STUDY TITLE
Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

DATA REQUIREMENTS
OECD Principles of Good Laboratory Practice, ENV/MC/CHEM(98)17, November 26, 1997

STUDY DIRECTOR
Emily R. Decker

STUDY COMPLETED ON
October 30, 2002

PERFORMING LABORATORY / TESTING FACILITY
Exygen Research
3058 Research Drive
State College, PA 16801
Phone: 814-272-1039

STUDY SPONSOR
3M Environmental Laboratory
Building 2-3E-09
St. Paul, MN 55133-3331
Phone: 651-778-6565

PROJECT
Study Plan Number: ExP-023-082
Exygen Study Number: 023-082
Sponsor Study Number: E02-1071

Total Pages: 111

000318

CONTAIN NO CBI
GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Exygen Study Number 023-082, entitled "Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071," conducted for 3M Environmental Laboratory, was performed in compliance with OECD Good Laboratory Practice Standards (as revised in 1997), ENV/MC/CHEM(98)17 by Exygen Research, with the following exceptions:

1. § 8.3 (5): The computerized system of data generation did not provide for the retention of a full audit trail to show all changes or to associate all changes to data to a timed and dated electronic signature.
2. § 6.2 (4): The stability of the test items under storage or the study test conditions was not known. Also the purity of C6 acid and THPFOS was not known.
3. § 5.2 (3): The date of receipt of for the calf serum sample ID 0204718 was not documented.
4. § 1.2.2 (g): The instrument used for the analysis has not been qualified.

Emily R. Decker
Study Director
Exygen Research

William K. Reagan, Ph.D.
Sponsor Representative
3M Environmental

Date
QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit of Exygen Research reviewed Exygen Study Number 023-082 entitled, "Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071." All phases were reviewed for conduct according to Exygen Research’s Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Date Inspected</th>
<th>Date Reported to Study Director</th>
<th>Date Reported to Exygen Management</th>
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<td>10/14/02</td>
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<td>10/25/02</td>
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<td>10/25-28/02</td>
<td>10/29/02</td>
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<td>4. Final Report Review</td>
<td>10/30/02</td>
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</tr>
</tbody>
</table>

Naomi Lovallo
Technical Lead-QA

10/30/02    Date

Exygen Research.
CERTIFICATION OF AUTHENTICITY

This report, for Exygen Study Number 023-082, is a true and complete representation of the raw data for the study.

Submitted by: Exygen Research
3058 Research Drive
State College, PA 16801
(814) 272-1039

Study Director, Exygen

Emily R. Decker
Scientist
Exygen Research

Exygen Research Facility Management:

John M. Flaherty
Vice President
Exygen Research

Sponsor Study Monitor, 3M:

William K. Reagan, Ph.D.
3M Environmental

Exygen Research.
STUDY IDENTIFICATION

Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

STUDY PLAN NUMBER: ExP-023-082
EXYGEN STUDY NUMBER: 023-082
SPONSOR STUDY NUMBER: E02-1071
TYPE OF STUDY: Residue
TEST SYSTEM: Human Serum, Human Plasma, and Monkey Serum
TEST ITEMS: perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundecanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluoroctane sulfonate (THPFOS), and tetrahydroperfluorodecane sulfonate (THPFDS)

SPONSOR: William K. Reagan- Sponsor Study Monitor
3M Environmental
Building 2-3E-09
St. Paul, MN 55133-3331

STUDY DIRECTOR: Emily R. Decker
Exygen Research
Phone: (814) 272-1039

TESTING FACILITY: Exygen Research
3058 Research Drive
State College, PA 16801

ANALYTICAL PHASE TIMETABLE:
Study Initiation Date: 10/09/02
Experimental Start Date: 10/15/02
Experimental Termination Date: 10/23/02
Study Completion Date: 10/30/02

Exygen Research.
**PROJECT PERSONNEL**

The Study Director for this project at Exygen Research was Emily R. Decker. The following personnel from Exygen Research were associated with various phases of the study:

<table>
<thead>
<tr>
<th>Name</th>
<th>Title</th>
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<tbody>
<tr>
<td>Paul Connolly</td>
<td>Technical Leader-LC/MS</td>
</tr>
<tr>
<td>Emily Decker</td>
<td>Scientist</td>
</tr>
<tr>
<td>Xiaoming Zhu</td>
<td>Technician</td>
</tr>
<tr>
<td>Rickey Keller</td>
<td>Sample Custodian</td>
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1.0 SUMMARY

Exygen Research conducted a quantitative screening on various human serum, human plasma, and monkey serum samples for the determination of perfluorooctane sulfonate (PFOS), perfluorohexanoate (C6), perfluoroheptanoate (C7), pentadecafluoroocotanoate (C8), heptadecafluorononanoate acid (C9), nonadecafluorodecanoate (C10), perfluoroundecanoate (C11), perfluorododecanoate (C12), tetrahydroperfluorooctane sulfonate (THPFO), and tetrahydroperfluorodecane sulfonate (THPFD) according to protocol ExP-023-082 (Appendix A). This screening was performed on an instrument that had not been used for routine fluorochemical analysis prior to this study. The method used for this study has not been validated at the levels reported for C8 and PFOS and not validated at any level for the other anions. These levels were completely dependent on instrument sensitivity.

Recoveries for fortified samples are given in Tables I-III. Residues of each anion in human serum are summarized in Table IV. Residues of each anion in human plasma are summarized in Table V. Residues of each anion in monkey serum are summarized in Table VI.

2.0 OBJECTIVE

The objective of this study was to screen human serum, human plasma, and monkey serum samples and quantitate to the lowest possible level according to instrument sensitivity.

3.0 INTRODUCTION

This report details the results of the analysis for perfluorooctane sulfonate (PFOS), perfluorohexanoate (C6), perfluoroheptanoate (C7), pentadecafluoroocotanoate (C8), heptadecafluorononanoate acid (C9), nonadecafluorodecanoate (C10), perfluoroundecanoate (C11), perfluorododecanoate (C12), tetrahydroperfluorooctane sulfonate (THPFO), and tetrahydroperfluorodecane sulfonate (THPFD) in human serum, human plasma, and monkey serum samples.

The study was initiated on October 09, 2002, when the study director signed study plan number ExP-023-082. The experimental start date was October 15, 2002, and the experimental termination date was October 23, 2002.

4.0 TEST SYSTEM

Pooled human serum samples were purchased by the sponsor from Sigma-Aldrich, Milwaukee, WI, Lampire Biological Laboratories, Pipersville, PA, Bioresource Exygen Research.
Technology, Inc., Fort Lauderdale, FL, and Golden West Biologicals, Temecula, CA. Pooled monkey serum samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA. Pooled human plasma samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA, Bioresource Technology, Inc., Fort Lauderdale, FL, Golden West Biologicals, Temecula, CA, and Innovative Research, Inc. Southfield, MI. In addition, blank matrix consisting of pooled human plasma collected in rural China was provided by the sponsor. Also, calf serum was purchased from Sigma-Aldrich by Exxygen.

<table>
<thead>
<tr>
<th>Exxygen ID</th>
<th>Sponsor ID</th>
<th>Matrix</th>
<th>Source</th>
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<tbody>
<tr>
<td>0203963</td>
<td>Lot 020821</td>
<td>Human Serum</td>
<td>BioResource</td>
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<tr>
<td>0203964</td>
<td>Lot 22K0965</td>
<td>Human Serum</td>
<td>Sigma-Aldrich</td>
</tr>
<tr>
<td>0203965</td>
<td>Lot G0140604</td>
<td>Human Serum</td>
<td>Golden West Biologicals</td>
</tr>
<tr>
<td>0204292</td>
<td>X328-A</td>
<td>Human Serum</td>
<td>Lampire</td>
</tr>
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<td>0204334</td>
<td>TCR-684</td>
<td>Human Plasma</td>
<td>Golden West Biologicals</td>
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<tr>
<td>0204335</td>
<td>TN-A-06332</td>
<td>Monkey Serum</td>
<td>Lampire</td>
</tr>
<tr>
<td>0204490</td>
<td>TCR-674</td>
<td>Human Plasma</td>
<td>3M (plasma from rural China)</td>
</tr>
<tr>
<td>0204991</td>
<td>TN-A-6337</td>
<td>Human Plasma</td>
<td>Lampire</td>
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<tr>
<td>0204492</td>
<td>TN-A-06333</td>
<td>Monkey Serum</td>
<td>Lampire</td>
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<td>0204493</td>
<td>TN-A-06336</td>
<td>Monkey Serum</td>
<td>Lampire</td>
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<tr>
<td>0204718</td>
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<tr>
<td>0204747</td>
<td>TCR-683</td>
<td>Human Plasma</td>
<td>Innovative Research</td>
</tr>
</tbody>
</table>

Samples were received frozen on dry ice and then placed in frozen storage (≤-10°C) until samples were logged in by Exxygen personnel. All records concerning sample receipt, processing and storage can be found in the raw data package associated with this study.

5.0 TEST ITEMS

The analytical standards PFOS, C6, C7, C8, C9, C10, C11, C12, THPFOS, and THPFDS were received at Exxygen on September 30, 2002 from 3M Environmental Technology and Services. The available information for the reference material is listed below. The reference material was stored frozen.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Exxygen Inventory No.</th>
<th>Lot No.</th>
<th>Purity (%)</th>
<th>Expiration Date</th>
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<tbody>
<tr>
<td>PHAA (C6)</td>
<td>SP0002086</td>
<td>NB 117735-32</td>
<td>TBD</td>
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<td>PFOA (C8)</td>
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<td>210002</td>
<td>&gt;97</td>
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<tr>
<td>PFNA (C9)</td>
<td>SP0002085</td>
<td>H7568</td>
<td>&gt;99</td>
<td>07/19/07</td>
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<tr>
<td>C10</td>
<td>SP0002090</td>
<td>R11K</td>
<td>98</td>
<td>12/01/10</td>
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<td>SP0002093</td>
<td>U11N</td>
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<td>SP0002092</td>
<td>PMR-269-83</td>
<td>94.7</td>
<td>08/22/12</td>
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</table>

Exxygen Research.
The molecular structures of the anions are given below.

