

---

**Analytical Report**

**Occupational and Environmental Health**

**Analysis of Perfluorooctanoic Acid (PFOA) in Human Serum Samples**

**Exygen Report No. L0003000**

---

**Testing Laboratory**

Exygen Research  
3058 Research Drive  
State College, PA 16801

---

**Requester**

Dr. Marsha Bailey  
Occupational and Environmental Health  
4 Rosemar Circle  
Parkersburg, WV 26101  
(304)-757-0270

EID871401

## 1 Introduction

Results are reported for the analysis of perfluorooctanoic acid (PFOA) in human serum samples received at Exogen from Dr. Marsha Bailey at Occupational and Environmental Health. The Exogen project number assigned to the samples is P959.

## 2 Sample Receipt

A total of twelve samples were received at Exogen in 5 mL screw-cap vials labeled with permanent marker. A copy of all sample log-in information is presented in Attachment A.

The twelve samples were received on 07/23/04. The samples were shipped frozen on dry ice via FedEx. The samples were stored frozen from time of receipt until analysis.

## 3 Methods - Analytical and Preparatory

### 3.1 Sample Preparation

The samples were extracted and analyzed according to the current revision of method ExM-008-211. Fifty microliters of sample was used for the extraction procedure. Using the Multiprobe apparatus, 500  $\mu$ L of acetonitrile was added to the sample and then passed through a protein precipitation column.

### 3.2 Sample Analysis by LC/MS and 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 compounds are observed and quantitated against extracted standards.

An HP1100 system interfaced to a PE Sciex API 4000 system was used to analyze the sample extracts. A gradient elution through a Jones Chromatography Genesis C-8 50 x 2.1 mm x 4 $\mu$ m 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	Flow Rate (mL/min)
0.0	40	60	0.3
3.0	40	60	0.3
3.5	0	100	0.3
3.7	0	100	0.5
7.0	0	100	0.5
7.5	40	60	0.5
9.0	40	60	0.5
9.5	40	60	0.3
12.0	40	60	0.3

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

Parameter	Setting
Ionization Mode	Electrospray
Polarity	Negative
Transitions Monitored	413->369 (PFOA), 415->370 ( <sup>13</sup> C-PFOA)
Gas Temperature	350°C
Drying Gas (N2)	7.0 L/min

## 4. Analysis

### 4.1 Calibration

A 6-point calibration curve was analyzed throughout the analytical sequence for the fluorocompounds. The calibration points were prepared at 0.5, 1, 5, 10, 20, 50 ng/mL for PFOA. 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  $r^2 \geq 0.985$ .

For the results reported here, calibration criteria were met.

### 4.2 Surrogates

Surrogate spikes were not a part of this analysis.

### 4.3 Laboratory Control Spikes

Laboratory control spikes in the analytical set were prepared by adding a known concentration of the analytes to control human serum. Laboratory control spikes are used to assess method accuracy. The laboratory control spikes must show recoveries between 80-120% for levels at the LOQ and 85-115% for levels greater than the LOQ or the data is rejected. For the results reported here, the spikes were within the acceptable range.

### 4.4 Matrix Spikes

Two matrix spikes in the analytical set were prepared by adding a known concentration of the target analyte to separate human serum samples. Matrix spikes are used to assess method accuracy in the matrix. The matrix spikes should show recoveries between 85-115%. For the results reported here, the matrix spikes were within the acceptable range.

**4.5 Sample Related Comments**

Three samples in the analytical set were extracted and analyzed in duplicate. Duplicate sample results are reported along with the sample results in Attachment B.

**5 Data Summary**

Please see Attachment B for a detailed listing of the analytical results. Results are reported in parts per billion (ng/mL) for the analytes on an as-received basis.



**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 Exogen. Hardcopy data is stored for a minimum of five years. Occupational and Environmental Health 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

**8 Signatures**

 Emily R. Decker, Principal Investigator	<u>7/29/04</u> Date
 John M. Flaherty, Vice President	<u>7/29/04</u> Date



3058 Research Drive  
State College, PA 16801

Phone: 814-272-1039  
Fax: 814-231-1580

**Login**

**Login Group: L0003000**

Login #: 3110  
Project: P0000959

Conform COC Sample: True  
Conform COC: True  
Conform Sample: True  
Conform Request: True

Company Name: DuPont de Nemours & Co, Inc.  
Submitted By: Charles R. Powley  
Login Type: Immediate Receipt of Samples  
Started: T

Date Start: 07/23/2004  
Due Date: 08/06/2004  
Received Date: 07/23/2004  
Received By: Ammerman, Mark  
Spread Sample:

Label:  
Exygen SD/PI: Decker, Emily  
Project Title/Type: Analysis for PFOA in Human Serum Samples by LC/MS/MS / ROUTINE  
Login Notes:  
Conform Notes:

**Packages / Containers**

Package	Canon	Mail Date / Condition	Shipper / ID	Temp. Control/Temp.	Direction / Handled By
PK0003722		7/23/04 3:38:30PM Package & Contents Uncompromised	FEDEX 8458 8093 4889	Dry Ice 0.0	RECEIVED Ammerman, Mark

Container #	Gross Weight	pH	Container Type	Preservative	Mfg. Lot	Mfg. ID
C0040826	4.00 g		5 ml serum tube	NONE		
C0040828	4.40 g		5 ml serum tube	NONE		
C0040829	4.70 g		5 ml serum tube	NONE		
C0040831	4.60 g		5 ml serum tube	NONE		
C0040834	4.60 g		5 ml serum tube	NONE		
C0040836	4.30 g		5 ml serum tube	NONE		
C0040832	4.80 g		5 ml serum tube	NONE		
C0040841	4.60 g		5 ml serum tube	NONE		
C0040843	5.00 g		5 ml serum tube	NONE		
C0040844	4.80 g		5 ml serum tube	NONE		
C0040845	5.30 g		5 ml serum tube	NONE		
C0040846	4.00 g		5 ml serum tube	NONE		

