without stirring) at 4 °C. The resulting white precipitate (lipovitellin) is then recovered by centrifugation at 106,000 x *g* at 4 °C for 1 hour and dissolved in buffer containing 1 M NaCl, 5 mM Tris-HCl (pH 7.8), 1 mM PMSF, and 2 µM leupeptin, and sodium azide added to a final concentration of 1 mM (i.e., dilute stock solution 1000 x). A final protein concentration of 8-10 mg/mL is desired.

LIPOVITELLIN, PHOSVITIN, & VLDL ISOLATION FROM EGG YOLK (MAY, 1994)



⊛ ALL BUFFERS CONTAINED 1mM PMSF and 2MM LP