Name: PFOS
Chemical Name: Perfluorooctanesulfonate
Molecular Weight: 499, as shown

Name: C6
Chemical Name: Perfluorohexanoate
Molecular Weight: 313, as shown

Name: C7
Chemical Name: Perfluoroheptanoate
Molecular Weight: 363, as shown

Name: C8
Chemical Name: Pentadecafluorooctanoate
Molecular Weight: 413, as shown
Name: C9
Chemical Name: Heptadecafluorononanoate
Molecular Weight: 463, as shown

Name: C10
Chemical Name: Nonadecafluorodecanoate
Molecular Weight: 513, as shown

Name: C11
Chemical Name: Perfluoroundecanoate
Molecular Weight: 563, as shown

Name: C12
Chemical Name: Perfluorododecanoate
Molecular Weight: 613, as shown
Name: THPPOS
Chemical Name: Tetrahydroperfluoroctane sulfonate
Molecular Weight: 427, as shown

Name: THPFDS
Chemical Name: Tetrahydroperfluorodecane sulfonate
Molecular Weight: 527, as shown

6.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Method of Analysis for the Determination of Perfluoroheptanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine" was used for this study. For this study, several modifications were made and have been documented in the protocol/protocol deviations.

6.1 Extraction Procedure

a. Measure 2 mL of serum sample into a 15 mL disposable centrifuge tube and fortify, if appropriate.

b. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.

c. Centrifuge tubes at ~3000 rpm for ~ 5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

d. Load the sample onto a conditioned SPE column. Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.

e. Elute with 5 mL of methanol and then evaporate to less than 1 mL using a nitrogen evaporator. Bring final volume up to 1 mL with methanol.

f. Analyze samples using electrospray LC/MS/MS.

The volume of sample used and the volume of methanol used for elution were different than those cited in the method. This was done to allow for lower quantification limits for the anions in this study.
6.2 Preparation of Standards and Fortification Solutions

Individual stock solutions of all of the anions were prepared on October 02, 2002, as specified in method ExM-023-071. The stock standard solutions were prepared at a concentration of ~100 μg/mL by dissolving ~10 mg of the standard (corrected for purity and salt content when appropriate) in methanol.

From these solutions, a 1.0 μg/mL mixed fortification standard solution was prepared by transferring the appropriate volume (~0.4 - 1 mL) of each of the stock solutions into a 100-mL volumetric flask and bringing the volume up to the mark with methanol.

The 0.1 μg/mL mixed fortification standard was prepared by transferring 10 mL of the 1.0 μg/mL mixed fortification standard into a volumetric flask and bringing the volume up to 100 mL with methanol.

A set of calibration standards were prepared by dilution in the following manner:

<table>
<thead>
<tr>
<th>Initial Conc. (ng/mL)</th>
<th>Volume (mL)</th>
<th>Diluted to (mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
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<tr>
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<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>100</td>
<td>0.5</td>
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<td>10</td>
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<td>10</td>
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</tr>
<tr>
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<td>1</td>
<td>10</td>
<td>0.1</td>
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</table>

The stock standard solutions and all fortification and calibration standard solutions were stored in a refrigerator (6° ± 2°C) when not in use.

6.3 Chromatography

Quantification was accomplished by electrospray LC/MS/MS analysis. An API 4000 Sciex system was used in this study because of its greater sensitivity and also because it had not been used for fluorochemical analysis prior to this study. Peaks were detected in the control matrices corresponding to some of the target anions, especially for C8.

6.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run had a concentration of 0.1 ng/mL, which corresponds to a concentration of 0.05 ng/mL (ppb) in the extracted samples. Residues were calculated below this level where the response of the anion was approximately three times the signal to noise ratio. The results were Exygen Research.
reported as Not Detected (ND) if the response was approximately less than three times the signal to noise ratio and Not Quantifiable (NQ) was used for negative results. All other responses were reported.

6.5 Description of Instrument and Operating Conditions

Instrument: PE SCIEX API 4000 Biomolecular Mass Analyzer, (LC/MS/MS #8) SCIEX Turbo Ion Spray Liquid Introduction Interface Turbo Ion spray temperature = 350 °C Auxiliary gas flow = ~ 7.0 L/min Harvard Infusion Pump

Computer: Dell OptiPlex GX 110

Software: PE Sciex Analyst 1.2

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

HPLC Column: Genesis C-8, 5 cm x 2.1 mm i.d. x 4 μ (Exygen ID: 71A) (JONESCHROMATOGRAPHY: Part No. FK5962E)

Column Temperature: 35°C

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

Mobile Phase (B): Methanol

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% A</th>
<th>% B</th>
<th>Flow Rate (mL/min)</th>
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<td>0.3</td>
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</tbody>
</table>

Injected Volume: 15 μL

Ions monitored:

<table>
<thead>
<tr>
<th>Anion</th>
<th>Parent ion</th>
<th>Daughter ion</th>
<th>Dwell (secs)</th>
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<th>Collision Energy</th>
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6.6 Quantitation and Example Calculation

Fifteen microliters of sample or calibration standard were injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x weighted linear regression) by Analyst software using seven concentrations of standards prepared in methanol. The residue concentration for the samples was determined from the following equations:

Use Equation 1 to calculate the amount of anion found (in ng/mL, based on peak area) using the standard curve (1/x weighted linear regression parameters) generated by the Analyst software program.

Equation 1:

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

Use Equation 2 to calculate the amount of analyte found (in ppb)

Equation 2:

\[
\text{Analyte found (ppb = ng/mL)} = \frac{(\text{analyte found (ng/mL) x FV (mL) x DF}}}{\text{sample volume (mL})}
\]

\[
\text{FV = final volume}
\]
\[
\text{DF = dilution factor}
\]

For samples fortified with known amounts of analyte prior to extraction, use Equation 3 to calculate the percent recovery (ppb = ng/mL)

Equation 3:

\[
\text{Recovery (%) =}
\]

\[
\frac{[\text{total analyte found (ppb)} - \text{analyte found in control or sample (ppb)]}}{\text{analyte added (ppb)}} \times 100
\]

**Note:** Any analyte found in the control was subtracted from analyte found. However, the response for the sample duplicate was not used.
An example of a calculation using an actual sample follows:

Human Serum Sample, Exygen ID 0204491 Spk J (Data Set: 101702A), fortified with 0.5 ng/mL (calculation is using values for C6):

Where:

- peak area = 54636
- intercept = 632.277
- slope = 59435.9
- dilution factor = 1
- ng/mL added (fort level) = 0.5 ng/mL
- avg. amt in controls = 0 (Not detected)
- final volume = 1 mL
- sample volume = 2 mL

From equation 1:

\[
\text{Analyte found (ng/mL)} = \frac{[54636 - 632.277]}{59435.9}
\]

\[
= 0.9 \text{ ng/mL}
\]

From equation 2:

\[
\text{Analyte found (ppb)} = \frac{0.9 \text{ ng/mL} \times 1 \text{ mL}}{2 \text{ mL}}
\]

\[
= 0.45 \text{ ppb (ng/mL)}
\]

From equation 3:

\[
\% \text{ Recovery} = \frac{(0.45 \text{ ng/mL} - 0 \text{ ng/mL}) \times 100}{0.5 \text{ ng/mL}}
\]

\[
= 90\%
\]

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the raw data.
7.0 EXPERIMENTAL DESIGN

For the screening of each sample, duplicate extractions were performed. Also, each sample was fortified at 0.5 ng/mL and 5.0 ng/mL and then taken through the extraction procedure. Two calibration curves were also taken through the extraction procedure, one using calf serum and one using human plasma. These were treated as quality control fortifications in the data set and were not used for the calibration curve. Since there was residue detected in the samples for THPFOS and THPFDS, an additional analysis in which a three-daughter ion confirmation was performed.

8.0 RESULTS

There was no significant residue detected in the reagent blank analyzed with these samples. Also, there was no carry-over present for any of the anions in the instrument blanks (methanol washes) analyzed in the analytical sets, except for C8 and C9, and this is most likely contributed to those analytes being present in the instrumental system, particularly in the mobile phase. This is especially evident with the absence of the anions (except for C8 and C9) in the methanol wash analyzed after the injection of the 10 ng/mL calibration standard. All fortifications were at a level equal to or less than the 10 ng/mL standard. Since there was no carry-over observed after the injection of this standard, the carry-over present after proceeding injections would be minimal. A representative chromatogram of a standard prepared in methanol can be found in Figure 1.

Recoveries for fortified samples are given in Tables I-III. Recoveries outside the suggested range of 70% to 130% were reported, however this method has not been validated at these low levels and some of the recoveries were outside of this range because the level of residue in the sample was significantly greater than the amount fortified, especially for C8 and PFOS. Example chromatograms of fortified samples are shown in Figure 2.

Residues of each anion in human serum are summarized in Table IV. Residues of each anion in human plasma are summarized in Table V. Residues of each anion in monkey serum are summarized in Table VI. Example chromatograms of a human plasma sample are given in Figure 3. The detection of THPFDS in some of the samples warranted further investigation. The presence of THPFOS and THPFDS was confirmed with a re-analysis with additional daughter ion confirmation. A chromatogram detailing the three daughter ion confirmation of THPFDS is given in Figure 4.
9.0 CONCLUSIONS

The quantitative screening of these serum and plasma samples produced levels of certain analytes at extremely low levels (< 100 ppt). These levels are based solely on the instrument sensitivity and not the method recovery. The results contained in this report should be evaluated as a quantitative screening. Contamination of these samples due to instrument conditions is very limited because the instrument used for the analyses had never been used for routine fluorochemical analysis prior to the initiation of this study. No carry-over was observed throughout the injections of the analytical sets, which was demonstrated with the absence of the target analytes in the methanol washes analyzed after the injection of the highest level of calibration standard (10 ng/mL).

Two people took a set of 64 samples through the sample preparation procedure in approximately 10 hours and the analysis by LC/MS/MS took approximately 48 hours.

10.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

The method used in this study has not been validated for C8 and PFOS at the levels given in this report and at any level for the rest of the anions. Residues were reported lower than the lowest calibration standard.

