

***Study Title***

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

***Amended Analytical Laboratory Report Title***

Determination of the Presence and Concentration of Perfluorooctanoate Fluorochemical  
in Liver, Serum, Urine and Feces Samples

***Data Requirement***

Not Applicable

***Author***

3M Environmental Laboratory

***Analytical Phase Completion Date***

June 11, 2001

***Performing Laboratories***

**Liver, Serum and Urine Analyses**

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**Feces Analyses**

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***Project Identification***

3M Medical Department Study: T-6889.3  
Covance In-Life Study: #6329-231  
Analytical Report: FACT TOX-026  
3M Laboratory Request No. U2782

***Total Number of Pages***

408

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## GLP Compliance Statement

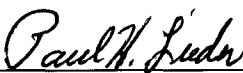
Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanoate Fluorochemical in Liver, Serum, Urine and Feces Samples

Study Identification Numbers: T-6889.3, FACT TOX-026, LRN U2782

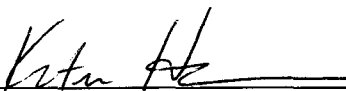
This study was conducted in compliance with United States Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards 40 CFR Part 792, with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

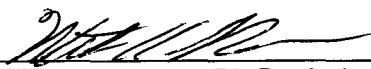
- There was a point during the course of this study in which there were two study directors assigned to the study. This was resolved by amending the protocol.
- Labels of reagents and solutions did not contain all of GLP requirements.
- It is unknown if an in-phase inspection was conducted. The audit report could not be located.

  
Paul Lieder, Ph.D., Study Director

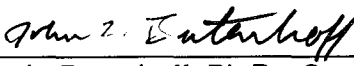
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**GLP Study—Quality Assurance Statement**

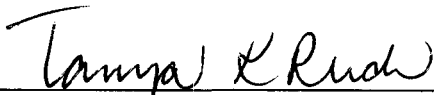
Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanoate Fluorochemical in Liver, Serum, Urine and Feces Samples

Study Identification Numbers: T-6889.3, FACT TOX-026, LRN U2782

This analytical study performed at 3M Environmental Laboratory has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and laboratory management.

| Inspection Dates                                                                  | Phase        | Date Reported to     |                      |
|-----------------------------------------------------------------------------------|--------------|----------------------|----------------------|
|                                                                                   |              | Management           | Study Director       |
| 10/26/98                                                                          | Protocol     | 10/27/98             | 10/27/98             |
| 9/25/00–9/29/00<br>10/2/00–10/6/00<br>10/9/00, 10/30/00                           | Data         | 10/9/00,<br>10/30/00 | 10/9/00,<br>10/30/00 |
| 12/14/00–12/15/00<br>12/18/00–12/20/00, 5/3/01,<br>5/4/01, 5/7/01, 5/8/01, 5/9/01 | Draft Report | 12/21/00,<br>5/10/01 | 12/21/00,<br>5/10/01 |

See Appendix H for individual contractor quality assurance statements.

  
\_\_\_\_\_  
QAU Representative

6/18/01  
\_\_\_\_\_  
Date

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### Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test substance and reference substance reserve samples, as well as the specimens pertaining to the analytical phase of this study are archived at the 3M Environmental Laboratory. Feces specimens will be returned from Centre Analytical Laboratories for archiving at the 3M Environmental Laboratory and will be maintained at 3M Environmental Laboratory according to GLP requirements.

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## Summary

Under the conditions of the present studies, the fluorochemical perfluorooctanoate (POAA) was observed in the serum, urine, and liver of most samples collected from cynomolgus monkeys dosed with the test substance during the in-life phase of the study.

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## Introduction and Purpose

The purpose of the analytical phase of this study is to determine the presence and concentration of perfluorooctanoate (POAA) in liver, serum, urine and feces samples taken at specified intervals from the 26-week capsule toxicity study in cynomolgus monkeys. The analytical phase of this study was initiated on November 12, 1998.

The text and tables in this report cover ONLY the analyses conducted at 3M, unless otherwise specified, and the analogous information on feces analysis can be found in the documents from Centre Analytical Laboratory (Appendix H).

## Test System

A total of 22 cynomolgus male monkeys were obtained from Covance Research Products, Inc. and acclimated for 35 days. The monkeys were 3 to 7 years old and weighed 3–5 kg at the initiation of the treatment. All monkeys were identified with a collar tag. Animals were assigned to the control or treatment group using a computerized blocking procedure designed to achieve body weight balance with respect to treatment groups. The in-life laboratory staff gave each study animal the specified dose orally once daily for at least 183 days, with the exceptions noted in the Covance in-life final report #6329-231. A select number of monkeys were then chosen from the main study to continue on in a recovery group study. The doses administered for each control and sub-treatment group are listed in Table 1. Doses were given in the form of Size No. 2 gelatin capsules.

**Table 1. Cynomolgus Monkey Population Demographics for Study (#6329-231)**

| Population                                             | Total | Selected for Study Group | Selected for Recovery Group |
|--------------------------------------------------------|-------|--------------------------|-----------------------------|
| Group 1<br>Control                                     | 6     | 6                        | 2                           |
| Group 2<br>Low-dose<br>(3 mg/kg/day)                   | 4     | 4                        | 0                           |
| Group 3<br>Mid-dose<br>(10 mg/kg/day)                  | 6     | 6                        | 2                           |
| Group 4<br>High-dose<br>(30/20 mg/kg/day) <sup>a</sup> | 6     | 6                        | 0                           |

<sup>a</sup>30 mg/kg/day was suspended on Day 12 and reinitiated on Day 22 at 20 mg/kg/day.

NOTE: This in-life information came from Covance in-life final report for study #6329-231.

## **Specimen Collection**

A total of 947 liver, serum, urine and feces specimens were collected from cynomolgus monkeys (beginning 7 October 1998) and sent to the 3M Environmental Laboratory to be analyzed for perfluorooctanoate (POAA). Serum, urine and feces specimens were collected approximately every two weeks and liver samples were collected at time of sacrifice. An additional 101 specimens of whole blood, plasma, red blood cells, kidneys and testes were collected by Covance (study #6329-231), but were not part of the scope of analysis determined by the study director. The number and type of specimens collected for analyses in the analytical phase of this study are presented below.

### **Specimens Collected from Study Groups 1 through 4 (through 3/31/99):**

**Serum Specimens**—292 specimens

**Liver Specimens**—19 specimens

**Urine Specimens**—272 specimens

**Feces Specimens**—272 specimens

### **Specimens Collected from the Recovery Group from 4/08/99 to 7/02/99:**

**Serum Specimens**—32 specimens

**Liver Specimens**—4 specimens

**Urine Specimens**—28 specimens

**Feces Specimens**—28 specimens

Sera, liver and urine samples were extracted beginning on 12 November 1998 using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE) or ethyl acetate. Sample extracts were analyzed using high-performance liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response monitoring mode. POAA levels in sera and liver were quantitated by external calibration (without an internal standard reference), while the urine analysis was performed by internal calibration (with an internal standard reference). Analytical details are included in this report.

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## **Specimen Receipt and Maintenance**

The 3M Environmental Laboratory received liver, serum, urine and feces specimens collected at predetermined time points of the *in-life* phase of this study (#6329-231) beginning 11/3/98 to 8/17/99 from Covance. All specimens were received frozen on dry ice and were immediately transferred to storage at  $-55^{\circ}\text{C} \pm 20^{\circ}\text{C}$  and  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . Feces specimens were shipped to Centre Analytical Laboratories frozen on dry ice for analysis.

Control matrices used in the liver, sera, and urine analyses were obtained from various sources and are presented in Appendix A.

The remaining portions of the samples analyzed at the 3M Environmental Laboratory will be maintained at the laboratory for a period of time as specified by the regulations and will be kept at  $-50^{\circ}\text{C} \pm 20^{\circ}\text{C}$  or  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

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## Chemical Characterization of Test Substance

### Ammonium Perfluorooctanoate CAS Number: 3825-26-1

Chemical Formula:  $\text{C}_7\text{F}_{15}\text{CO}_2^-\text{NH}_4^+$

Molecular Weight: 431.1

Chemical characterization information on the test substance and reference substances used in this study is presented in tabular form in Appendix A. Purity determinations are on-going.

### Procurement

One lot of ammonium perfluorooctanoate (Lot number 332) was obtained from 3M Specialty Chemicals.

### Stability Studies

No stability samples were sent for analysis.

### Dose Confirmation Analyses

Dose confirmation analyses were not performed on the test substance dose samples. Since the test material was not mixed with a carrier, dose analyses were not required.

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## Method Summaries

### Centre Analytical Laboratories

The methods and analytical equipment settings used by Centre Analytical Laboratories for feces analysis are presented in the contract lab report (see Appendix H).

### 3M Environmental Laboratory

Following is a brief description of the methods used during this analytical phase by the 3M Environmental Laboratory. Detailed descriptions of the methods used in this phase are located in Appendix C.

As the present analytical phase progressed, more advanced methods evolved and earlier methods were used with deviations until amendments to the protocol were written. A summary of protocol and method deviations is presented in Appendix B of this report.

#### **PREPARATORY METHODS**

- **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"

Liver samples were homogenized in water. An aliquot of each homogenate was spiked with surrogate and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. The extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter using a 3 cc disposable plastic syringe into glass autosampler vials.

- **ETS-8-4.1**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry"

Serum samples were extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. The MtBE extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, then filtered through a 0.2 µm nylon filter using a 3 cc plastic syringe into glass autovials.

- **ETS-8-96.0**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry"

Urine samples were extracted using an ion-pairing extraction procedure. An ion-pairing reagent is added to 2 mL of the sample, and the analyte-ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 0.5 mL of methanol, then filtered through a 0.2 µm nylon filter using a 3 cc plastic syringe into glass autovials.

**ANALYTICAL METHODS**

- **ETS-8-7.0**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-5.1**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-97.0**, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry"

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC/ES/MS/MS. For example, molecular ion 413, selected as the primary ion for POAA ( $C_7F_{15}CO_2^-$ ) analysis, was fragmented to produce ion 169, 119, and 219. The characteristic ion 169 was monitored for quantitative analysis. In liver and sera analyses, each curve is plotted using linear regression with 1/x weighting. In urine analyses, each curve is plotted with an internal standard using a quadratic fit and 1/x weighting.

