

AR 226-1871



Analytical Report

Occupational and Environmental Health

Analysis of Perfluorooctanoic Acid (PFOA) in Human Serum Samples

Exxygen Report No. L0003000

Testing Laboratory

**Exxygen Research
3058 Research Drive
State College, PA 16801**

Requester

**Dr. Marsha Bailey
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EID871401

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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 P858.

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 Method Preparation

3.1 Sample Preparation

The samples were extracted and analyzed according to the current revision of method Ed4-008-211. Fifty microliters of sample was used for the extraction procedure. Using the Multiprobe apparatus, 500 μ 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 μ 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 (¹³ C-PFOA)
Gas Temperature	350°C
Drying Gas (N2)	7.0 L/min

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 analyte 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.

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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 Ezyen. 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
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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 PFQA 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 8063 4888	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	4.80 g		5 ml serum tube	NONE		
C0040834	4.80 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	4.80 g		5 ml serum tube	NONE		
C0040843	5.00 g		5 ml serum tube	NONE		
C0040844	4.80 g		5 ml serum tube	NONE		
C0040846	5.30 g		5 ml serum tube	NONE		
C0040848	4.00 g		5 ml serum tube	NONE		

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Instance:

R0113316



Login

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	C0040826	LIQUID	Human Serum	CONFIDENTIAL	07/22/2004	07/23/2004	08/06/2004
L0003000-0003	C0040829	LIQUID	Human Serum	INFORMATION	07/22/2004	07/23/2004	08/06/2004
L0003000-0004	C0040831	LIQUID	Human Serum	REDACTED	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	C0040836	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	C0040848	LIQUID	Human Serum		07/22/2004	07/23/2004	08/06/2004

EID871406

7/29/04
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Instance: R0113316



[illegible]

PLEASE FAX THE COMPLETED FORM to Oxygen 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:

Received by	Date	Time
<i>[Signature]</i>	07/11/64	12:30

Note: When samples arrive at Brygan, chain-of-custody procedures follow Brygan SOP V401, "Contract Research Sample Handling."

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RS 1.1.03

EID871407

7.23.01

7.23.01

FedEx. USA Air Mail

845880934889

FCU 14143

if No. - Call 1

1. From: Dr. Martha Bailey 201 751.0220

2. To: Occupational & Environmental Health

3. Address: #4 Rossmore Circle

4. City: Baltimore Md 21201

5. Zip: 21201

6. Name: Eugen Research

7. Name: 305 Research Dr.

8. Name: State College PA 16801

9. Special Handling: ☐ Fragile ☐ Perishable ☐ Other

10. Insurance: ☐ Insured ☐ Not Insured

11. Signature: [Signature]

12. Date: 10

13. Tracking Number: 845880934889

14. Barcode: 

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

Sponsor ID	PFOA (ppb)
CONFIDENTIAL INFORMATION REDACTED	103
	103
	85.0
	15.7
	61.3
	80.4
	86.4
	60.6
	40.1
	128
	61.2
	83.1
	73.7
	78.8
	42.6

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**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
	18.2	10	107
	1040	1000	103
	1090	1000	99
	4890	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	5.67

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