11.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Exygen Research will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the archives of Exygen Research for the lifetime of the product. Sponsor permission will be obtained before discarding.
Table I  Summary of Recoveries for Calibration Curve in Calf Serum Compared to Standards in Methanol

<table>
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AVG: 96 109 122 124 121 107 107 148 131

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RELATIVE STANDARD DEVIATION: 6.5 7.5 15.6 12.1 15.4 7.6 10.7 5.2 7.3

Table II  Summary of Recoveries for Calibration Curve in Human Plasma Compared to Standards in Methanol

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AVG: 93 113 126 102 81 114 111 146 130

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RELATIVE STANDARD DEVIATION: 10.9 8.5 11.5 10.7 35.8 9.9 5.6 8.5 9.0

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.
Table III  Summary of Recoveries for Laboratory Fortified Matrix Spikes

Calf Serum

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AVG: 91 98 96 112 105 96 103 ** 131 117
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RELATIVE STANDARD DEVIATION: 10.9 18.1 5.2 18.4 13.5 0.7 3.4 ** 14.0 10.9

Human Serum

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AVG: 83 111 197 129 114 124 125 ** 195 141
STANDARD DEVIATION: 24.3 17.6 113.1 32.6 14.0 17.7 17.8 ** 73.7 31.4
RELATIVE STANDARD DEVIATION: 29.3 15.9 57.3 25.3 12.4 14.3 14.2 ** 37.8 22.3

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.
Table III (cont') Summary of Recoveries for Laboratory Fortified Matrix Spikes

Human Plasma

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<th>Sponsor ID</th>
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<th>C8</th>
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AVG: 86 95 162 119 105 96 97 ** 119 111

STANDARD DEVIATION: 9.4 13.9 108.0 35.3 17.8 15.3 11.1 ** 17.0 15.7

RELATIVE STANDARD DEVIATION: 11.0 14.7 66.7 29.7 17.0 16.0 11.4 ** 14.3 14.1

Monkey Serum

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sponsor ID</th>
<th>Fort Level (ng/mL)</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
<th>C11</th>
<th>C12</th>
<th>PFOS</th>
<th>THPFOS</th>
<th>THPFDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0204335 Spk I</td>
<td>TN-A-06332</td>
<td>0.5</td>
<td>106</td>
<td>91</td>
<td>68</td>
<td>52</td>
<td>88</td>
<td>85</td>
<td>95</td>
<td>**</td>
<td>114</td>
<td>129</td>
</tr>
<tr>
<td>0204492 Spk K</td>
<td>TN-A-06333</td>
<td>0.5</td>
<td>122</td>
<td>112</td>
<td>129</td>
<td>134</td>
<td>117</td>
<td>129</td>
<td>125</td>
<td>**</td>
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<td>155</td>
</tr>
<tr>
<td>0204493 Spk L</td>
<td>TN-A-06336</td>
<td>0.5</td>
<td>123</td>
<td>98</td>
<td>97</td>
<td>102</td>
<td>115</td>
<td>93</td>
<td>94</td>
<td>**</td>
<td>132</td>
<td>120</td>
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<tr>
<td>0204335 Spk S</td>
<td>TN-A-06332</td>
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<td>100</td>
<td>93</td>
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<td>107</td>
<td>108</td>
<td>105</td>
<td>109</td>
<td>**</td>
<td>107</td>
<td>104</td>
</tr>
<tr>
<td>0204492 Spk U</td>
<td>TN-A-06333</td>
<td>5.0</td>
<td>88</td>
<td>85</td>
<td>100</td>
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<td>97</td>
<td>93</td>
<td>101</td>
<td>**</td>
<td>124</td>
<td>116</td>
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AVG: 109 97 106 101 106 102 105 ** 130 124

STANDARD DEVIATION: 13.7 9.3 20.6 27.4 11.1 15.5 11.5 ** 19.8 17.2

RELATIVE STANDARD DEVIATION: 12.5 9.6 20.4 27.2 10.5 15.2 10.9 ** 15.3 13.9

** Recovery not applicable because the residues detected in sample were significantly greater than the amount fortified.
Table IV  Summary of Residues for Human Serum Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sponsor ID</th>
<th>C6</th>
<th>C7</th>
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<th>C9</th>
<th>C10</th>
<th>C11</th>
<th>C12</th>
<th>PFOS</th>
<th>TPHPFOS</th>
<th>THPFDS</th>
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<tbody>
<tr>
<td>0203963</td>
<td>Lot 020821</td>
<td>ND</td>
<td>ND</td>
<td>2.83</td>
<td>0.683</td>
<td>0.228</td>
<td>0.371</td>
<td>0.0333</td>
<td>32.0</td>
<td>ND</td>
<td>0.0743</td>
</tr>
<tr>
<td>0203963 Dup</td>
<td>Lot 020821</td>
<td>ND</td>
<td>ND</td>
<td>2.93</td>
<td>0.684</td>
<td>0.22</td>
<td>0.373</td>
<td>0.0282</td>
<td>33.3</td>
<td>ND</td>
<td>0.0599</td>
</tr>
<tr>
<td>0203964</td>
<td>Lot 22K0965</td>
<td>ND</td>
<td>ND</td>
<td>0.0940</td>
<td>1.45</td>
<td>0.307</td>
<td>0.107</td>
<td>0.0942</td>
<td>0.0122</td>
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<td>ND</td>
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<tr>
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<td>Lot 22K0965</td>
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<td>ND</td>
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<td>0.276</td>
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<td>0.0172</td>
<td>8.95</td>
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<tr>
<td>0203965</td>
<td>Lot G0140604</td>
<td>0.0687</td>
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<td>0.214</td>
<td>0.207</td>
<td>0.0199</td>
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<td>0.0846</td>
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<tr>
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<td>Lot G0140604</td>
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<tr>
<td>0204292</td>
<td>X328-A</td>
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<td>0.634</td>
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<td>0.137</td>
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<tr>
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<td>4.21</td>
<td>0.772</td>
<td>0.221</td>
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<td>20.6</td>
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Table V  Summary of Residues for Human Plasma Samples

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<th>Sample ID</th>
<th>Sponsor ID</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
<th>C11</th>
<th>C12</th>
<th>PFOS</th>
<th>TPHPFOS</th>
<th>THPFDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0204334</td>
<td>TCR-684</td>
<td>ND</td>
<td>ND</td>
<td>0.0925</td>
<td>2.57</td>
<td>0.174</td>
<td>0.108</td>
<td>0.0797</td>
<td>ND</td>
<td>14.8</td>
<td>0.0140</td>
</tr>
<tr>
<td>0204334 Dup</td>
<td>TCR-684</td>
<td>ND</td>
<td>ND</td>
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<td>0.198</td>
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<td>0.0695</td>
<td>ND</td>
<td>14.9</td>
<td>ND</td>
</tr>
<tr>
<td>0204490</td>
<td>TCR-674</td>
<td>ND</td>
<td>ND</td>
<td>0.0887</td>
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<td>0.0252</td>
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<td>0.115</td>
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<td>0.130</td>
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<td>0204747</td>
<td>TCR-683</td>
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<td>0.178</td>
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<td>19.4</td>
<td>0.0292</td>
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Table VI  Summary of Residues for Monkey Serum Samples

<table>
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<th>Sample ID</th>
<th>Sponsor ID</th>
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<th>C7</th>
<th>C8</th>
<th>C9</th>
<th>C10</th>
<th>C11</th>
<th>C12</th>
<th>PFOS</th>
<th>TPHPFOS</th>
<th>THPFDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0204335</td>
<td>TN-A-06332</td>
<td>ND</td>
<td>ND</td>
<td>1.25</td>
<td>3.24</td>
<td>0.286</td>
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<td>17.2</td>
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<td>0.0141</td>
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<tr>
<td>0204335 Dup</td>
<td>TN-A-06332</td>
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<td>ND</td>
<td>1.72</td>
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<td>0.0464</td>
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<td>ND</td>
</tr>
<tr>
<td>0204492</td>
<td>TN-A-06333</td>
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<td>ND</td>
<td>NQ</td>
<td>NQ</td>
<td>NQ</td>
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<td>0.137</td>
<td>ND</td>
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<td>ND</td>
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<tr>
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<td>TN-A-06333</td>
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<td>ND</td>
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<td>NQ</td>
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</tr>
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<td>ND</td>
<td>ND</td>
<td>NQ</td>
<td>NQ</td>
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<td>ND</td>
</tr>
<tr>
<td>0204493 Dup</td>
<td>TN-A-06336</td>
<td>ND</td>
<td>ND</td>
<td>NQ</td>
<td>NQ</td>
<td>NQ</td>
<td>0.106</td>
<td>0.0321</td>
<td>18.1</td>
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<td>ND</td>
</tr>
</tbody>
</table>

ND = Not Detected
NQ = Not Quantifiable (negative residue calculated)
Figure 1  Chromatogram Representing 0.1 ng/mL Calibration Standard
Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard
Figure 1 (cont) Chromatogram Representing 0.1 ng/mL Calibration Standard
Figure 1 (cont)  Chromatogram Representing 0.1 ng/mL Calibration Standard
Figure 2 Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb (Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)
Figure 2 (cont') Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb
(Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)
Figure 2 (cont') Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb
(Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)
Figure 2 (cont')  Chromatogram Representing a Fortified Human Plasma Sample at 0.5 ppb
(Exygen ID: 0204490 Spk J, Sponsor ID: TCR-674)
Figure 3
Chromatogram Representing a Human Plasma Sample (Exogen ID: 0204490, Sponsor ID: TCR-674)
Figure 3 (cont')  Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)
Figure 3 (cont')  Chromatogram Representing a Human Plasma Sample (Exygen ID: 0204490, Sponsor ID: TCR-674)
Figure 4  Chromatogram Representing a Sample Analyzed for Three Daughters for THPFDS
(Exygen ID: 0204292 Dup, Sponsor ID: X328-A).
APPENDIX A

Exygen Study Plan
ExP-023-082
(Exygen Study No. 023-082)
and
Deviations
STUDY PLAN

Study Title:
Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082

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

Study Sponsor:
3M Environmental Laboratory
Building 2-3E-09
St. Paul, MN 55133-3331
Phone: (651) 778-6565
DISTRIBUTIONS:

1) Emily R. Decker, Study Director, Exygen Research
2) William K. Reagen, Sponsor Study Monitor, 3M
3) Exygen Research Quality Assurance Unit
STUDY PLAN APPROVAL

Study Title: Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082

This study plan was audited by the Quality Assurance Unit of Exygen Research.