- The method validation study for the extraction and analysis of POAA in sera was not complete at the start of data collection. For method parameters characterized during validation, see Appendix D.

**ANALYTICAL EQUIPMENT**

The actual analytical equipment settings used in the analysis of liver, sera and urine varied slightly during actual data collection. The following is representative of the settings used during the analytical phase of this study. (Please see Appendix H for the instrument settings used during feces analysis.

**Liquid Chromatograph:** Hewlett-Packard® Series 1100 Liquid Chromatograph system

Analytical column: Keystone® Betasil™ C<sub>18</sub> 2x50 mm (5 µm)

Column temperature: Ambient

Mobile phase components:

Component A: 2mM ammonium acetate

Component B: methanol

Flow rate: 300 µL/min

Injection volume: 10 µL

Solvent Gradient:

| <i>Time (minutes)</i> | <i>%B</i> |
|-----------------------|-----------|
| 0.0                   | 40%       |
| 1.0                   | 40%       |
| 4.5                   | 95%       |
| 6.5                   | 95%       |
| 7.0                   | 40%       |

**Mass Spectrometer:** Micromass® API/Mass Spectrometer Quattro II™ Triple

Quadrupole system

Software: Mass Lynx™ 3.2–3.4

Cone Voltage: 15–60 V

Collision Gas Energy: 20–40 eV

Mode: Electrospray Negative

Source Block Temperature: 150°C ±10°C

Electrode: Z-spray

Analysis Type: Multiple Reaction Monitoring (MRM)

**Table 2. Target Ions Monitored in 3M Environmental Laboratory Analyses**

| Target Analyte | Primary Ion (AMU) | Product Ion (AMU)   |
|----------------|-------------------|---------------------|
| POAA           | 413.0             | 119.0, 169.0, 219.0 |
| THPFOS         | 427.0             | 80.0                |

\*THPFOS was used as a surrogate standard.

## Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Earlier methods were used with deviations until amendments to the protocol were written.

Deviations from the original protocol and methods are documented in Appendix B.

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## Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated in the protocol for this study:

- **Linearity:** The coefficient of determination ( $r^2$ ) equal to or greater than 0.980
- **Limits of Quantitation (LOQ):** The Limit of Quantitation (LOQ) is equal to the lowest acceptable standard in the calibration curve
- **Acceptable Precision:** Inter-assay, intra-assay and system are within 30% for the method
- **Acceptable Spike Recoveries:** 70–130% for sera, 60–140% for liver and urine
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and mass spectral daughter ion characterization.

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## Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory protocol for FACT TOX-026 (see Appendix B) were met with the exceptions noted in this report.

### Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination ( $r^2$ ) of the standard curve was  $\geq 0.980$ .
- **Calibration Standards:** For sera and liver analyses, quantitation of the target analyte used linear regression analysis, weighted  $1/x$ , of two extracted matrix curves bracketing each group of samples, or of a single curve preceding the samples. Urine data was determined using a  $1/x$  weighted quadratic fit calibration curve with an internal standard.

For sera, liver and urine analyses, high or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier was deactivated. Quantitation of the analyte was based on the response of three specific product ions using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).

- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within  $\pm 30\%$  of the theoretical value), and is at least two times the analyte peak area detected in the matrix blanks. Unless otherwise noted, the LOQ for liver is approximately 75 ng/g, for sera the LOQ is approximately 14 ng/mL and for urine the LOQ is approximately 20 ng/mL.

- **Blanks:** All blanks in liver assays were below the lower limit of quantitation for the compound of interest. In some instances for sera and urine data, the blanks were above the LOQ (refer to Appendix F for clarification). To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed monkey sera and monkey liver, rabbit sera and liver were selected as a suitable surrogate matrix. Monkey urine was used for standardization and QC samples in urine analysis.
- **Precision:** Not specifically determined within the course of this phase of the study.
- **Matrix Spikes:** Sera—Matrix spike samples were prepared from control monkey and/or rabbit sera along with each batch of sera samples. Samples were spiked with a final concentration of approximately 250–500 ng/mL, levels that approximate the background levels detected in the Group 1 samples.  
Liver—Matrix spike samples were prepared from control monkey liver along with each batch of liver samples. Samples were spiked at approximately 250 ng/g, levels that approximate the background levels detected in the Group 1 samples.  
Urine—Matrix spike samples were prepared from control monkey urine along with each batch of urine samples. Samples were spiked at approximately 60 ng/mL, levels that approximate the background levels detected in the Group 1 samples.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation for liver and sera analysis, but was used to monitor for gross instrument failure. THPFOS was used as an internal standard for quantitation of POAA in the urine samples.

### Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera, liver, urine, and feces data can be considered to be accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery for sera was 94% with a standard deviation of 11%. The average fortified sample recovery for liver was 90% with a standard deviation of 26%. The average fortified sample recovery for urine was 88% with a standard deviation of 17%. The average fortified sample recovery for feces was 117% with a standard deviation of 22%.

### Summary of Sample Results

- **Samples from Control Animals:** Low levels of POAA were often detected in the sera, liver, and urine of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, POAA levels found in the sera, liver, and urine of the test animals increased with dose groups. Detailed sample data tables are presented in Appendices E and F.

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### Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix G for example calculations used to generate the serum, urine, and liver sample data in FACT TOX-026. Data calculations used for feces sample data are included in the Centre Analytical Laboratories report.

- **Blanks:** All blanks in liver assays were below the lower limit of quantitation for the compound of interest. In some instances for sera and urine data, the blanks were above the LOQ (refer to Appendix F for clarification). To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed monkey sera and monkey liver, rabbit sera and liver were selected as a suitable surrogate matrix. Monkey urine was used for standardization and QC samples in urine analysis.
- **Precision:** Not specifically determined within the course of this phase of the study.
- **Matrix Spikes:** Sera—Matrix spike samples were prepared from control monkey and/or rabbit sera along with each batch of sera samples. Samples were spiked with a final concentration of approximately 250–500 ng/mL, levels that approximate the background levels detected in the Group 1 samples.  
Liver—Matrix spike samples were prepared from control monkey liver along with each batch of liver samples. Samples were spiked at approximately 250 ng/g, levels that approximate the background levels detected in the Group 1 samples.  
Urine—Matrix spike samples were prepared from control monkey urine along with each batch of urine samples. Samples were spiked at approximately 60 ng/mL, levels that approximate the background levels detected in the Group 1 samples.
- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation for liver and sera analysis, but was used to monitor for gross instrument failure. THPFOS was used as an internal standard for quantitation of POAA in the urine samples.

### Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera, liver, urine, and feces data can be considered to be accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery for sera was 93% with a standard deviation of 11%. The average fortified sample recovery for liver was 90% with a standard deviation of 26%. The average fortified sample recovery for urine was 88% with a standard deviation of 17%. The average fortified sample recovery for feces was 117% with a standard deviation of 22%.

### Summary of Sample Results

- **Samples from Control Animals:** Low levels of POAA were often detected in the sera, liver, and urine of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, POAA levels found in the sera, liver, and urine of the test animals increased with dose groups. Detailed sample data tables are presented in Appendices E and F.

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### Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix G for example calculations used to generate the serum, urine, and liver sample data in FACT TOX-026. Data calculations used for feces sample data are included in the Centre Analytical Laboratories report.

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## **Statement of Conclusion**

Under the conditions of the present studies, the fluorochemical perfluorooctanoate was observed in the serum, urine, and liver of most samples collected from cynomolgus monkeys dosed with the test substance during the in-life phase of the study.

## Appendix A: Chemical Characterization and Control Matrices

**Table 3. Characterization of the Control Matrices Used for Urine, Liver and Sera Analyses in Study FACT TOX-026**

| Control Matrix       | Rabbit Serum<br>TN-A-2052 | Rabbit Serum<br>TN-A-2307 | Rabbit Liver<br>TN-A-0808 | Rabbit Serum<br>TN-A-2391 | Rabbit Serum<br>TN-A-2573 |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Source               | Sigma Chemicals           | Sigma Chemicals           | Corning Hazelton          | Sigma Chemicals           | Sigma Chemicals           |
| Expiration Date      | 01/01/2010                | 01/01/2010                | 01/01/2010                | 01/01/2010                | 01/01/2010                |
| Storage Conditions   | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              |
| Chemical Lot #       | 107H8409                  | 118H8418                  | F00011                    | 118H8418                  | 118H8418                  |
| Physical Description | Rabbit Serum              | Rabbit Serum              | Rabbit Liver              | Rabbit Serum              | Rabbit Serum              |

| Control Matrix       | Rabbit Liver<br>TN-A-0806 | Rabbit Liver<br>TN-A-0802 | Rabbit Serum<br>99062-055 | Monkey Urine<br>99062-067 | Monkey Urine<br>99062-076 |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Source               | Corning Hazelton          | Corning Hazelton          | Sigma Chemicals           | N/R                       | Covance                   |
| Expiration Date      | 01/01/2010                | 01/01/2010                | 01/01/2010                | 01/01/2010                | 01/01/2010                |
| Storage Conditions   | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              | -20°C ± 10°C              |
| Chemical Lot #       | F00009                    | F00005                    | 118H8418                  | I06015                    | 6329-262                  |
| Physical Description | Rabbit Liver              | Rabbit Liver              | Rabbit Serum              | Monkey Urine              | Monkey Urine              |

| Control Matrix       | Monkey Urine<br>99062-078 |
|----------------------|---------------------------|
| Source               | Covance                   |
| Expiration Date      | 01/01/2010                |
| Storage Conditions   | -20°C ± 10°C              |
| Chemical Lot #       | 6329-262                  |
| Physical Description | Monkey Urine              |