EID871405

# Login

## Samples

Sample ID	Container	Matrix	Fraction	Sample	Date Sampled	Date Received	Date Due
L0003000-0001	C0040826	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0002	C0040828	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0003	C0040829	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0004	C0040831	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0005	C0040834	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0006	C0040836	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0007	C0040839	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0008	C0040841	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0009	C0040843	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0010	C0040844	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0011	C0040845	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004
L0003000-0012	C0040846	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004

CONFIDENTIAL

INFORMATION

REDACTED

EID871406





# CHAIN OF CUSTODY

Exygen Research Sample Receiving • 3048 Research Drive • State College, PA 16801  
T: 814.231.8032 • www.exygenresearch.com FAX: 814.231.1580

COLLECTION SITE INFORMATION	STAFF INVOLVED IN SAMPLE SHIPMENT
Collection Site (name & address): <u>Dr. Marsha Bailey</u> <u>#4 Rosemar Circle</u> <u>Barbersburg, WV 26601</u>  Phone: <u>304-757-0270</u> Fax: <u>304-757-0268</u> Email: <u>mbaileyjoeb@exygen.net</u>	1. <u>Lugh Ann Painter</u> <small>(Printed Name)</small> <u>[Signature]</u> <u>JAP 7-22-04</u> <small>Signature Initials/Date</small> 2. <u>Terri L. Thewen</u> <small>(Printed Name)</small> <u>[Signature]</u> <u>TLT 7-22-04</u> <small>Signature Initials/Date</small> 3. _____ <small>(Printed Name)</small> _____ <small>Signature Initials/Date</small>

**IMPORTANT:** Each person who enters information on this form must add their name, signature, initials, and date above. Line out unused prompts. Specify AM or PM for time entries. Include month, day, and year for dates. Contents of package must be verified by someone other than the packager; attest to the verification by initialing and dating the prompt. (\*) Thoroughly document the reason for any time gap between the time the serum preparation is completed and the time packaged by adding a footnote to the bottom of the page.

Sample Identification	Blood Sample Collection Date & Time	Serum Sample Prep. Completed Date & Time
	7-22-04 8:10 am	7-22-04 1:35 pm
	7-22-04 9:55 am	7-22-04 2:15 pm
	7-22-04 10:20 am	7-22-04 1:56 pm
	7-22-04 11:00 am	7-22-04 2:17 pm
	7-22-04 10:45 am	7-22-04 1:54 pm
	7-22-04 10:00 am	7-22-04 1:47 pm
	7-22-04 10:05 pm	7-22-04 1:45 pm
	7-22-04 10:35	7-22-04 2:28 pm
	7-22-04 8:40 am	7-22-04 2:26 pm
	7-22-04 11:20 am	7-22-04 2:23 pm
	7-22-04 8:10 am	7-22-04 1:38 pm
	7-22-04 8:20 am	7-22-04 1:30 pm

CONFIDENTIAL INFORMATION  
REDACTED

PLEASE FAX THE COMPLETED FORM to Exygen Sample Receiving prior to shipping samples.

Cooler Contents Verified Initial & Date → TLT 7/22/04

KEEP THE PINK COPY and include the other two with sample shipment.

Cooler ID #:

Relinquished by	Date	Time	Received by	Date	Time
			[Signature]	7/22/04	10:50

Note: When samples arrive at Exygen, chain-of-custody procedures follow Exygen SOP V401, "Contract Research Sample Handling." Page 1 of 1 REV. 1.11/03

EID871407

FedEx. USA Airbill  
Express  
845880934889

1 From  
On 7.22.04

To  
Dr. Martha Bailey  
Occupational & Environmental Health  
#11 Posner Circle  
Baltimore MD 21201

2 Your Internal Billing Reference  
To  
Eugen Bismarck  
3058 Bismarck Dr.  
State College PA 16801

3 To  
Eugen Bismarck  
3058 Bismarck Dr.  
State College PA 16801



Recipients Copy

4a Special Packaging Services  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

4b Special Freight Services  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

5 Packaging  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

6 Special Handling  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

7 Payment  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

8 Signature  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

9 Insurance  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

10 Tracking  
 Retail Ready Overlay  
 Retail Ready Overlay  
 Retail Ready Overlay

EID871408



**Summary of Residue Found (ng/mL) for PFOA  
in Human Serum Samples**

<b>Sponsor ID</b>	<b>PFOA (ppb)</b>
	103
	103
CONFIDENTIAL INFORMATION	85.0
	15.7
REDACTED	61.3
	90.4
	86.4
	50.8
	40.1
	128
	51.2
	83.1
	73.7
	78.5
	42.6

EID871409

**Summary of Recoveries (%) for PFOA  
in Human Serum Samples**

Sponsor ID	PFOA (ppb)	Amount Fortified (ppb)	Recovery (%)
CONFIDENTIAL INFORMATION REDACTED	5.10	5.0	102
	16.2	10	107
	1040	1000	103
	1090	1000	99
	4590	5000	90
AVERAGE:			100
STD DEV:			6.4
% RSD:			6.4

**Summary of PFOA (ng/mL)  
in Human Serum Control Samples**

Sponsor ID	PFOA (ng/mL)
Control A	5.47

EID871410