[Signature] [Date]
Naomi Lovajo
Technical Lead-QA

APPROVALS

[Signature] [Date]
Emily R. Decker, Study Director
Exygen

[Signature] [Date]
John Flaherty, Vice President, Facility Management
Exygen

[Signature] Date
William Reagen, Sponsor Study Monitor
3M
Study Plan: ExP-023-082
Exygen Study No: 023-082

Study Title: Analysis of Pooled Human Sera and Plasma and Monkey Sera for Fluorocarbons Using Exygen Method ExM-023-071

Study Plan Number: ExP-023-082
Exygen Study Number: 023-082

This study plan was audited by the Quality Assurance Unit of Exygen Research.

Ramini Lovallo
Technical Lead-QA

APPROVALS

Emily R. Decker, Study Director
Exygen

John Flaherty, Vice President, Facility Management
Exygen

William Reagen, Sponsor Study Monitor
3M

Date

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Received Time: Oct. 3, 4:13PM  Print Time: Oct. 9, 4:14PM
Exygen Research

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The purpose of this study is to perform analysis for perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundeccanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOs), and tetrahydroperfluordecanesulfonate (THPFDs) in pooled human serum and plasma and monkey sera using Exygen method ExM-023-071 entitled "Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluoroctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine."

The study will be audited for compliance with OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHRM(98)17 by the Quality Assurance Unit of Exygen Research.

The test items are perfluorooctane sulfonate (PFOS), perfluorohexanoic acid (C6), perfluoroheptanoic acid (C7), pentadecafluorooctanoic acid (C8), heptadecafluorononanoic acid (C9), nonadecafluorodecanoic acid (C10), perfluoroundeccanoic acid (C11), perfluorododecanoic acid (C12), tetrahydroperfluorooctane sulfonate (THPFOs), and tetrahydroperfluordecanesulfonate (THPFDs). All test items were received from the Sponsor.

Name: PFOS
Chemical Name: Perfluoroctanesulfonate
Molecular Weight: 499, as shown

\[
\begin{align*}
&\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{SO}_3^- \\
&\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{F} &\text{F}
\end{align*}
\]
Name: C6
Chemical Name: Perfluorohexanoic acid
Molecular Weight: 313, as shown

Name: C7
Chemical Name: Perfluorohexanoic acid
Molecular Weight: 363, as shown

Name: C8
Chemical Name: Pentadecafluorooctanoic acid
Molecular Weight: 413, as shown

Name: C9
Chemical Name: Heptadecafluorononanoic acid
Molecular Weight: 463, as shown
Name: C10
Chemical Name: Nonadecasfluorodecanoic acid
Molecular Weight: 513, as shown

Name: C11
Chemical Name: Perfluoroundecanoic acid
Molecular Weight: 563, as shown

Name: C12
Chemical Name: Perfluorododecanoic acid
Molecular Weight: 613, as shown

Name: THPFOs
Chemical Name: Tetrahydroperfluoroctane sulfonate
Molecular Weight: 427, as shown
Name: THPFDS
Chemical Name: Tetrahydroperfluorodecane sulfonate
Molecular Weight: 527, as shown

![Molecular Structure](image)

**OBJECTIVE**

The purpose of this study is to perform analysis on four different lots of pooled human serum, four different lots of pooled human plasma, and three lots of pooled monkey serum for the target fluorocompounds using the analytical method, "Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine."

**TESTING FACILITY**

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

**STUDY DIRECTOR**

Emily Decker
Scientist
Exygen Research
Phone: (814) 272-1039
emily.decker@exygen.com

**SPONSOR**

3M Environmental Laboratory
Building 2-3E-09
St. Paul, MN 55133-3331
Phone: (651) 778-6565
It is proposed that the analytical portion of this study be conducted from October 14 to October 21, 2002. The actual experimental start and termination dates will be included in the final report.

All communications between the Testing Facility, method developers, and the Sponsor will be directed through the Study Director (or designate) and the Sponsor Study Monitor. Communications will be fully documented by the Study Director.

Pooled human sera and plasma and monkey sera are used as the test systems in this study. The matrices will be provided by the sponsor. The matrices will be representative of that for which this analytical method was designed.

Pooled human serum samples were purchased by the sponsor from Sigma-Aldrich, Milwaukee, WI, Lampire Biological Laboratories, Pipersville, PA, Bioresource Technology, Inc., Fort Lauderdale, FL, and Golden West Biologicals, Temecula, CA. Pooled monkey serum samples were purchased by the sponsor from Lampire Biological Laboratories, Pipersville, PA. Pooled human plasma samples were purchased by the sponsor from Lampire Biological Laboratories.
In addition to the calibration standards described in Section 3.5.3, a set of calibration standards will be processed through the extraction procedure identical to the samples, using bovine serum and also a set using human plasma. The fortification of the standards before extraction is done according to the following table:

<table>
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<tr>
<th>Conc. Of Mixed Fortification Solution (ng/mL)</th>
<th>Fortification Volume (µL)</th>
<th>Volume of Control Sample (mL)</th>
<th>Conc. of Extracted Calibration Standard (ng/mL)</th>
</tr>
</thead>
<tbody>
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<td>0.2</td>
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</table>

**Verification of Analytical Procedure**

The testing facility will establish the relationship between the instrument response and the concentration of analyte in order to assess the linearity of the system. A standard curve should be constructed with at least five standards. The testing facility should also verify the endogenous levels of analyte in the matrix control samples. This should be accomplished by analysis of a control sample for each matrix and examination of the region of analyte retention. The potential exists for interference from fluorochromes introduced from dietary material and other exogenous sources. Samples are fortified with the target analytes. The compounds will be made into solutions as per the method, and added to the matrices via a micropipette. Fortified samples will be processed through the described procedures to ensure method accuracy and to check for bias.

Recoveries should be between 70% and 130% of the fortified levels. The sponsor may accept occasional recoveries outside of this range. The relative standard deviation (RSD) for each fortification level as well as the overall RSD, should be less than or equal to 20%.

Any modifications to the analytical method will be documented in the study raw data. Modifications deemed significant by the Sponsor or Study Director will necessitate revision of the method.
Control of bias will be addressed by taking representative sub-samples from a homogeneous mixture of each matrix for untreated control samples, and by analyzing at least two levels of fortifications.

Statistics will be limited to those specified in the subject method and to the calculation of average recoveries, as applicable.

All aspects of this study shall be performed and reported in compliance with OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17. The final report or data package (supplied to the Sponsor) shall contain a statement that the study was conducted in compliance with current and applicable GLP standards and will outline any deviations in the study from those standards. This statement will be signed by the Study Director and Sponsor.

A final report will be prepared by the study director or their designee at the conclusion of the study. The report will include, but will not be limited to, the following:

- The name and address of the Study Director, Sponsor Monitor and of the testing facility.
- A statement of GLP compliance (any related documentation, such as chain-of-custody records, must be in the study records).
- The signed and dated statement by the Exygen Research Quality Assurance Unit regarding dates of study inspections and dates findings were reported to the Study Director and Management.
A description of the exact analytical conditions employed in the study. If the subject method was followed exactly, it is necessary to include only a copy of the analytical method. Any modifications to this method will be incorporated into the report. If the method is photo-reduced, the project number and page number must be included on each page.

- Any steps considered critical, i.e. steps where little variation is allowable or directions must be followed precisely.

- Description of the instrumentation used and operating conditions.

- The number of worker-hours or calendar days required to complete one set of samples.

- All results from all sets analyzed. Identify all control and fortified samples, and in the data table include sample number, fortification level, and unique identification number by sample set.

- Representative chromatograms for each analyte in each matrix, including chromatograms of a standard and a control sample, and a chromatogram at a fortification level. The location of the analyte peaks will be clearly identified in all chromatograms.

- All circumstances that may have affected the quality or integrity of the data will be documented in the report.

- Locations where raw data and the final report are to be archived.

- Additions or corrections to the final report shall be in the form of an amendment by the Study Director. The amendment shall clearly identify that part of the report that is being altered and the reasons for the alterations. The amendment will be signed and dated by the Study Director and the Sponsor Study Monitor.

**SAFETY AND HEALTH**

- Laboratory personnel will practice good sanitation and health habits.

- Any health condition of laboratory personnel that may be considered to adversely affect the study will be reported to the Study Director.

- Any injury to laboratory personnel occurring during the conduct of this study will be reported to the study director.
• Every reasonable precaution shall be taken to prevent inadvertent exposure of personnel and the environment to the test or reference substance(s).

**AMENDMENT TO STUDY PLAN**

All significant changes to the study plan outlined here will be expressed in writing, signed and dated by the Study Director and Sponsor Study Monitor. Amendments usually will be issued prior to initiation of study plan change. However, when a change is required without sufficient time for the issue of a written amendment, that change may be effected verbally with supporting documentation signed and dated by the Study Director and followed with a written amendment as soon as possible. In this case, the effective date of the written amendment will be the date of the documented change. Copies of the signed amendments will be appended to all distributed study plan copies. The original amendment will be maintained with the original study plan. Any deviations from the study plan or from the analytical method as provided will be documented and reported promptly to the Sponsor Study Monitor.

**DATA RECORD-KEEPING**

Records to be maintained include the following (as appropriate):

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

**Chromatograms** - All chromatograms will contain the following:

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

• Additionally, fortifications will include the amount of analyte added and the sample number of the sample that was fortified.
• Analytical standard chromatograms will additionally include the concentration (e.g., μg/mL).

• As part of the documentation the following sheets will be included in each analytical set: a run sheet listing the samples to be run in the set, and an instrument conditions sheet describing the instrument type and operating conditions.

QUALITY ASSURANCE

The QA Unit of Exogen Research will inspect the study at intervals adequate to assure compliance with GLP's, and will report the findings of audits to the Study Director, Exogen Management, and the Sponsor Study Monitor.

RETENTION OF DATA AND RECORDING

All hard copy raw data, including, but not limited to, the original chromatograms, worksheets, correspondence, and results shall be included with the data package submitted to the Sponsor. These will be archived with the original study plan, amendments, final report, and all pertinent information from the Sponsor.