N/R—not recorded

Table 4. Characterization of Test and Reference Substances in Study FACT TOX-026

|                      | Test Substance                                             | Reference Substances                                      |                                                          |                                                    |
|----------------------|------------------------------------------------------------|-----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------|
| Laboratory           | Covance Laboratories, Inc.                                 | 3M Environmental Lab                                      | 3M Environmental Lab                                     | Centre Analytical Laboratories                     |
| Chemical Name        | APFO (Ammonium Perfluorooctanoate)<br><i>TCR # missing</i> | APFO (Ammonium Perfluorooctanoate)<br>TN-A-1479<br>SE-030 | APFO (Ammonium Perfluorooctanoate)<br>TN-A-0497<br>SD022 | APFO (Ammonium Perfluorooctanoate)<br>TCR-99030-30 |
| Source               | 3M Specialty Chemical                                      | 3M Specialty Chemicals                                    | 3M Specialty Chemicals                                   | 3M Specialty Chemicals                             |
| Expiration Date      | N/R                                                        | 2010                                                      | 1/1/2010                                                 | N/R                                                |
| Storage Conditions   | Ambient temperature                                        | Ambient temperature                                       | Ambient temperature                                      | Ambient temperature                                |
| Chemical Lot #       | 332                                                        | 245                                                       | N/R                                                      | 332                                                |
| Physical Description | White powder                                               | White powder                                              | Light-colored powder                                     | White powder                                       |
| Purity               | 95.0–95.2%                                                 | TBD**                                                     | TBD**                                                    | 95.0%                                              |

| Analytical Reference Substances |                                |                      |
|---------------------------------|--------------------------------|----------------------|
| Laboratory                      | Centre Analytical Laboratories | 3M Environmental Lab |
| Chemical Name                   | THPFOS<br>SE037                | THPFOS<br>SD028      |
| Source                          | ICN Biomedicals                | ICN Biomedicals      |
| Expiration Date                 | 1/01/2010                      | 1/1/2010             |
| Storage Conditions              | Ambient temperature            | Ambient temperature  |
| Chemical Lot #                  | 53406                          | 59909                |
| Physical Description            | Brown waxy solid               | Brown powder         |
| Purity                          | TBD**                          | NA*                  |

N/R—Not recorded

\*None remaining; purity assumed to be 100%.

\*\*Unless otherwise indicated, at the time of quantitation, the purity for all analytes was assumed to be 100%.

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## **Appendix B: Protocol, Protocol Amendments and Deviation Summary**

**Study Title**

**6-Month Capsule Toxicity Study with  
Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys**

**PROTOCOL**

**Author**

Lisa Clemen

**Date:**

October 12, 1998

**Performing Laboratory**

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**

FACT-TOX-026

## Study Identification

### 6-Month Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys

|                                                                                                       |                                                                                                                           |
|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| <b>Test Material</b>                                                                                  | Ammonium perfluorooctanoate<br>(APFO/POAA)                                                                                |
| <b>Sponsor</b>                                                                                        | 3M Toxicology Services - Medical Department<br>3M Center, Building 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220 |
| <b>Sponsor Representative</b>                                                                         | Paul Lieder, Ph.D., DABT<br>3M Toxicology Services<br>Building 220-2E-02<br>651-737-2678                                  |
| <b>Study Director</b>                                                                                 | Kristen Hansen, Ph.D.<br>3M Environmental Technology and Safety<br>Services<br>Building 2-3E-09<br>651-778-6018           |
| <b>Study Location(s)</b><br><i>In vivo Testing Facility</i>                                           | Covance Laboratories, Inc.<br>3301 Kinsman Boulevard<br>Madison, Wisconsin 53704                                          |
| <i>Analytical Testing Laboratory</i>                                                                  | 3M Environmental Laboratory<br>Building 2-3E-09<br>935 Bush Avenue<br>St. Paul, MN 55106                                  |
| <b>Proposed Study Timetable</b><br><i>Analytical Start Date</i><br><i>Analytical Termination Date</i> | November 12, 1998<br>May 12, 1999                                                                                         |

## **1. STUDY**

Six month capsule toxicity study with ammonium perfluorooctanoate (APFO/POAA) in cynomolgus monkeys.

## **2. PURPOSE**

The analytical portion of this study is designed to determine levels of (APFO/POAA) in the liver and serum of cynomolgus monkeys. Based on these results additional tissues or fluids may be analyzed. The in-life portion of this study was conducted at Covance Laboratories, study #6329-231.

## **3. REGULATORY COMPLIANCE**

This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practices Standards, 40 CFR 792. However, analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

## **4. QUALITY ASSURANCE**

The 3M Environmental Laboratory Quality Assurance Unit will audit the protocol, study conduct, and final report in accordance with the Good Laboratory Practice Standards and 3M Environmental Laboratory Standard Operating Procedures.

## **5. TEST MATERIAL**

**5.1 Identification** Ammonium perfluorooctanoate (APFO/POAA)

**5.2 Source** 3M Specialty Chemical Division

**5.3 Physical Description** Gelatin capsules

**5.4 Purity and Stability** Determined by the Sponsor

**5.5 Storage Conditions** Room temperature

**5.6 Reserve Samples** Responsibility of the Sponsor

**5.7 Disposition** Specimens will be retained per GLP regulation

**5.8 Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

## 6. CONTROL MATERIAL

- 6.1 **Identification** Monkey liver and serum
- 6.2 **Source** Covance Laboratories, Inc.
- 6.3 **Physical Description** Liver and serum
- 6.4 **Purity and Stability** Not applicable
- 6.5 **Storage Conditions** Frozen at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$
- 6.6 **Reserve Samples** Not applicable
- 6.7 **Disposition** Biological tissues and fluids are retained per GLP regulation
- 6.8 **Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

## 7. REFERENCE MATERIAL

- 7.1 **Identification** Ammonium perfluorooctanoate (APFO/POAA), lot #377 or #245
- 7.2 **Source** 3M Specialty Chemicals
- 7.3 **Physical Description** White powder
- 7.4 **Purity and Stability** Responsibility of the Sponsor
- 7.5 **Storage Conditions** Room temperature
- 7.6 **Reserve Samples** Not applicable
- 7.7 **Disposition** Retained as per GLP regulation and 3M Environmental Laboratory policy
- 7.8 **Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

## 8. TEST SYSTEM

Cynomolgus monkeys were used as the test system, and were maintained and dosed as described in Covance protocol #6329-231. Group 1 control animals did not receive the test substance. Groups 2, 3, and 4 received the test substance daily for 26 weeks, in increasing concentration per group. Two animals each from Groups 1, 3, and 4 were designated as recovery animals and were allowed a 13 week recovery period after cessation of treatment.

## 9. SPECIMEN RECEIPT

The 3M Environmental Laboratory will receive samples of the following body tissues and fluids from the indicated points in the study:

| Body tissue/fluid               | Collected                                         | Shipped                                     | Expected # of samples                              |
|---------------------------------|---------------------------------------------------|---------------------------------------------|----------------------------------------------------|
| Serum – all animals             | 7 days post treatment, every two weeks thereafter | Packed on dry ice for shipping              | 264 from main study<br>78 additional from recovery |
| Urine, feces – recovery animals | After 6, 30, and 90 days recovery                 | Packed on dry ice for shipping              | 18 urine, 18 feces                                 |
| Liver – all animals             | At termination of the study                       | Shipped with final serum samples on dry ice | 22                                                 |

Total number of test animals: 16

Total number of control animals: 6

Samples sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

## 10. PREPARATORY METHODS

- 10.1** FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.2** FACT-M-3.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.3** If preparatory methods other than those listed above are used, an amendment to this protocol will be written.

## 11. ANALYTICAL METHODS

- 11.1** FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.2** FACT-M-4.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.3** If analytical methods other than those listed above are used, an amendment to this protocol will be written.

## **12. DATA QUALITY OBJECTIVES**

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators is included in the analytical methods. In addition, the following criteria will be met:

**12.1 Linearity**  $r^2 \geq 0.980$

### **12.2 Limits of detection / quantitation**

**12.2.1** Method Detection Limit (MDL) for APFO/POAA

**a.** Serum: 5 ppb

**b.** Liver: 24 ppb

**12.2.2** Practical Quantitation Limit (PQL) – Equal to the lowest standard in the calibration curve

**12.3 Duplicate acceptable precision** < 30% for the method

**12.4 Spike acceptable recoveries** 70% - 130%

**12.5 Use of confirmatory methods** Indeterminate samples will be re-analyzed

**12.6 Demonstration of specificity** Chromatographic retention time, mass spectral daughter ion characterization

## **13. SUB-CONTRACTED ANALYSIS**

All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

## **14. STATISTICAL ANALYSIS**

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

**14.1 Data transformations and analysis** Data will be reported as the concentration (weight/weight or weight/vol) of APFO/POAA or metabolite per tissue or fluid, or of APFO/POAA or metabolite per unit of tissue or fluid.

**14.2 Statistical analysis** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

## **15. REPORT**

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

**15.1** Name and address of the facility performing the study

**15.2** Dates upon which the study was initiated and completed

- 15.3** A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards
- 15.4** Objectives and procedures as stated in the approved protocol, including any changes in the original protocol
- 15.5** The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor
- 15.6** Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor
- 15.7** A description of the methods used to conduct the test(s)
- 15.8** A description of the test system
- 15.9** A description of any circumstances that may have affected the quality or the integrity of the data
- 15.10** The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study
- 15.11** A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses
- 15.12** Statistical methods used to evaluate the data, if applicable
- 15.13** The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable
- 15.14** The location where raw data and the final report are to be stored
- 15.15** A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

## **16. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT**

Original data, or copies thereof, will be available at 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

**16.1** The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

**16.1.1** Approved protocol and amendments

**16.1.2** Study correspondence

**16.1.3** Shipping records

**16.1.4** Raw data

**16.1.5** Approved final report (original signed copy)

**16.1.6** Electronic copies of data

**16.2** The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

**16.2.1** Training records

**16.2.2** Calibration records

**16.2.3** Instrument maintenance logs

**16.2.4** Standard Operating Procedures, Equipment Procedures, and Methods

## **17. SAMPLE RETENTION**

Specimens will be maintained in the laboratory specimen archives for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

## **18. PROTOCOL AMENDMENTS AND DEVIATIONS**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

## **19. ATTACHMENTS**

**19.1 Attachment A** Preparatory and analytical methods

**20. SIGNATURES**

Paul H. Lieder 11/30/98  
Paul Lieder, Ph.D, DABT, Sponsor Representative Date

Kristen Hansen 11/12/98  
Kristen Hansen, Ph.D., 3M Environmental Laboratory Study Director Date

***Study Title***

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 1**

***Amendment Date:***

July 23, 1999

***Performing Laboratory***

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

***Laboratory Project Identification***

ET&SS FACT-TOX026  
LIRN U2782

**This amendment modifies the following portion(s) of the protocol:**

1. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-3.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-2.0 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-4.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

**AMEND TO READ:** The extraction and analytical methods FACT-M-3.1 and FACT-M-4.1, respectively, were updated on 04/27/99 to:

ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** The methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.