The testing facility shall keep all electronic raw data and any instrument, equipment, and storage logs for the lifetime of the product and shall obtain permission of the sponsor before discarding.
APPENDIX I

METHOD

"Method of Analysis for the Determination of Perfluorohexanesulfonate (PFHS), Perfluorooctanesulfonate (PFOS) and Pentadecafluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine"
TITLE
Method of Analysis for the Determination of Perfluorobutanesulfonate (PFBS), Perfluorooctanesulfonate (PFOS) and Perfluorodecanoic Acid (PFDA) in Rat Liver, Serum and Urine

AUTHORS
John Fishery, Karen Riska, and Emily Docker

DATE ISSUED
July 30, 2002

SPONSOR
3M Medical Department Corporate Toxicology
3M Center, Building 220-2E-32
St. Paul, MN 55144-1000

PERFORMING LABORATORY
Exygen Research
3058 Research Drive
State College, PA 16801

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MANAGEMENT APPROVAL

Oliver Flaherty
Laboratory Manager
Exogen Research

Date

Richard A. Grazhdal, Ph.D.
President
Exogen Research

Date

John L. Brunehoff, July 25, 2002
Sponsor Representative
3M Medical Department Corporate Toxicology
TABLE OF CONTENTS

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MANAGEMENT APPROVAL
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I. SUMMARY

This report details a method of analysis for residues of Perfluorooctanesulfonate (PFOS), Perfluorooctanesulfonate (PFOS) and Perfluorooctanoic Acid (PFOA) in Rat Liver, Serum and Urine.

Residues of PFHxS, PFOS and PFOA are extracted from each matrix with acetonitrile. The acetonitrile extract is added to water and loaded onto a conditioned C18 solid phase extraction (SPE) cartridge. Analyte residues are eluted with 2 mL of methanol. Quantification of PFHxS, PFOS and PFOA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using multiple reaction monitoring (MRM).

The proposed limit of quantification (LOQ) is the lowest quantification specified by the method, which gives adequate recovery according to EPA guidelines. For this method, it is 10 ng/g (parts-per-billion) each for PFHxS, PFOS, and PFOA.

The theoretical limit of detection (LOD) will be based on the signal to noise ratio and will be at least greater than 3 times the level of noise, based on the instrumentation system used. For all analyses, the lowest analytical standard corresponds to 0.1 ng/mL.

This method was developed using rat liver, serum and urine. Typical percent recoveries ± standard deviations (at 10 and 50 ng/g) are shown below:

<table>
<thead>
<tr>
<th>Fortification Level (ng/g)</th>
<th>PFHxS Recovery in Rat Liver</th>
<th>Fortification Level (ng/mL)</th>
<th>PFHxS Recovery in Rat Serum</th>
<th>PFHxS Recovery in Rat Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>115% ± 8.9% (n=3)</td>
<td>10</td>
<td>10% ± 4.7% (n=3)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>111% ± 5.5% (n=3)</td>
<td>50</td>
<td>111% ± 5.5% (n=3)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fortification Level (ng/g)</th>
<th>PFOS Recovery in Rat Liver</th>
<th>Fortification Level (ng/mL)</th>
<th>PFOS Recovery in Rat Serum</th>
<th>PFOS Recovery in Rat Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>86% ± 2.5% (n=3)</td>
<td>10</td>
<td>86% ± 2.5% (n=3)</td>
<td>53% ± 6.1% (n=3)</td>
</tr>
<tr>
<td>50</td>
<td>88% ± 1.5% (n=3)</td>
<td>50</td>
<td>120% ± 2.1% (n=3)</td>
<td>70% ± 2.0% (n=3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fortification Level (ng/g)</th>
<th>PFOA Recovery in Rat Liver</th>
<th>Fortification Level (ng/mL)</th>
<th>PFOA Recovery in Rat Serum</th>
<th>PFOA Recovery in Rat Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>94% ± 3.1% (n=3)</td>
<td>10</td>
<td>117% ± 1.5% (n=3)</td>
<td>89% ± 2.3% (n=3)</td>
</tr>
<tr>
<td>50</td>
<td>94% ± 2.5% (n=3)</td>
<td>50</td>
<td>111% ± 4.0% (n=3)</td>
<td>87% ± 2.1% (n=3)</td>
</tr>
</tbody>
</table>

Representative calibration curves are shown in Figures 1-3. Representative chromatograms are shown in Figures 4 to 39.
2. EXPERIMENTAL COMPOUNDS

The structures for PFHS, PFOS and PFOA are given below.

**PFHS**
- Chemical Name = Perfluorohexanesulfonate
- Molecular weight = 399, as shown

**PFOS**
- Chemical Name = Perfluorooctanesulfonate
- Molecular weight = 499, as shown

**PFOA**
- Chemical Name = Perfluorooctanoic Acid
- Molecular weight = 413, as shown
3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Grade</th>
<th>Source</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (MeOH)</td>
<td>HPLC</td>
<td>EM Science MX24051-1</td>
<td></td>
</tr>
<tr>
<td>Ammonium Acetate</td>
<td>Reagent</td>
<td>JT Baker 0596-01</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Type I</td>
<td>Oxygen NA</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC</td>
<td>EM Science AX0145-1</td>
<td></td>
</tr>
</tbody>
</table>

Type I water = electrical resistivity, minimum of 16.67 MΩ cm at 25 °C, from a Labconco Waterpro™ workstation.

3.2. STANDARDS

<table>
<thead>
<tr>
<th>Standard</th>
<th>TCR Number</th>
<th>Purity (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfluorooctanesulfonate (PFOS)</td>
<td>SE-036</td>
<td>99.99% all isomers</td>
<td>3M</td>
</tr>
<tr>
<td>Perfluorooctanesulfonate (PFOS)</td>
<td>SD-018</td>
<td>84.36 straight chain</td>
<td>3M</td>
</tr>
<tr>
<td>Pentafluorooctanoic acid (PFOA)</td>
<td>Lot No: 96</td>
<td>Aldrich</td>
<td></td>
</tr>
</tbody>
</table>

3.3. EQUIPMENT AND SUPPLIES

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balance, analytical (display as least 0.0001 g)</td>
<td>Mettler</td>
</tr>
<tr>
<td>125-mL LDPE square bottom bottles</td>
<td>Nalgene</td>
</tr>
<tr>
<td>Disposable glass micropipette (50-100 &amp; 100-200 μL)</td>
<td>Drummond (VWR)</td>
</tr>
<tr>
<td>Tissuenser</td>
<td>Thermo</td>
</tr>
<tr>
<td>Wrist action shaker</td>
<td>Burrell Scientific</td>
</tr>
<tr>
<td>Sorvall RC 5C plus Centrifuge</td>
<td>DuPont</td>
</tr>
<tr>
<td>50 mL disposable polypropylene centrifuge tubes</td>
<td>VWR</td>
</tr>
<tr>
<td>15 mL disposable polypropylene centrifuge tubes</td>
<td>VWR</td>
</tr>
<tr>
<td>Vacuum pump manifold</td>
<td>Supelco</td>
</tr>
<tr>
<td>Sep Pak Vac 6 cc (1g) tC18 cartridges (part # WAT 035795)</td>
<td>Waters</td>
</tr>
<tr>
<td>2-μL clear HPLC vial Kit (cat # 5181-3400)</td>
<td>HPLC Scientific</td>
</tr>
<tr>
<td>Class A pipets and volumetric flasks</td>
<td>various suppliers</td>
</tr>
<tr>
<td>Standard lab equipment (graduated cylinders, disposable tubes etc.)</td>
<td>various suppliers</td>
</tr>
<tr>
<td>Stand-alone drop-In guard cartridge holder (part #444017-400)</td>
<td>Keystone Scientific</td>
</tr>
<tr>
<td>Hyperscar drop-In guard column (4 mm) (part #444017-400)</td>
<td>Keystone Scientific</td>
</tr>
<tr>
<td>HPLC Pump (LC-10AD)</td>
<td>Shimadzu</td>
</tr>
<tr>
<td>LC/MS/MS and HPLC systems</td>
<td>As described in section 4.5,</td>
</tr>
</tbody>
</table>

Exxygen Research
Notes:
1. In order to avoid contamination, the use of disposable labware (containers, tubes, pipettes, etc.) is highly recommended.
2. Teflon or Teflon-lined containers or equipment should not be used.
3. It may be necessary to check the solvents (acetonitrile, methanol) for the presence of contaminants (especially PFOA) by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
4. Use disposable micropipettes or pipettes to aliquot standard solutions when preparing standards and samples for extraction.
5. Equivalent materials may be substituted for those specified in this method.

3.4. SOLUTIONS

(1) 2 mM ammonium acetate in water is prepared by weighing 0.154 g of ammonium acetate and dissolving in 1 L of water.

(2) Hypercarb filtered type I water is prepared by filtering type I water through a Hypercarb guard column using a HPLC pump at 2-3 mL/min. Before use, wash the guard cartridge with ~25 mL of HPLC grade acetonitrile, then ~ 25 mL of type I water, then begin collecting the filtered type I water in the extraction. Repeat the wash after filtering ~2L of water.

Note: The aforementioned example is provided for guidance, alternative volumes may be prepared as long as the appropriate ratio of the solvent to solute are maintained.

3.5. PREPARATION OF STANDARD SOLUTIONS

Analytical standards are used for three purposes:
1. Calibration Standards — These standards are prepared in methanol and are used to calibrate the detector used in the analysis.
2. Laboratory Control Spikes — These fortifications are prepared at concentrations corresponding to the LOQ and 5x LOQ and are used to determine analytical recovery. Laboratory control spikes are prepared in control matrix.
3. Matrix Spikes — These fortifications are prepared by spiking into the field samples at a known concentration. Matrix spikes are used to evaluate the effect of the sample matrix on analytical recovery and are prepared at the client's request.

The analyst may vary the absolute volumes of the standards as long as the correct proportions of solute to solvent are maintained.
3.5.1. Stock Solution

Prepare individual stock solutions at 100 μg/mL for PFHS, PFOS and PFOA by weighing out 10 mg of each analytical standard (corrected for purity and if necessary, salt content) and dilute to 100 mL with methanol in separate 100-mL volumetric flasks. The stock solutions (in 125-mL LDPE bottles) are to be stored in a refrigerator at 2°C to 6°C and are stable for a maximum period of one year from the date of preparation.

3.5.2. Fortification Solutions

a. Prepare a mixed fortification standard at 1.0 μg/mL (1000 ng/mL) of PFHS, PFOS and PFOA by adding 1.0 mL of each of the 100 μg/mL stock solutions into a 100-mL volumetric flask and bring up to volume with methanol.

b. Prepare a mixed fortification standard at 0.1 μg/mL (100 ng/mL) of PFHS, PFOS and PFOA by diluting 10.0 mL of the 1.0 μg/mL mixed fortification solution to 100 mL with methanol in a volumetric flask.