The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-2.0 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

**AMEND TO READ:** The extraction and analytical methods FACT-M-1.0 and FACT-M-2.0, respectively, were updated on 07/22/99 to:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** The methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE, POAA and Monester were removed from the standard mix, and M556 was added to the standard mix.

The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

3. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

**AMEND TO READ:** Additional extraction and analytical methods, listed below, were added to the protocol:

ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or other fluorochemical compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** These methods were developed and validated for urine extraction and analysis after the original protocol was written and approved.

4. **PROTOCOL READS:** Section 2.0 lists serum and liver as the matrices of interest for determination of POAA.

**AMEND TO READ:** Urine will be included for determination of POAA.

**REASON:** The methods were developed and validated for extraction and analysis of urine after the original protocol was written and approved.

5. **PROTOCOL READS:** Section 6.0 lists monkey serum and liver as the control matrices received from Covance Laboratories.

**AMEND TO READ:** Control matrix monkey urine was obtained from Covance Laboratories and is maintained at a temperature of  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . All traceability information for this matrix will be included in the final report.

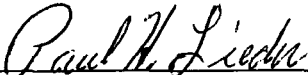
**REASON:** Addition of control urine matrix to the analytical protocol.

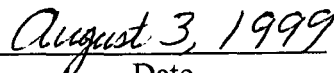
6. **PROTOCOL READS:** Section 12.2 lists monkey serum and liver method detection limits.

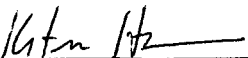
**AMEND TO READ:** Method detection limit for urine is 6 ppb.

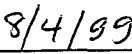
**REASON:** Addition of method detection limit for urine matrix.

### Amendment Approval

  
Paul Lieder, Ph.D., DABT, Sponsor Representative

  
Date

  
Kris J. Hansen, Ph.D., Study Director

  
Date

***Study Title***

6-Month Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 2**

***Amendment Date:***

20 January 2000

***Performing Laboratory***

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

***Laboratory Project Identification***

ET&SS LRN-U2782  
FACT TOX-026  
Covance Study: 6329-231  
3M Medical Department Study: T-6889.3

**This amendment modifies the following portion(s) of the protocol:**

**1. PROTOCOL READS:**

The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.

**AMEND TO READ:**

The role of study director for the present study was reassigned to Paul Lieder, Ph.D., as of 20 January 2000. The previous study director, Kristen Hansen, has been reassigned to the role of Principle Analytical Investigator.

**REASON:**

The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 40 CFR Part 792).

**2. PROTOCOL READS:**

The sponsor for the present study was identified as Paul Lieder.

**AMEND TO READ:**

The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.

**REASON:**

To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

**3. PROTOCOL READS:**

**17. Sample Retention:**

Specimens will be maintained in the laboratory specimen archives for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

**AMEND TO READ:**

**17. Specimen Retention:**

Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Any specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. Specimens analyzed at sub-contract laboratories will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

**REASON:**

To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

**4. PROTOCOL READS:**

Section 16 states that the following raw data and records will be retained in the study folder in the archives according to AMDT-S-8: Approved protocol and amendments; study correspondence; shipping records; raw data; approved final report (original signed copy); and electronic copies of data. Additionally, Section 16 states that supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following: Training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.

**AMEND TO READ:**

Section 16 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

**REASON:**

To direct subcontract laboratories in the disposition of the items listed above.

## Amendment Approval

*John P. Butenhoff*

*John Butenhoff, Ph.D., Sponsor Representative*

*February 10, 2000*

Date

*Kristen Hansen*

*Kristen J. Hansen, Ph.D., Outgoing Study Director*

*11-Feb-2000*

Date

*Paul H. Lieder*

*Paul Lieder, Ph.D., Incoming Study Director*

*Feb 10, 2000*

Date

***Study Title***

6-Month Capsule Toxicity Study with Ammonium Perfluorooctanoate  
(APFO/POAA) in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 3**

***Amendment Date:***

20 April 2000

***Performing Laboratories***

***LIVER, SERUM, AND URINE ANALYSES***

3M Environmental Technology and Safety Services  
Fluorine Analytical Chemistry Team  
Building 2-3E-09, 935 Bush Avenue  
St. Paul, MN 55106

***FECES ANALYSES***

Centre Analytical Laboratories, Inc.  
3048 Research Drive  
State College, PA 16801

***Laboratory Project Identification***

ET&SS LRN-U2782  
FACT TOX-026  
Covance Study: 6329-231  
3M Medical Department Study: T-6889.3

**This amendment modifies the following portion(s) of the protocol:**

**1. PROTOCOL READS:**

The amended section 2.0 text states that this study is designed to determine levels of APFO/POAA in the liver, serum, and urine of cynomolgus monkeys.

**AMEND TO READ:**

This study is designed to determine levels of APFO/POAA in the liver, serum, feces, and urine of cynomolgus monkeys.

**REASON:**

The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

**2. PROTOCOL READS:**

The amended section 6.0 lists monkey liver, serum, and urine.

**AMEND TO READ:**

Add: monkey feces with a physical description of feces.

**REASON:**

Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

**3. PROTOCOL READS:**

The amended Section 12.2.1 items a., b., and c., list the Method Detection Limits for matrices analyzed in this study.

**AMEND TO READ:**

The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

**REASON:**

The method detection limits are specific to the 3M Environmental Laboratory. This statement was added to allow for sub-contracted analyses, revised methods, and added matrices.

**4. PROTOCOL READS:**

Section 13. lists the laboratories that will be conducting analyses for this study.

**AMEND TO READ:**

Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801

**REASON:**

Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

**5. PROTOCOL READS:**

Sections 10.3 and 11.3 state that if methods other than those listed are used in this study, an amendment will be written to include the new methods.

**AMEND TO READ:**

The feces extraction and analytical method used by **Centre Analytical Laboratories** will be; 00M-023-003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."

**REASON:**

The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

**Amendment Approval**

*John L. Butenhoff*

*April 19, 2000*

---

John L. Butenhoff, Ph.D., Sponsor Representative

Date

*Paul H. Lieder*

*April 19, 2000*

---

Paul Lieder, Ph.D., Study Director

Date



*Study Title*

**26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys**

**PROTOCOL AMENDMENT NO. 4**

*Amendment Date:*

August 2, 2000

*Performing Laboratories*

Covance Laboratories Inc.  
3301 Kinsman Boulevard  
Madison, WI 53704

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

*Laboratory Project Identification*

FACT-TOX-026  
LIMS U2782  
Covance 6329-231  
3M Medical Department Study: T-6889.3

**This amendment modifies the following portion(s) of the protocol:**

**1. PROTOCOL READS:**

**STUDY TITLE:** "6-Month Capsule Toxicity [..]" (on the title page and page 2 of the protocol for the analytical phase of the study).

**AMEND TO READ:**

**STUDY TITLE:** "26-Week Capsule Toxicity [..]" (on the title page and page 2 of the protocol for the analytical phase of the study).

**REASON:**

To correct the title for the analytical phase of the study.

**2. PROTOCOL READS:**

DATA QUALITY OBJECTIVES (Section 12., page 6)

**AMEND TO READ:**

Add to this section: LOQ in feces of 10 ng/g

**REASON:**

To specify the analytical limits for feces analyses.

**3. PROTOCOL READS: (page 2)**

**STUDY DIRECTOR:** Peter J. Thomford **and** Paul Lieder

**TESTING FACILITY:** Covance Laboratories

**SPONSOR:** APME AdHoc APFO Toxicology Working Group **and** 3M Toxicology Services - Medical Department

**SPONSOR REPRESENTATIVE:** David Farrar **and** John Butenhoff

**AMEND TO READ:**

**STUDY DIRECTOR:** Paul Lieder

**TESTING FACILITY:** 3M Toxicology Services - Medical Department

**SPONSOR:** APME AdHoc APFO Toxicology Working Group (including 3M Toxicology Services - Medical Department)

**SPONSOR REPRESENTATIVE:** David Farrar (in-life) **and** John Butenhoff (analytical)

**REASON:**

To reassign the testing facility, in order to abide by the GLP requirement for one study director. To clarify the responsibilities of all parties included in this study.

**4. PROTOCOL READS:**

**PRINCIPAL ANALYTICAL INVESTIGATOR:** Kristen Hansen, Ph.D. (Amendment No 2 to TOX 026, Section 1.)

**AMEND TO READ:**

Add: **PRINCIPAL ANALYTICAL INVESTIGATORS:** Kristen Hansen, Ph.D. and Enaksha Wickremesinhe, Ph.D.

**REASON:**

To specify the PAI at Centre.

**5. PROTOCOL READS:**

**ANALYTICAL TERMINATION DATE:** May 12, 1999 (under Proposed Study Timetable, page 2)

**AMEND TO READ:**

**ANALYTICAL TERMINATION DATE:** September 30, 2000

**REASON:**

To allow for the analyses of all samples.

**6. PROTOCOL READS:**

**LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT** (Section 16., page 7)

**AMEND TO READ:**

Add: After issuing their final report, Centre will forward all original study-specific raw data to 3M Environmental Laboratories, together with copies of appropriate facility-specific raw data applicable to this study. Centre will maintain a copy of the applicable study-specific raw data, protocol and analytical report in the Centre archives.

**REASON:**

To specify the archival requirement for the portion of the data developed by Centre.

**7. PROTOCOL READS:**

**SAMPLE RETENTION** (Section 17., page 8)

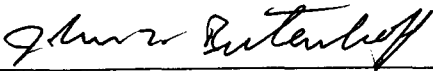
**AMEND TO READ:**

After the analytical report on feces is signed by the PAI, all feces specimens of this study will be returned to 3M Environmental Laboratory. These specimens may then be discarded by written direction of the study director. Specimens of blood, plasma, red blood cells, serum, bile, and urine may also be discarded by written direction of the study director after

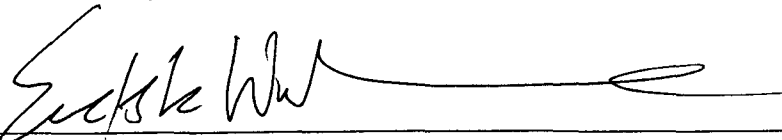
the analytical report for the 3M Environmental Laboratory analyses is signed by the study director.

***REASON:*** To specify the handling of the above biological specimens, and to define when the quality assurance verification is considered complete.

### Amendment Approval

 19 SEPT 2000  
\_\_\_\_\_  
John Butenhoff, Ph.D. Date  
Sponsor Representative, Analytical Phase

 19 SEPT 2000  
\_\_\_\_\_  
Paul Lieder, Ph.D., DABT Date  
3M Study Director

 9/26/00  
\_\_\_\_\_  
Enaksha Wickremesinha, Ph.D. Date  
Centre Analytical Laboratories, Principal Analytical Investigator

**Study Title**  
26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 5**

**Amendment Date:**  
October 12, 2000

**Performing Laboratory**  
3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**  
FACT-TOX-026  
ET&SS LRN U2782  
Covance 6329-231  
3M Medical Department Study: T-6889.3

**This amendment modifies the following portion(s) of the protocol:**

**1. *PROTOCOL READS:***

Principal Analytical Investigators: Kristen Hansen, Ph.D. and Enaksha Wickremesinhe, Ph.D.

**2. *AMEND TO READ:***

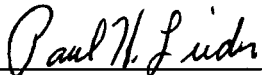
Principal Analytical Investigators: Kristen Hansen, Ph.D. and Emily Stauffer.

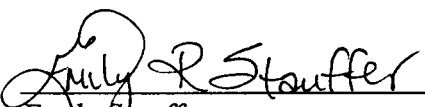
***REASON:***

To change role of the Principal Analytical Investigator at Centre Analytical Laboratories.

## Amendment Approval

 10/18/00  
\_\_\_\_\_  
John L. Butenhoff, Ph.D. Date  
Sponsor Representative, Analytical Phase

 10/23/00  
\_\_\_\_\_  
Paul Lieder, Ph.D., DABT Date  
3M Study Director

 11/2/00  
\_\_\_\_\_  
Emily Stauffer Date  
Centre Analytical Laboratories, Principal Analytical Investigator

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TOX-026

Covance 6329-231

Deviation Type

☐ SOP

☒ Method

☐ Equipment Procedure

(Check one)

☐ Protocol

☐ Other:

Document Number FACT-M-2.0, FACT-M-4.1

ETS-8-5.1, ETS-8-97.0

Date(s) of occurrence 11/16/98, 4/30/99, 5/05/99, 5/10/99, 5/17/99, 5/19/99, 5/20/99, 5/25/99, 5/26/99, 5/28/99, 6/03/99, 6/16/99, 8/13/99, 8/17/99, 8/23/99, 8/24/99, 9/22/99

## II. Description:

Required Procedure/process:

These methods state: Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted....

Actual Procedure/process:

The extracted matrix standards were analyzed prior to and following each set of extracts. Only the 1st standard curve was plotted.... The 2nd curve was not used in plotting the standard curve.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

Lisa A. Clemen

Date

11/14/00

## IV. Impact on Study / Project

All other QC for the run was acceptable & all results were bracketed. No adverse effect.

gh 11/17/00

Authorized By (Study Director / Project Lead)

Paul H. Lieder

11/24/00

John Z. Butenhoff 4 DEC 00

Date

Sponsor: John Butenhoff

Study Director: Paul Lieder

3M Environmental Laboratory

Form ETS-4-8.0

Deviation No. 1

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TDX-026

Covance 6329-231

Deviation Type

(Check one)

☐ SOP

☒ Method

☐ Equipment Procedure

☐ Protocol

☐ Other:

Document Number

FACT-M-4.1

Date(s) of occurrence 11/16/98, 11/24/98, 12/03/98

01/04/99, 01/21/99, 01/27/99

## II. Description:

Required Procedure/process:

Method FACT-M-4.1 states the initial curve should be plotted using linear regression without being weighted.

Actual Procedure/process:

The initial curve was plotted using linear regression, weighted 1/x.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written

Recorded By

Jim A. Clemen

Date

11/14/00

## IV. Impact on Study / Project

The use of a weighted curve is an improvement. No adverse effect.

by 11/17/00

Authorized By (Study Director / Project Lead)

Paul H. Lieder 11/20/00

John Z. Butenloff 4 DEC 00

Date

Sponsor: John Butenloff  
3M Environmental Laboratory  
Form ETS-4-8.0

Study Director: Paul Lieder

Deviation No. 2

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No. TDX026 Covance 6329-231

Deviation Type ☐ SOP ☐ Method ☐ Equipment Procedure  
(Check one) ☒ Protocol ☐ Other:

Document Number FACT-m-3.1 + FACT-m-4.1 Date(s) of occurrence 4/29/99

## II. Description:

Required Procedure/process:

The extraction and analytical methods listed are FACT-m-3.1 and FACT-m-4.1.

Actual Procedure/process:

The extraction and analytical methods which will be followed are ETS-8-4.1 and ETS-8.5.1

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

An amendment to the protocol will be issued.

Recorded By

Olson A Clemen

Date

4/29/99

## IV. Impact on Study / Project

This deviation will not affect the outcome of this study.

Authorized By (Study Director) / Project Lead)

Kirtan H

Date

4/29/99

# Record of Deviation

## I. Identification

Study / Project No. TOX026 Covance 6329-231

Deviation Type ☐ SOP ☒ Method ☐ Equipment Procedure  
(Check one) ☐ Protocol ☐ Other:

Document Number ETS-8-4.1 + ETS-8-5.1 Date(s) of occurrence  
4/29/99

## II. Description:

Required Procedure/process:

ETS-8-4.1 and ETS-8-5.1 The extraction and analytical methods do not list perfluorooctanoate as a target analyte.

Actual Procedure/process:

Perfluorooctanoate (PDAA) will be the target analyte for the extraction and analysis of serum.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

The methods will be revised to include this component.

Recorded By

John A. Clemen

Date

4/29/99

## IV. Impact on Study / Project

This deviation will have no affect on the outcome of this study

Authorized By (Study Director) / Project Lead)

Kriter HB

Date

4/29/99

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TDX-026

Covance 6329-231

Deviation Type

☐ SOP

☒ Method

☐ Equipment Procedure

(Check one)

☐ Protocol

☐ Other:

Document Number

FACT-M-1.0

Date(s) of occurrence

05/07/99, 05/18/99, 06/21/99, 07/13/99

## II. Description:

Required Procedure/process:

Method FACT-M-1.0 states that ethyl acetate will be used as the extraction solvent.

Actual Procedure/process:

Methyl tert-butyl ether (MTBE) was used as the extraction solvent.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written. Methods were validated using MTBE as the extraction solvent.

Recorded By

Lisa A. Clemens

Date

11/14/00

## IV. Impact on Study / Project

The use of MTBE is an improvement. No adverse affect.

gh 11/17/00

Authorized By (Study Director / Project Lead)

Paul W. Lieder

Study Director: Paul Lieder

Date

gh 11/20/00 / ghr 1. Revalidated 4 DEC 00

# Record of Deviation

| <b>I. Identification</b>                                                                                                                                                                                          |                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Study / Project No. <u>FACT-TOX-026</u> <span style="float: right;"><u>Covance 6329-231</u></span>                                                                                                                |                                                                                                                                |
| Deviation Type (Check one) <input type="checkbox"/> SOP <input checked="" type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure <input type="checkbox"/> Protocol <input type="checkbox"/> Other: |                                                                                                                                |
| Document Number<br><u>FACT-m-2.0</u>                                                                                                                                                                              | Date(s) of occurrence <u>5/10/99, 5/11/99, 5/12/99, 5/13/99, 5/27/99, 6/16/99, 6/22/99, 6/23/99, 6/24/99, 7/13/99, 7/21/99</u> |
| <b>II. Description:</b>                                                                                                                                                                                           |                                                                                                                                |
| Required Procedure/process:                                                                                                                                                                                       |                                                                                                                                |
| <u>Method FACT-m-2.0 states the initial curve will be plotted using linear regression.</u>                                                                                                                        |                                                                                                                                |
| Actual Procedure/process:                                                                                                                                                                                         |                                                                                                                                |
| <u>The initial curve was plotted using linear regression weighted 1/x.</u>                                                                                                                                        |                                                                                                                                |
| <b>III. Actions Taken:</b>                                                                                                                                                                                        |                                                                                                                                |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                    |                                                                                                                                |
| <u>This deviation was written.</u>                                                                                                                                                                                |                                                                                                                                |
| Recorded By<br><u>Aisha A. Clemen</u>                                                                                                                                                                             | Date<br><u>11/14/00</u>                                                                                                        |
| <b>IV. Impact on Study / Project</b>                                                                                                                                                                              |                                                                                                                                |
| <u>The use of a weighted curve is an improvement. No adverse affect to the study.</u>                                                                                                                             |                                                                                                                                |
| <u>lgh 11/17/00</u>                                                                                                                                                                                               |                                                                                                                                |
| Authorized By (Study Director / Project Lead)<br><u>Paul H. Lieder 11/20/00 / John Z. Butenhoff &amp; REC 00</u>                                                                                                  | Date                                                                                                                           |

Sponsor: John Butenhoff      Study Director: Paul Lieder  
 3M Environmental Laboratory  
 Form ETS-4-8.0

Deviation No. 6

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TDX-026

Covance 6329-231

Deviation Type

(Check one)

☐ SOP

☐ Method

☐ Equipment Procedure

☒ Protocol

☐ Other:

Document Number

FACT-TDX-026

Date(s) of occurrence 5/17/99, 5/18/99,

5/19/99, 5/20/99, 5/22/99

## II. Description:

Required Procedure/process:

The protocol states method ETS-8-5.1 will be used to analyze the sera extracts.