Example: one hundred microliters of the 0.1 μg/mL solution spiked into 1 g of liver or 1 mL of serum/urine is equivalent to a 10 ppb (10 ng/g or ng/mL) fortification.

Store all fortification standard solutions in a refrigerator (in 125-mL LDPE bottles) at 2°C to 6°C for a maximum period of one year from the date of preparation. Note also that additional concentrations may be prepared if necessary.

3.5.3. Calibration Standards

LCMS/MS calibration standards containing PFHS, PFOS and PFOA are prepared at 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0 ng/mL in methanol via dilution of the 0.1 μg/mL mixed fortification solution (section 3.5.2.b).

The following is a typical example; additional concentrations may be prepared as needed:

<table>
<thead>
<tr>
<th>Initial Conc. (ng/mL)</th>
<th>Volume (mL)</th>
<th>Diluted to (mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>5.0</td>
<td>100</td>
<td>5.0</td>
</tr>
<tr>
<td>100</td>
<td>2.0</td>
<td>100</td>
<td>2.0</td>
</tr>
<tr>
<td>100</td>
<td>1.0</td>
<td>100</td>
<td>1.0</td>
</tr>
<tr>
<td>5.0</td>
<td>10.0</td>
<td>100</td>
<td>0.5</td>
</tr>
<tr>
<td>2.0</td>
<td>10.0</td>
<td>100</td>
<td>0.2</td>
</tr>
<tr>
<td>1.0</td>
<td>10.0</td>
<td>100</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The standards may be used for a period of one year (in 125-mL LDPE bottles) when stored refrigerated (at 2°C to 6°C).
4. METHOD

4.1. FLOW DIAGRAM

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

**Method Flow Diagram**

- Weigh 1 g of liver or measure 1 mL of serum or urine (fortify samples designated as matrix spikes and laboratory control spikes)
- Add 9 mL of water to liver, 19 mL of water to serum and urine, homogenize
- Remove 1 mL and add 5 mL of ACN, shake
- Centrifuge
- Decant supernatant into 35 mL of water
- Load onto conditioned SPE
- Elute with 2 mL methanol
- LC/MS/MS Analysis

4.2. SAMPLE PROCESSING

For liver samples, place frozen samples in a food processor and homogenize with dry ice. Then place samples in containers and leave open in frozen storage overnight to allow for CO₂ sublimation. Seal and place the samples in frozen storage below –10°C until time of extraction. No sample processing is needed for serum and urine samples. However, frozen serum and urine samples must be allowed to completely thaw to room temperature before use.
4.3. **Batch Setup**

a. A batch of samples should not contain more than 20 field samples.

b. Each batch of samples analyzed must include at least one control (method blank using control matrix) and two matrix controls fortified at known concentrations (typically 10 and 50 ng/g for liver or ng/mL for serum and urine) to verify procedural recovery for the batch.

c. At least one field sample in each batch must also be separately fortified at a known concentration and carried through the procedure to verify recovery. Additional samples in the batch may also be fortified if desired.

d. All samples require duplicate injections.

4.4. **Sample Extraction**

4.4.1. **Liver Extraction**

a. Weigh 1 g of liver sample into a 50 mL disposable centrifuge tube and fortify, if appropriate.

b. Add water to the sample for a final volume of 5 mL. Cap tightly.

c. Homogenize sample using a tissuemizer for ~1 minute.

d. Transfer 1 mL of the sample using a disposable pipette into 15 mL disposable centrifuge tubes. Add 5 mL of ACN and shake for ~20 minutes on a wrist action shaker.

e. Centrifuge tubes at ~3000 rpm for ~5 minutes. Carefully decant supernatant into a 50 mL disposable centrifuge tube and add 35 mL of water.

f. Load the sample onto a conditioned SPE column (for conditioning details, see section 4.4.3). Discard the eluate. Any analyte residues will be trapped on the SPE column at this point.

g. Elute with 2 mL of methanol. Collect 2 mL of elute into a graduated 15 mL centrifuge tube.

h. Analyze samples using electrospray LC/MS/MS.

4.4.2. **Serum and Urine Extraction**

a. Measure 1 mL of serum or urine sample into a 50 mL disposable centrifuge tube and fortify, if appropriate.

b. Add 19 mL of water to sample. Cap tightly and vortex for ~1 minute. Then continue with steps d-h in section 4.4.1.
4.4.3. SPE Column Conditioning

Place the unconditioned SPE columns on the vacuum manifold. Condition the SPE columns by passing ~10 mL of methanol through the column followed by ~5 mL of water. The washes may be pulled through the SPE column using vacuum at a flow rate of ~1 drops/sec or may be allowed to pass through the column unaided. Discard all washes. Do not allow the column to dry.

4.5. Quantitation

4.5.1. LC/MS/MS System and Operating Conditions

<table>
<thead>
<tr>
<th>Mass Spec:</th>
<th>Micromass Quattro Ultima (Micromass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interface:</td>
<td>Electrospray (Micromass)</td>
</tr>
<tr>
<td></td>
<td>Harvard infusion pump (Harvard Instruments), for tuning</td>
</tr>
<tr>
<td>Computer:</td>
<td>COMPAQ Professional Workstation AP200</td>
</tr>
<tr>
<td>Software:</td>
<td>Windows NT, Masslynx 3.3</td>
</tr>
<tr>
<td>HPLC:</td>
<td>Hewlett Packard (HP) Series 1100</td>
</tr>
<tr>
<td></td>
<td>HP Quat Pump</td>
</tr>
<tr>
<td></td>
<td>HP Vacuum Degasser</td>
</tr>
<tr>
<td></td>
<td>HP Autosampler</td>
</tr>
<tr>
<td></td>
<td>HP Column Oven</td>
</tr>
</tbody>
</table>

Note: A 4 x 10 mm hypercarb drop in guard cartridge is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

<table>
<thead>
<tr>
<th>HPLC Column:</th>
<th>General C18 (Jones Chromatography), 2.1 mm x 50 mm, 4µ μ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column Temperature:</td>
<td>25°C</td>
</tr>
<tr>
<td>Injection Volume:</td>
<td>15 µL</td>
</tr>
<tr>
<td>Mobile Phase A:</td>
<td>2 mM Ammonium Acetate in Type I water</td>
</tr>
<tr>
<td>Mobile Phase B:</td>
<td>Methanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>% A</th>
<th>% B</th>
<th>Flow Rate (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>50</td>
<td>10</td>
<td>0.3</td>
</tr>
<tr>
<td>2.0</td>
<td>50</td>
<td>10</td>
<td>0.3</td>
</tr>
<tr>
<td>5.0</td>
<td>10</td>
<td>90</td>
<td>0.3</td>
</tr>
<tr>
<td>9.0</td>
<td>10</td>
<td>90</td>
<td>0.3</td>
</tr>
<tr>
<td>9.5</td>
<td>0</td>
<td>100</td>
<td>0.3</td>
</tr>
<tr>
<td>14.0</td>
<td>0</td>
<td>100</td>
<td>0.3</td>
</tr>
<tr>
<td>14.5</td>
<td>90</td>
<td>10</td>
<td>0.3</td>
</tr>
<tr>
<td>20.0</td>
<td>90</td>
<td>10</td>
<td>0.3</td>
</tr>
</tbody>
</table>

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 x 30) and also columns
from different manufacturers (Keystone Brixel C18 etc.) could be used, provided equivalent chromatography is obtained.

**Ions monitored:**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mode</th>
<th>Transition Monitored</th>
<th>Approximate Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHS</td>
<td>Negative</td>
<td>399 → 80</td>
<td>~8.2 min.</td>
</tr>
<tr>
<td>PFOS</td>
<td>Negative</td>
<td>499 → 99</td>
<td>~8.6 min</td>
</tr>
<tr>
<td>PFOA</td>
<td>Negative</td>
<td>413 → 369</td>
<td>~8.6 min</td>
</tr>
</tbody>
</table>

The retention times may vary, on a day to day basis, depending on the batch of mobile phase etc. Drift in retention times (up to ± 4%) is acceptable within an analytical run, as long as the drift continues through the entire analysis and the standards are included at the beginning and end of the analytical run.

**Note:** An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

The mass spectrometer is tuned for each analyte by infusing a - 1.0 μg/mL standard solution (at 10 μL/min, using an infusion pump) via a "T" into a stream of mobile phase containing 50% methanol and 50% 2 mM ammonium acetate in water at 0.2 mL/min flow rate. Each analyte is 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.

### 4.5.2. Calibration Curve Procedures

a. Inject the same aliquot (between 10 to 50 μL) of each calibration standard (ranging from the lowest level standard to the highest level prepared), into the LC/MS/MS.

b. Use weighted linear standard curves for quantification. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using MetAlign (or equivalent) software system. Any calibration standard found to be a statistical outlier by using an appropriate outlier test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that may be excluded must not exceed 20% of the total number of standards injected.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.9925 (R² ≥0.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.
Typical calibration curves for PFBS, PFOS and PFOA can be found in Figures 1-3.

4.5.3 Sample Analysis

a. Inject the same aliquot (between 10 to 50 µL) of each standard, sample, recovery, control, etc. into the LC/MS/MS system.

b. Standards corresponding to at least five or more concentration levels (starting with the LOQ level or below) must be included in an analytical set.

c. An entire set of calibration standards should be injected at the beginning of a set followed by calibration standards interspersed approximately every 5-10 samples (to account for a second set of extracted standards). As an alternative, an entire set of calibration standards may be included at the beginning and at the end of a sample set. In either case, calibration standards must be the first and last injection in a sample set.

d. The concentration of each sample/fortification/control is determined from the standard curve, based on the peak area of each analyte. The standard responses should bracket responses of the residue found in each sample set. Results may be quantified up to 10% outside the curve by extrapolation. If necessary, dilute the samples to give a response within the standard curve range.

e. Fortification recoveries falling within 70 to 130% are considered acceptable.

f. Samples must be stored refrigerated between 2°C to 6°C until analysis.

g. Samples in which either no peaks are detected or peaks less than the lowest concentration of the calibration standards are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the lowest concentration of the calibration standards will be reported as NQ, (not quantifiable).