Actual Procedure/process:

Method ETS-8-5.D was listed as the method used to analyze these sera sample extracts.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

Lisa A. Clemen

Date

11/14/00

## IV. Impact on Study / Project

No adverse affects.

h/h 11/17/00

Authorized By (Study Director / Project Lead)

Paul H. Lieder

11/20/00 / John Z. Butenloff 4 DEC 00

Date

Sponsor: John Butenloff

Study Director: Paul Lieder

3M Environmental Laboratory

Form ETS-4-8.0

Deviation No. 7

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TOX-026

Covance 6329-231

Deviation Type

(Check one)

☐ SOP

☐ Method

☐ Equipment Procedure

☒ Protocol

☐ Other:

Document Number

FACT-M-2.0

Date(s) of occurrence 5/19/99, 5/27/99, 6/16/99,  
6/22/99, 6/23/99, 6/24/99, 7/13/99, 7/31/99

## II. Description:

Required Procedure/process:

The protocol lists method FACT-M-2.0 for analyzing liver extracts.

Actual Procedure/process:

Methods ETS-8-5.1 (a sera analytical method) and FACT-M-2.1 were listed as the methods used when analyzing these liver extracts.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

Lisa A. Clemens

Date

11/14/00

## IV. Impact on Study / Project

No adverse affects, FACT-M-2.1 is an improvement over FACT-M-2.0.

by 11/17/00

Authorized By (Study Director / Project Lead)

Paul H. Lieder 11/20/00 / John Z. Butenhoff 4 DEC 00

Date

Sponsor: John Butenhoff Study Director: Paul Lieder  
3M Environmental Laboratory  
Form ETS-4-8.0

Deviation No. 8

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TOX-026

Covance 6329-231

Deviation Type

☐ SOP

☐ Method

☐ Equipment Procedure

(Check one)

☒ Protocol

☐ Other:

Document Number

FACT-TOX-026

Date(s) of occurrence

06/21/99, 07/13/99

## II. Description:

Required Procedure/process:

The protocol lists the liver extraction method as FACT-M-1.0

Actual Procedure/process:

Followed method FACT-M-1.1

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

Lisa A. Clemen

Date

11/14/00

## IV. Impact on Study / Project

FACT-M-1.1 is an improvement. No adverse effect.

h/n 11/17/00

Authorized By (Study Director / Project Lead)

Paul W. Lieder 11/20/00 / John Z. Butenhoff 4 DEC 00

Date

Sponsor: John Butenhoff Study Director: Paul Lieder  
3M Environmental Laboratory  
Form ETS-4-8.0

Deviation No. 9

(assigned by Study Director or Project Lead at the end of study or project)

## Record of Deviation

### I. Identification

Study / Project No.

FACT-TOX-026

Covance 6329-231

Deviation Type  
(Check one)

☐ SOP

☒ Method

☐ Equipment Procedure

☐ Protocol

☐ Other:

Document Number

ETS-8-97.0

Date(s) of occurrence

08/06/99 + 08/10/99

### II. Description:

Required Procedure/process:

Method states the internal standard will be used for calculating concentrations and the curve for POAA will be plotted using a quadratic fit.

Actual Procedure/process:

Concentrations were calculated using external standard and POAA curve was plotted using linear regression.

### III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

No actions were taken since these data were used for screening purposes - to determine dilutions, and were not reported.

Recorded By

John A. Clemen

Date

08/11/99

### IV. Impact on Study / Project

This deviation will not adversely affect the outcome of this study.

Authorized By (Study Director / Project Lead)

Kifer H.

Date

8/11/99

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TDX-026

Covance 6329-231

Deviation Type

☐ SOP

☒ Method

☐ Equipment Procedure

(Check one)

☐ Protocol

☐ Other:

Document Number

ETS-8-97.0

Date(s) of occurrence

8/24/99

## II. Description:

Required Procedure/process:

The method states - analyze a midrange continuing calibration verification at least every 10 samples, with a minimum of one per batch.

At least one continuing calibration verification per 10 samples must show a percent recovery within  $\pm 30\%$  of the spiked concentration.

Actual Procedure/process:

One continuing calibration verification per 15 samples had a percent recovery within  $\pm 30\%$  of the spiked concentration.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

Data will be flagged in the Excel spreadsheets.

Recorded By

Lisa A. Clemen

Date

11/16/00

## IV. Impact on Study / Project

Data will be flagged to denote added uncertainty.

by 11/17/00

Authorized By (Study Director / Project Lead)

Paul W. Liden 11/24/00 / Glen Z. Butenloff 4 DEC 00

Date

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TOX-026

Conance 6329-231

Deviation Type

(Check one)

☐ SOP

☐ Method

☐ Equipment Procedure

☒ Protocol

☐ Other:

Document Number

ETS-8-97.0

Date(s) of occurrence

9/07/99, 9/08/99, 9/09/99, 9/10/99

## II. Description:

Required Procedure/process:

The protocol lists method ETS-8-97.0 for analyzing urine extracts.

Actual Procedure/process:

Methods ETS-8-5.1 (a sera analytical method) and ETS-8-7.0 (a liver analytical method) were listed as the methods used when analyzing the urine extracts.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written

Recorded By

Lisa A. Clemens

Date

RE UC

11/08/00

11/14/00

## IV. Impact on Study / Project

Although the incorrect methods were documented, the correct method (ETS-8-97.0) was followed. No adverse affect.

4h 11/17/00

Authorized By (Study Director / Project Lead)

Paul W. Lieder 11/20/00 / John Z. Bartenhoff 4 DEC 00

Date

Sponsor: John Bartenhoff Study Director: Paul Lieder  
3M Environmental Laboratory  
Form ETS-4-8.0

Deviation No. 12

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

FACT-TOX-026

Covance 6329-231

Deviation Type

(Check one)

☐ SOP

☒ Method

☐ Equipment Procedure

☐ Protocol

☐ Other:

Document Number

ETS-8-97.0

Date(s) of occurrence

9/28/99

## II. Description:

Required Procedure/process:

Method ETS-8-97.0 states the initial curve will be plotted using a quadratic fit, weighted  $1/x$ , using internal standard.

Actual Procedure/process:

The curve was plotted using a quadratic fit, weighted  $1/x$ , using external standard.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

A. A. Clemen

Date

11/14/00

## IV. Impact on Study / Project

A variable injection volume was used, this invalidates the use of the internal standard. Although the data is not expected to be affected, these results will be flagged.

km 11/17/00

Authorized By (Study Director / Project Lead)

Date

Paul H. Lieder, 11/20/00 / John Z. Butenhoff 4 DEC 00

Sponsor: John Butenhoff, Study Director: Paul Lieder  
3M Environmental Laboratory  
Form ETS-4-8.0

Deviation No. 13

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.

TOX006

Deviation Type  
(Check one)

☐ SOP

☒ Method ☐ Equipment Procedure

☐ Protocol

☐ Other:

Document Number

ETS-8-97.0

Date(s) of occurrence

9-27-00

## II. Description:

Required Procedure/process:

The area of the method blank needs to be less than half that of the LOD.

\* Although this is not stated in the method, it is part of the control of bias documentation that is considered part of the analytical method.

Actual Procedure/process:

The area count for MKU080499-H2OBLK-1 is greater than half the LOD. The area counts for the other nine H2O blanks is less than half that of the LOD.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

Notified PI.

Recorded By

mmx

Date

9-29-00

## IV. Impact on Study / Project

The one anomalously high blank appears to have been affected by a low concentration of surrogate, rather than a particularly high concentration of PIAA. As the remaining blanks were acceptable, this anomaly is not indicative of a systematic error. No adverse effect on the study. kjh (PAI) 10/02/00

Authorized By (Study Director / Project Lead)

Date

John R. Buten 11/7/00

Paul H. Lieder 11/8/00

Sponsor Rep. John Butenhiott  
3M Environmental Laboratory  
Form ETS-4-8.0

Study Director- Paul Lieder

Deviation No. 14

(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

|                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>I. Identification</b>                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                    |
| Study / Project No. FACT-TOX-026                                                                                                                                                                                                                                                                   | Covance 6329-231                                                                                                                                                                                   |
| Deviation Type<br>(Check one)                                                                                                                                                                                                                                                                      | <input type="checkbox"/> SOP<br><input type="checkbox"/> Protocol<br><input checked="" type="checkbox"/> Method<br><input type="checkbox"/> Equipment Procedure<br><input type="checkbox"/> Other: |
| Document Number(s): ETS-8-5.1, ETS-8-7.0, and ETS-8-97.0                                                                                                                                                                                                                                           | Date(s) of occurrence:<br>Entire study                                                                                                                                                             |
| <b>II. Description:</b>                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                    |
| Required Procedure/process:                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                    |
| The method describes the calculations that should be used to convert extract concentration to matrix concentration.                                                                                                                                                                                |                                                                                                                                                                                                    |
| Actual Procedure/process:                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                    |
| The actual calculation used varied somewhat from that written in the method. One additional factor was added for salt correction. This accommodates the mass difference between the analytical standard (C7F15COONH4) and the target analyte (C7F15COO-) determined after the study was completed. |                                                                                                                                                                                                    |
| <b>III. Actions Taken:</b>                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                    |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                                                                                                     |                                                                                                                                                                                                    |
| This deviation was written.                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                    |
| Recorded By                                                                                                                                                                                                                                                                                        | Date                                                                                                                                                                                               |
| <i>John A. Clemmer</i>                                                                                                                                                                                                                                                                             | 02/12/01                                                                                                                                                                                           |
| <b>IV. Impact on Study / Project</b>                                                                                                                                                                                                                                                               |                                                                                                                                                                                                    |
| The updated calculations accommodate new information and are an improvement. No adverse affect on the study.                                                                                                                                                                                       |                                                                                                                                                                                                    |
| <i>hjh 02/12/01</i>                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                    |
| Authorized By (Study Director / Project Lead)                                                                                                                                                                                                                                                      | Date                                                                                                                                                                                               |
| <i>John A. Clemmer 19 FEB 01</i> <i>Paul H. Lieder</i>                                                                                                                                                                                                                                             | 19 Feb 01                                                                                                                                                                                          |

Sponsor Representative: John Butenhoff

Study Director: Paul Lieder

# Record of Deviation

| I. Identification                                                                                                                                                                                                    |                                     |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Study / Project No. FACT-TOX-026 Covance 6329-231                                                                                                                                                                    |                                     |
| Deviation Type <input type="checkbox"/> SOP <input checked="" type="checkbox"/> Method <input type="checkbox"/> Equipment Procedure<br>(Check one) <input type="checkbox"/> Protocol <input type="checkbox"/> Other: |                                     |
| Document Number(s): ETS-8-7.0, ETS-8-97.0                                                                                                                                                                            | Date(s) of occurrence: Entire Study |
| II. Description:                                                                                                                                                                                                     |                                     |
| Required Procedure/process:                                                                                                                                                                                          |                                     |
| These methods state that matrix spike percent recoveries must be within $\pm 30\%$ of the spiked concentration.                                                                                                      |                                     |
| Actual Procedure/process:                                                                                                                                                                                            |                                     |
| The matrix spike percent recoveries for liver and urine data were $\pm 40\%$ of the spiked concentration.                                                                                                            |                                     |
| III. Actions Taken:                                                                                                                                                                                                  |                                     |
| (such as amendment issued, SOP revision, etc.)                                                                                                                                                                       |                                     |
| This deviation was written.                                                                                                                                                                                          |                                     |
| Recorded By<br><i>Lisa A. Clemen</i>                                                                                                                                                                                 | Date<br><i>01/11/01</i>             |
| IV. Impact on Study / Project                                                                                                                                                                                        |                                     |
| Data quality stated in the final report will reflect actual recovery of the matrix spikes.                                                                                                                           |                                     |
| No adverse impact on the study.                                                                                                                                                                                      |                                     |
| <i>Kh 01/11/01</i>                                                                                                                                                                                                   |                                     |
| Authorized By (Study Director / Project Lead)<br><i>John Z. Butenith 19 FEB 01</i>                                                                                                                                   | Date<br><i>19 Feb 01</i>            |

Sponsor Representative: John Butenith

Study Director: Paul Lieder

# Record of Deviation

## I. Identification

Study / Project No. FACT-TOX-026 Covance 6329-231

Deviation Type ☐ SOP ☐ Method ☐ Equipment Procedure  
(Check one) ☒ Protocol ☐ Other:

Document Number(s):  
Protocol FACT-TOX-026

Date(s) of occurrence:  
Entire Study

## II. Description:

Required Procedure/process:

Methods ETS-8-7.0 and ETS-8-97.0 state that matrix spike percent recoveries must be within  $\pm$  30% of the spiked concentration.

Actual Procedure/process:

The matrix spike percent recoveries for liver and urine data were  $\pm$  40% of the spiked concentration.

## III. Actions Taken:

(such as amendment issued, SOP revision, etc.)

This deviation was written.

Recorded By

*John A. Clemen*

Date

*05/15/01*

## IV. Impact on Study / Project

Data quality stated in the final report will reflect actual recovery of the matrix spikes.  
No adverse impact on the study.

*John 05/15/01*

Authorized By (Study Director / Project Lead)

*Paul H. Lieder 5/18/01*

Date

*John Z. Butenhoff May 24, 2001*

Study Director: Paul Lieder

Sponsor Representative: John Butenhoff

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## Appendix C: Extraction and Analytical Methods

This appendix includes the following 3M Environmental Laboratory methods:

### *Liver*

**FACT-M-1.1**, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry, (15 pages)

**FACT-M-2.1**, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry, (9 pages)

**ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry", (14 pages)

**ETS-8-7.0**, "Analysis of Potassium Perfluorooctane-sulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry", (10 pages)

### *Serum*

**ETS-8-4.1**, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry, (14 pages)

**FACT-M-3.1**, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry, (17 pages)

**ETS-8-5.1**, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry, (9 pages)

**FACT-M-4.1**, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry, (9 pages)

### *Urine*

**ETS-8-96.0**, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry, (14 pages)

**ETS-8-97.0**, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry, (10 pages)

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-1.1

Adoption Date: 05/26/98

Revision Date: 06/03/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

DJ Bm  
Laboratory Manager

6/3/99  
Date

Kler Hz  
Group Leader

6/1/99  
Date

Lisa A Clemen  
Technical Reviewer

6/01/99  
Date

Initial PSD  
Date 4/5/01  
Exact Copy of Original

#### 0 SCOPE AND APPLICATION

1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

Matrices: Rabbit, rat, bovine, and monkey liver or other liver as designated in the validation report.

## 2.0 SUMMARY OF METHOD

---

- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemicals from liver homogenate using an ion pairing reagent and 5.0 ml of ethyl acetate. In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, POAA, PFOSEA, and FC-807 monoester (see 3.0 *Definitions*). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into ethyl acetate. Four ml of extract are removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 ml of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

## 3.0 DEFINITIONS

---

- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt)  $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide  $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol  
 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 POAA: perfluorooctanoate (anion of ammonium salt)  $C_7F_{15}COO^-$
- 3.6 PFOSEA: perfluorooctane sulfonyl ethylamide  $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.7 FC-807 monoester  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O-PO_3H$
- 3.8 Surrogate standard 1H,1H,2H,2H perfluorooctane sulfonic acid

## 4.0 WARNINGS AND CAUTIONS

---

### 4.1 Health and safety warnings:

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, it may contain pathogens.

## 5.0 INTERFERENCES

---

- 5.1 There are no known interferences at this time.

## 6.0 EQUIPMENT

---

- 6.1 The following equipment is used while carrying out this method. Equivalent equipment is acceptable.
- 6.1.1 Ultra-Turrax with T25 grinder attachment for grinding/dispersing/emulsifying
  - 6.1.2 Vortex mixer, VWR, Vortex Genie 2
  - 6.1.3 Centrifuge, Mistral 1000 or IEC
  - 6.1.4 Shaker, Eberbach or VWR
  - 6.1.5 Nitrogen evaporator, Organomation
  - 6.1.6 Balance, ( $\pm 0.100$  g)

## **7.0 SUPPLIES AND MATERIALS**

---

- 7.1 Gloves
- 7.2 Eppendorf or disposable pipettes
- 7.3 Nalgene bottles, capable of holding 250 ml and 1 L
- 7.4 Wheaton 6 ml Plastic Sample Vials
- 7.5 Glass, type A, volumetric flasks
- 7.6 40 ml glass I-CHEM vials
- 7.7 Polypropylene centrifuge tubes, 15 ml
- 7.8 Labels
- 7.9 Syringes, capable of measuring 5  $\mu$ L to 50  $\mu$ L
- 7.10 Glass, type A, volumetric pipettes
- 7.11 Graduated pipettes
- 7.12 Electronic pipettor, Eppendorf or equivalent
- 7.13 Timer
- 7.14 Disposable plastic 3 cc syringes
- 7.15 Filters, nylon syringe filters, 0.2  $\mu$ m, 25 mm
- 7.16 Crimp cap autovials

**Note:** Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q<sup>TM</sup> water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

## **8.0 REAGENTS AND STANDARDS**

---

- 8.1 ASTM Type I reagent grade water, Milli-Q<sup>TM</sup> or equivalent; all water used in this method should be Milli-Q<sup>TM</sup> water and may be provided by a Milli-Q TOC Plus<sup>TM</sup> system.
- 8.2 Sodium hydroxide (NaOH), J.T. Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO<sub>3</sub>), J.T. Baker or equivalent
- 8.6 Ethyl acetate, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Liver tissue, frozen from supplier
- 8.9 Control matrix or blank matrix for standards, QC checks, blanks, etc.
- 8.10 **Fluorochemical standards**
  - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
  - 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
  - 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

- 8.10.4** EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 571
- 8.10.5** POAA (3M Specialty Chemical Division), molecular weight = 431
- 8.10.6** PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.7** FC-807 monoester (3M Specialty Chemical Division). FC-807 is a mixture of triester, diester, and monoester fluorochemical components. The monoester molecular weight = 650
- 8.10.8** Surrogate Standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H,2-H  $C_8F_{13}SO_3H$ ) molecular weight = 428
- 8.10.9** Other fluorochemicals, as appropriate

## **8.11 Reagent preparation**

- 8.11.1** 10 N sodium hydroxide (NaOH): Weigh approximately 200g NaOH. Pour into a 1000 ml beaker containing 500 ml Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.11.2** 1 N sodium hydroxide (NaOH): Dilute 10N NaOH 1:10. Measure 10 ml of 10N NaOH solution into a 100 ml volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 ml Nalgene bottle.
- 8.11.3** 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 ml Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 ml of 10N NaOH and dilute to volume with Milli-Q™ water. While adding the last few ml's of NaOH, add slowly because the pH changes abruptly. Store in a 1 L Nalgene bottle.
- 8.11.3.1** TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.
- 8.11.4** 0.25M Sodium carbonate/sodium bicarbonate buffer ( $Na_2CO_3/NaHCO_3$ ): Weigh approximately 26.5 g of sodium carbonate ( $Na_2CO_3$ ) and 21.0 g of sodium bicarbonate ( $NaHCO_3$ ) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

## **8.12 Standards**

- 8.12.1** Prepare PFOS standards for the standard curve.
- 8.12.2** Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (e.g. one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.12.3** Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight.
- 8.12.4** Bring to volume with methanol for a stock standard of approximately 1000 ppm ( $\mu g/ml$ ).
- 8.12.5** Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

### 8.13 Surrogate stock standard preparation

8.13.1 Prepare a surrogate stock standard. Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H,2-H, C<sub>8</sub>F<sub>13</sub>SO<sub>3</sub>H into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 0.5 ml of surrogate stock to a 50 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

### 8.14 Liver homogenate preparation

*Note: The following procedure will be much easier to perform with frozen liver tissue. Prevent tissue from thawing; keep stored on ice until excising a portion of it.*

8.14.1 Weigh 40 g of blank or control liver into a 250 ml Nalgene bottle containing 100 mls Milli-Q™ water. Record the actual weight of liver and total volume of water used. Grind the liver into a finely dispersed homogenate with an Ultra-Turrax T25 grinder (high speed for approximately 3 minutes or until sufficiently homogenized). Rinse grinder with an additional 100 ml of Milli-Q™ water, to bring the total volume of water added to 200 ml.