The analysis performed during the method development included fortifications at 10 and 50 ng/g of PFHS in rat liver, 10 and 50 ng/mL of PFHS in serum, 10 and 50 ng/g of PFOS and PFOA in rat liver and 10 and 50 ng/mL of PFOS and PFOA in serum and urine. Typical chromatograms can be found in Figures 4-39.
4.6. ACCEPTANCE CRITERIA
The following criteria must be met to assure the presence of PFHS, PFOA, and FFOA:

1. The chromatogram must show a peak of a daughter ion at 80 amu from a parent of 399 amu for PFHS, a daughter ion at 99 amu from a parent of 499 amu for PFOA, and a daughter ion at 369 amu from a parent of 413 amu for FFOA.

2. Method blanks must not contain analyte at levels greater than the LOQ. If a blank contains the analyte at levels greater than 10 ng/mL, then a new blank sample must be obtained and the entire set must be re-extracted.

3. Recoveries of control spikes and matrix spikes (if any) must be between 70-150% of their known values. If a control spike falls outside the acceptable limits, the entire set of samples should be re-extracted. Any matrix spikes outside 70-130% should be evaluated by the analyst to determine if re-extraction is warranted.

4. Any calibration standard found to be a statistical outlier by using the Hauge Error Test, may be excluded from the calculation of the calibration curve. However, the total number of calibration standards that could be excluded must not exceed 20% of the total number of standards injected.

5. The correlation coefficient (R) for calibration curves generated must be 20.9925 (R² 20.985). If calibration results fall outside these limits, then appropriate steps must be taken to adjust instrument operation, and the standards or the relevant set of samples should be reanalyzed.

6. Retention times between standards and samples must not drift more than ± 4% within an analytical run. If retention time drift exceeds this limit within an analytical run then the set must be reanalyzed.

4.7. PERFORMANCE CRITERIA
The following two criteria must be performed once as a system suitability test, before the commencement of analysis, when using an instrumentation set-up that has not been used for this method.

First Criterion:
Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (10 ng/mL) in matrix and obtain a signal to noise ratio for the analyte transition of at least 9:1, compared to a reagent blank. If this criterion cannot be met, optimize and change instrument operating parameters (or increase the injection volume, if appropriate).
Second Criterion:
Run a set of standards of five or more concentration levels, from at or below the LOQ, up to the highest concentration level to be included in the analysis. 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 is met, samples may be analyzed with standards interspersed.

4.8. TIME REQUIRED FOR ANALYSIS

One person can take a set of 20 samples through the sample preparation procedure in approximately 4 hours. The LC/MS/MS analysis of the set (containing 20 field samples, 1 matrix blank, 2 laboratory control spikes, 1 matrix spike and 12 standard injections) will take approximately 14 hours.

5. CALCULATIONS

a. Use Equation 1 to calculate the amount of analyte found (in ng/mL, based on peak area) using the standard curve (1/x weighted linear regression parameters) generated by the Masslynx software program.

Equation 1:

\[
\text{Analyte found (ng/mL) = (peak area - intercept) \times DF \times aliquot factor \times slope}
\]

DF = factor by which the final volume was diluted, if necessary. 
Aliquot factor = 9 for liver, 20 for serum and urine.

b. For samples fortified with known amounts of analyte prior to extraction, use Equation 2 to calculate the percent recovery.

Equation 2:

\[
\text{Recovery (\%) = }
\]

\[
\left[ \frac{\text{total analyte found (ng/mL) - analyte found in control (ng/mL)}}{\text{analyte added (ng/mL)}} \right] \times 100
\]

Note: Subtract analyte found in control (ng/mL) from analyte found (ng/mL), if ng/mL in control is greater than LOQ.
c. Use Equation 3 to calculate the amount of analyte found (in ppb)

\[
\text{Equation 3:}
\]

\[
\text{Analyte found (ppb or ppt/ml.)} = \frac{\text{analyte found (mg/ml.)} \times FV \times 10^3}{\text{sample weight (g) or sample volume (ml.)}}
\]

\[FV = \text{final volume}\]

For reporting purposes, samples in which either no peaks are detected or peaks less than the lowest concentration of the calibration standards are detected at the corresponding analyte retention time will be reported as ND (not detected). Samples in which peaks are detected at the corresponding analyte retention time that are less than the LOQ and greater than or equal to the lowest concentration of the calibration standards will be reported as NQ (not quantifiable).

6. SAFETY

The analyst should read the material safety data sheet for all standards and reagents before performing this method. Use universal precautions when handling standards and reagents, including working in fume hoods and wearing laboratory coats, safety glasses, and gloves.
Table 1. Recovery Summary of PFHS in Rat Liver and Serum

**Recovery Summary of PFHS in Rat Liver**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/g)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201684 Spk A</td>
<td>10</td>
<td>108</td>
</tr>
<tr>
<td>0201684 Spk B</td>
<td>10</td>
<td>110</td>
</tr>
<tr>
<td>0201684 Spk C</td>
<td>10</td>
<td>118</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>115</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>9.9</strong></td>
</tr>
<tr>
<td>Sample ID</td>
<td>Analyte Added (ng/g)</td>
<td>Percent Recovery (%)</td>
</tr>
<tr>
<td>0201684 Spk D</td>
<td>50</td>
<td>101</td>
</tr>
<tr>
<td>0201684 Spk E</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>0201684 Spk F</td>
<td>50</td>
<td>94</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>99</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>3.5</strong></td>
</tr>
</tbody>
</table>

**Recovery Summary of PFHS in Rat Serum**

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201682 Spk A</td>
<td>10</td>
<td>112</td>
</tr>
<tr>
<td>0201682 Spk B</td>
<td>10</td>
<td>103</td>
</tr>
<tr>
<td>0201682 Spk C</td>
<td>10</td>
<td>110</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>109</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>4.7</strong></td>
</tr>
<tr>
<td>Sample ID</td>
<td>Analyte Added (ng/mL)</td>
<td>Percent Recovery (%)</td>
</tr>
<tr>
<td>0201682 Spk D</td>
<td>50</td>
<td>107</td>
</tr>
<tr>
<td>0201682 Spk E</td>
<td>50</td>
<td>104</td>
</tr>
<tr>
<td>0201682 Spk F</td>
<td>50</td>
<td>122</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>111</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>9.6</strong></td>
</tr>
</tbody>
</table>
Table 2. Recovery Summary of PFOS in Rat Liver, Serum and Urine

### Recovery Summary of PFOS in Rat Liver

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk A</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>0201888 Spk B</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>0201888 Spk C</td>
<td>10</td>
<td>103</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>96</strong></td>
<td><strong>96</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>4.8</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk D</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>0201888 Spk E</td>
<td>50</td>
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<tr>
<td>0201888 Spk F</td>
<td>50</td>
<td>87</td>
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<tr>
<td><strong>Average</strong></td>
<td><strong>92</strong></td>
<td><strong>92</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>1.8</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Recovery Summary of PFOS in Rat Serum

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk A</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>0201888 Spk B</td>
<td>10</td>
<td>88</td>
</tr>
<tr>
<td>0201888 Spk C</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>88</strong></td>
<td><strong>88</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>3.5</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk D</td>
<td>50</td>
<td>118</td>
</tr>
<tr>
<td>0201888 Spk E</td>
<td>50</td>
<td>122</td>
</tr>
<tr>
<td>0201888 Spk F</td>
<td>50</td>
<td>121</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>120</strong></td>
<td><strong>120</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>2.1</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Recovery Summary of PFOS in Rat Urine

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk A</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>0201888 Spk B</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td>0201888 Spk C</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>97</strong></td>
<td><strong>97</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>4.7</strong></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/mL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201888 Spk D</td>
<td>50</td>
<td>78</td>
</tr>
<tr>
<td>0201888 Spk E</td>
<td>50</td>
<td>78</td>
</tr>
<tr>
<td>0201888 Spk F</td>
<td>50</td>
<td>78</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>78</strong></td>
<td><strong>78</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>1.3</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Recovery Summary of PFOA in Rat Liver, Serum and Urine

### Recovery Summary of PFOA in Rat Liver

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/μL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0201684 Spk A</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>0201684 Spk B</td>
<td>10</td>
<td>101</td>
</tr>
<tr>
<td>0201684 Spk C</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>98</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>3.1</strong></td>
</tr>
</tbody>
</table>

### Recovery Summary of PFOA in Rat Serum

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/μL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0201682 Spk A</td>
<td>10</td>
<td>116</td>
</tr>
<tr>
<td>0201682 Spk B</td>
<td>10</td>
<td>117</td>
</tr>
<tr>
<td>0201682 Spk C</td>
<td>10</td>
<td>115</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>117</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>1.5</strong></td>
</tr>
</tbody>
</table>

### Recovery Summary of PFOA in Rat Urine

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyte Added (ng/μL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0201682 Spk A</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td>0201682 Spk B</td>
<td>10</td>
<td>91</td>
</tr>
<tr>
<td>0201682 Spk C</td>
<td>10</td>
<td>92</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td><strong>92</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td></td>
<td><strong>3.5</strong></td>
</tr>
</tbody>
</table>
Figure 1. Calibration Curve for PFHS
Figure 2. Calibration Curve for PFOS

- Compound name: PFOS
- Coefficient of Determination: 0.997008
- Calibration curve: 321.120 \* x = 48,928
- Response type: External Std, Area
- Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Figure 3. Calibration Curve for PFOA

- Compound 1 name: PFOA
- Coefficient of Determination: 0.999945
- Calibration curve: STZZ0.9 * x + 39773.26
- Response type: External Std, Area
- Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None
Figure 4. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFBS

Figure 5. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFOS
Figure 6. Representative Chromatogram of a 0.1 ng/mL Standard Containing PFOA

Figure 7. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFHS
Figure 8. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFOS

Figure 9. Representative Chromatogram of a 0.5 ng/mL Standard Containing PFOA
Figure 10. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFHS

Figure 11. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFOS
Figure 12. Representative Chromatogram of a 5.0 ng/mL Standard Containing PFOA

Figure 13. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFHS
Figure 14. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFOS

Figure 15. Representative Chromatogram of a Reagent Blank Sample Analyzed for PFOA
Figure 16. Representative Chromatogram of a Control Liver Sample Analyzed for PFBS

Figure 17. Representative Chromatogram of a Control Liver Sample Analyzed for PFOS
Figure 18. Representative Chromatogram of a Control Liver Sample Analyzed for PFOA

Figure 19. Representative Chromatogram of a Control Serum Sample Analyzed for PFHS
Figure 20. Representative Chromatogram of a Control Serum Sample Analyzed for PFOS

Figure 21. Representative Chromatogram of a Control Serum Sample Analyzed for PFOA
Figure 22. Representative Chromatogram of a Control Urine Sample Analyzed for PFOA

Figure 23. Representative Chromatogram of a Control Urine Sample Analyzed for PFOS
Figure 24. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFHS

Figure 25. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFOS
Figure 26. Representative Chromatogram of a Control Liver Sample Fortified at 10 ng/g with PFOS

Figure 27. Representative Chromatogram of a Control Liver Sample Fortified at 50 ng/g with PFBS
Figure 28. Representative Chromatogram of a Control Liver Sample
Fortified at 50 ng/g with PFOS

Figure 29. Representative Chromatogram of a Control Liver Sample
Fortified at 50 ng/g with PFOA
Figure 30. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFHS

Figure 31. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFOS
Figure 32. Representative Chromatogram of a Control Serum Sample Fortified at 10 ng/mL with PFOA

Figure 33. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFHxS
Figure 34. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFOS

Figure 35. Representative Chromatogram of a Control Serum Sample Fortified at 50 ng/mL with PFOA
Figure 36. Representative Chromatogram of a Control Urine Sample
Fortified at 10 ng/mL with PFOS

Figure 37. Representative Chromatogram of a Control Urine Sample
Fortified at 10 ng/mL with PFOA
Figure 38. Representative Chromatogram of a Control Urine Sample Fortified at 50 ng/mL with PFOS

Figure 39. Representative Chromatogram of a Control Urine Sample Fortified at 50 ng/mL with PFOA
**PROTOCOL DEVIATION**

- **Deviation Number:** 1
- **Date of Occurrence:** 10/13/02
- **Exygen Study Number:** 023-082
- **Protocol Number:** ExP-023-082

**DESCRIPTION OF DEVIATION**

- Analytical Procedure Summary—II: Calibration Standards

Did not prepare extracted standards in human plasma at 1.5 and 2.5 ppb levels.

**ACTIONS TAKEN**

- Protocol deviation issued.

**Recorded By:**

- **Date:** 10/23/02

**IMPACT ON STUDY**

No negative impact because only had limited sample available and the linear range of the curve was still established without those two levels.

**Principal Investigator Signature:**

- **Date:** 10/30/02

**Study Director Signature:**

- **Date:** 10/30/03

**Management Signature:**

- **Date:**

**Exygen QAU Review:**

- **Date:** 11/5/02

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**Exygen Research:**

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PROTOCOL DEVIATION

Deviation Number: 2
Date of Occurrence: 10/17/02

Exygen Study Number: 023-082
Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

Analytical Procedure Summary- I. Method Summary:

Eluted with 5 mL of methanol and evaporated to less than 1 mL using a nitrogen evaporator and then brought final volume up to 1 mL with methanol.

ACTIONS TAKEN

Protocol deviation issued.

Recorded By: [Signature]
Date: 10/17/02

IMPACT ON STUDY

No negative impact because final concentration remained the same, but complete elution of the analytes was established.

[Signature]
Date: 10/17/02

Exygen OAU Review: [Signature]
Date: 10/17/02

Principal Investigator Signature

Study Director Signature

Management Signature

(Sponsor)
## PROTOCOL DEVIATION

**Deviation Number:** 3  
**Date of Occurrence:** throughout study

**Eoxygen Study Number:** 023-082  
**Protocol Number:** Exp-023-082

### DESCRIPTION OF DEVIATION

1. Analytical Procedure Summary-Method Section 4.5.3.d—several samples were quantified above 10% of the curve.

2. Analytical Procedure Summary-Method Section 4.5.3.e—the acceptance criteria for QC spike recoveries could not always be met.

### ACTIONS TAKEN


---

**Recorded By:** [Signature]  
**Date:** 10/30/02

**IMPACT ON STUDY**

1. No negative impact because residue was < than 15% above the curve for C6 and C8 for samples 020493 Spk V and for C8 for samples 0203965 and 0203965 Dup. Also, the samples that were quantified outside the curve for THPP and THPFS were only fortification recoveries and the majority of the recoveries were > 100%.

2. Impact not determined only documented since this method has not been validated for these analytes at these levels.

---

**Principal Investigator Signature:** [Signature]  
**Date:** 10/30/02

**Study Director Signature:** [Signature]  
**Date:** 10/30/02

**Management Signature (Sponsor):**  
**Date:**

**Eoxygen QAU Review:** [Signature]  
**Date:** 10/30/02
PROTOCOL DEVIATION

Deviation Number: 4
Date of Occurrence: throughout study

Exygen Study Number: 023-082 Protocol Number: ExP-023-082

DESCRIPTION OF DEVIATION

1. Analytical Procedure Summary-Method Section 4.5.3.3- several samples were quantified below the curve and ND was used for when no peak was detected and NQ was used for negative results. All other results were reported.

2. Analytical Procedure Summary-Method Section 4.5.3.3-a second set of calibration standards was not analyzed in a set.

ACTIONS TAKEN

for example - deviation issued, SOP revision, etc.


Recorded By: [Signature] Date: 6/30/02

IMPACT ON STUDY

1. No negative impact because no claim of quantitative accuracy has been established.

2. No negative impact because the extracted standards were analyzed throughout the set which provide a QC check on the internal calibration.

Principal Investigator Signature

[Signature] Date: 6/30/02

Study Director Signature

[Signature] Date: 6/30/02

Management Signature

[Signature] Date: 6/30/02

Exygen QAU Review: NLL 10/5/02

Exygen Research.
PROTOCOL DEVIATION

Deviation Number: 5
Date of Occurrence: Throughout study

Exygen Study Number: 023-082
Protocol Number: Exp-023-082

DESCRIPTION OF DEVIATION
1. Analytical Procedure Summary-Method Section 4.5.1—the transition monitored for PFOS was 499 → 80

ACTIONS TAKEN
for example—deviation issued, SOP revision, etc.
1. Protocol deviation issued.

Recorded By: Emily R. Deck
Date: 10/24/02

IMPACT ON STUDY
1. No negative impact.

Principal Investigator Signature
Emily R. Deck
Date: 10/30/02

Study Director Signature
John M. Rek
Date: 10/30/02

Management Signature
(Sponsor)
(Date)

Exygen QAU Review
MCL 10/30/02

Exygen Research.
PROTOCOL DEVIATION

Deviation Number: 1
Date of Occurrence: 10/15/02

Protocol Number: Exp. 023-082

EXYGEN STUDY NUMBER: 023-082

DESCRIPTION OF DEVIATION

Animetals Procedure Summary: Diluted Standards

Did not prepare extracted standards in human plasma at 1.5 and 2.5 ppb levels.

ACTIONS TAKEN

New action taken: Recordings were reviewed.

Recorded by: [Signature]
Date: 10/31/02

IMPACT ON STUDY

No negative impact because only limited sample available and the linear range of the curve was still established without those two levels.

Principal Investigator Signature: [Signature]
Date: 10/30/02

Research Director: [Signature]
Date: 10/30/02

Management Signature: [Signature]
Date: 10/30/02

Exygen QA Review: [Signature]
Date: 10/30/02
## Protocol Deviation

### Description of Deviation

<table>
<thead>
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**Analytical Procedure Summary:**

Eluted with 5 mL of methanol and evaporated to less than 1 mL using a nitrogen evaporator and then brought final volume up to 1 mL with methanol.

### Action Taken

**Protocol deviation issued:**

No negative impact because final concentration remained the same, but complete solution of the analytes was established.

### Verification

**Protocol Investigator Signature:**

Signed 10/20/03

**Study Director Signature:**

Signed 10/20/03

**Management Signature (Sponsor):**

Signed 10/20/03

**Exogen GAU Review:**

Signed 10/20/03

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**Exogen Research**

**000424**

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PROTOCOL DEVIATION

Protocol Number: 002-082

1. Analytical Procedure Summary-Method Section 4.3.3.4. Several samples were quantified above 10% of the curve.

2. Analytical Procedure Summary-Method Section 4.3.3.6. The acceptance criteria for QC spike recoveries could not always be met.

ACTIONS TAKEN

1.2. Protocol deviation letter.

Reported by: [Signature] Date: [Date]

1. No negative impact because residue was less than 10% above the curve for C9 and C8 for sample 0204493 Spx V and for C9 for samples 0213963 and 0213965 Dup. Also, the samples that were quantified outside the curve for TPFCS and TPFCS were only fortification recoveries and the majority of the recoveries were > 100%.

2. Impact not determined only documented since this method has not been validated for these analytes at these levels.

[Signatures]

Exogen QA Review

[Signature] Date: [Date]
PROTOCOL DEVIATION:

Exygen Study Number: 023-082
Protocol Number: Def-013-001

DESCRIPTION OR DEVIATION:
1. Analytical Procedure Summary: Method Section 4.3.a. several samples were quantified below the curve and HQ was used for when no peak was detected and HQ was used for negative results. All other results were included.

2. Analytical Procedure Summary: Method section 4.5.3.a. a second set of calibration standards was not analyzed in a set.

ACTION TAKEN:
- All samples reanalyzed with HQ reagent, etc.

1-2. Protocol deviation issue:

Recorded By: [Signature]
Date: 03/04/02

IMPACT ON STUDY:
1. No negative impact because no significant change in accuracy has been established.
2. No negative impact because the corrected standards were analyzed throughout the test which provide a QC check on the initial calibration.

Principal Investigator Signature: [Signature]
Date: 03/04/02

Study Director Signature: [Signature]
Date: 03/04/02

Management Signature: [Signature]
Date: 03/04/02

Exygen RAV Review: [Signature]
Date: 03/04/02

Exygen Research.

000426
### PROTOCOL DEVIATION

#### Protocol Number: 290-082

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<td>290-082</td>
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#### Description of Deviation

1. Analytical Procedure Summary: Section 4.2.3, the transition mentioned for F05 was 409 = 50

#### Action Taken

- Date: 07/06/2000

#### Impact

- Impact: Negative
- Date: 07/06/2000

#### Signature

- Signature: [Signature]
- Date: 07/06/2000

#### Management

- Manager: [Name]
- Date: 07/06/2000

- Sponsor: [Name]
- Date: 07/06/2000

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**Exygen GAI Review:**

[Signature]

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Exygen Research.