8.14.2 To determine the concentration of the blank liver homogenate, transfer ten 1.0 ml aliquots of the homogenate to tared polypropylene tubes, and weigh each aliquot on a balance. The average density of these aliquots is determined and then the concentration (g of liver/ml of homogenate) can be calculated as follows:

8.14.3

$$\frac{[\text{grams (g) of liver}] \times [\text{avg. weight of 1.0 ml of homogenate (density) (g/ml)}]}{\{[\text{grams (g) of liver}] + [\text{grams (g) of water}]\}}$$

8.14.3 Prepare sample livers as described in 8.3.1, but weigh out 1 g of liver, homogenize with 2.5 ml of Milli-Q™ water, and rinse with another 2.5 ml of Milli-Q™ water. Use Wheaton 6 ml plastic sample vials or appropriate receptacle. Rinse grinder unit after every sample with water and then with methanol. Label vials appropriately including study number, sample ID, liver weight, date, and analyst. Record all weights and volumes used. (Do not perform 8.3.2 for the sample liver homogenates).

## **9.0 SAMPLE HANDLING**

---

**9.1** All livers are received frozen and must be kept frozen until the extraction is performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Matrix blanks and method blanks**

**10.1.1** Extract two 1.0 ml aliquots of the liver homogenate (prepared in 8.14.1-2) following this procedure and use as matrix blanks. See Section 11.1.2.

**10.1.2** Extract two 1.0 ml aliquots of Milli-Q™ water following this procedure and use as method blanks.

### **10.2 Matrix spikes**

**10.2.1** Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

**10.2.2** Prepare each spike using liver chosen by the analyst, usually the control liver received with each sample set.

**10.2.3** Expected concentrations fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

**10.2.4** Prepare one matrix spike and one matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

### **10.3 Continuing calibration checks**

**10.3.1** Prepare and analyze continuing calibration check samples to ensure the accuracy of the initial calibration curve. If the percent difference between the initial curve and the continuing check differ by >30%, reanalyze samples analyzed after the last acceptable check.

**10.3.2** Prepare one check per group of ten samples. For example, if a sample set = 34, prepare and extract four checks.

**10.3.3** Prepare each continuing calibration check from the same blank liver homogenate used to prepare the initial curve.

**10.3.4** The expected concentrations fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (e.g. 10 ppb – 100 ppb, rather than 10 ppb – 1000 ppb).

## **11.0 CALIBRATION AND STANDARDIZATION**

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### **11.1 Prepare liver homogenate standards**

**11.1.1** Transfer 1 ml aliquots of blank/control liver homogenate prepared in 8.14.1-2 to 15 ml centrifuge tubes.

- 11.1.2** If the volumes of sample liver homogenates are limited, extract standards with liver homogenate volumes equal to the sample volumes. Do not extract below 0.50 ml of liver homogenate. Record the sample volume on the extraction sheet.
- 11.1.3** While preparing a total of twenty aliquots of liver homogenate in 15 ml centrifuge tubes, mix or shake between aliquots.
- 11.1.4** Two 1 ml, or appropriate aliquots, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1 (at the end of this section) to spike, in duplicate, two standard curves, for a total of eighteen standards and two matrix blanks.
- 11.1.5** Refer to the validation reports **FACT-M-1.1-V-1** and **FACT-M-2.1-V-1** which list the working ranges and Linear Calibration Range (LCR) for calibration curves.
- 11.1.6** Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2** To each standard, blank, or QC check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 10 ppb – 1000 ppb.
- 11.3** Extract spiked liver homogenate standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

| <b>Table 1</b><br><b>Approximate Spiking Amounts for Standards and Spikes</b><br><b>Using 1.0 ml of Liver</b> |    |                                         |
|---------------------------------------------------------------------------------------------------------------|----|-----------------------------------------|
| Working Standard<br>(Approx. Conc.)                                                                           | μL | Approx. final conc. of<br>PFOS in liver |
| -                                                                                                             | -  | Blank                                   |
| 0.500 ppm                                                                                                     | 4  | 0.012 ppm                               |
| 0.500 ppm                                                                                                     | 10 | 0.030 ppm                               |
| 0.500 ppm                                                                                                     | 20 | 0.060 ppm                               |
| 0.500 ppm                                                                                                     | 40 | 0.120 ppm                               |
| 5.00 ppm                                                                                                      | 10 | 0.300 ppm                               |
| 5.00 ppm                                                                                                      | 20 | 0.600 ppm                               |
| 5.00 ppm                                                                                                      | 30 | 0.900 ppm                               |
| 50.0 ppm                                                                                                      | 4  | 1.20 ppm                                |
| 50.0 ppm                                                                                                      | 6  | 1.80 ppm                                |

## **12.0 PROCEDURES**

---

- 12.1** Obtain frozen liver samples and homogenize as described in **8.14.3**.
- 12.2** Vortex mix homogenate for 15 seconds, then transfer 1.0 ml or other appropriate volume to a 15 ml polypropylene centrifuge tube.
- 12.3** Return liver homogenate samples to freezer after extraction amount has been removed.

- 12.4 Record the liver homogenate volume on the extraction worksheet. The final methanol volume will equal the initial homogenate volume. For example, if 1 ml of homogenate is transferred for extraction, then the final reconstitution methanol volume equals 1 ml.
- 12.5 Label the tube with the study number, liver ID, date, and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike blank liver homogenate aliquots with the appropriate amount of standard as described in Section 11.1 or Table I in that section for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.7 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in Section 11.2.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 To each sample, add 1 ml 0.5 M TBA and 2 ml of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.10 Using a volumetric pipette, add 5 ml ethyl acetate.
- 12.11 Cap each sample and put on the shaker for 20 minutes.
- 12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.
- 12.13 Transfer 4 ml of organic layer, using a 5 ml graduated glass pipette, to a clean 15 ml centrifuge tube. Label this fresh tube with the same information as in 12.5.
- 12.14 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.
- 12.15 Add 1.0 ml or appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume equals the initial volume of liver homogenate used for the extraction.
- 12.16 Vortex mix for 30 seconds.
- 12.17 Attach a 0.2  $\mu$ m nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 ml glass autovial (or low-volume autovial when necessary).
- 12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) who performed the extraction.
- 12.19 Cap and store extracts at approximately 4° C until analysis.
- 12.20 Complete the extraction worksheet, attached to this document, and tape to page of study notebook or include in study binder, as appropriate.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

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#### **13.1 Calculations:**

- 13.1.1 Calculate actual concentrations of PFOS, or other appropriate fluorochemical, in calibration standards using the following equation:

$$\frac{\text{ml of Standard} \times \text{Concentration of Standard } (\mu\text{g/ml})}{\text{Concentration of Blank Liver Homogenate (g/ml) (see 8.14.2)}} = \text{Final Concentration } (\mu\text{g/g}) \text{ of PFOS in Liver}$$

See **Attachment D** for a sample form to calculate the concentrations of standards.

#### **14.0 METHOD PERFORMANCE**

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- 14.1** The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).
- 14.2** The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
- 14.2.1** Method blanks and matrix blanks
  - 14.2.2** Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction
  - 14.2.3** Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

#### **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

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- 15.1** Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

#### **16.0 RECORDS**

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- 16.1** Complete the extraction worksheet attached to this method, and tape into the study notebook or include into study binder, as appropriate.

#### **17.0 ATTACHMENTS**

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- 17.1** Attachment A, Extraction worksheet
- 17.2** Attachment B, MDL/LOQ values
- 17.3** Attachment C, LOQ summary
- 17.4** Attachment D, Calibration standard concentration worksheet

#### **18.0 REFERENCES**

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- 18.1** The validation reports associated with this method are **FACT-M-1.1 and 2.1-V-1**.

#### **19.0 AFFECTED DOCUMENTS**

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- 19.1** FACT-M-2.1, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## 20.0 REVISIONS

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| <u>Revision<br/>Number.</u> | <u>Reason For Revision</u>                                                                                                                                                                                               | <u>Revision<br/>Date</u> |
|-----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 1                           | <i>Validation of method to include 7 fluorochemicals, new API/MS(MS) systems, monkey liver cross validation, improvements to ion pairing extraction, MDL study, updates in record keeping and storing policies, etc.</i> | 08/01/98                 |

|                                                                                       |                   |                            |
|---------------------------------------------------------------------------------------|-------------------|----------------------------|
| Blank                                                                                 | Std #             | amount =                   |
| <b>Extraction Method/Revision:</b>                                                    |                   | <b>Date &amp; Initials</b> |
| Add Surrogate, Vortex 15 sec.                                                         |                   |                            |
| Pipette Sample                                                                        | Volume            | ml                         |
| Pipette 1 ml of 0.5 M TBA, pH 10. PH =                                                | Std. #            |                            |
| Pipette 2 ml of 0.25 Na <sub>2</sub> CO <sub>3</sub> /0.25M NaHCO <sub>3</sub> buffer | Std. #            |                            |
| Pipette 5 ml of ethyl acetate                                                         | TN-A-             |                            |
| Shake 20 min.                                                                         | Shaker Speed      |                            |
| Centrifuge 20-25 min.                                                                 | Centrifuge speed: |                            |
| Remove a 4 ml aliquot of organic layer                                                |                   |                            |
| Put on Nitrogen Evaporator to dryness                                                 | Temperature:      |                            |
| Add methanol                                                                          | Volume            | ml TN-A-                   |
| Vortex 30 sec.                                                                        |                   |                            |
| Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 ml autosample vial  |                   |                            |

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**MDL/LOQ values for Rabbit Liver:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate Concentrations to be used for preparing the<br/>Standard Calibration Curve</b> |
|-----------------|------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 11.8             | 37.4             | 38 ppb – 1000 ppb                                                                                                                |
| PFOSA           | 6.06             | 19.3             | 20 ppb – 1200 ppb                                                                                                                |
| PFOSAA          | 55.7             | 177              | 180 ppb – 1000 ppb                                                                                                               |
| EtFOSE-OH       | 58.7             | 187              | 190 ppb – 1800 ppb                                                                                                               |
| POAA            | 23.7             | 75.5             | 76 ppb – 1800 ppb                                                                                                                |
| PFOSEA          | 16.2             | 51.7             | 62 ppb – 1200 ppb                                                                                                                |
| Monoester       | n/v              | n/v              | Monoester was not detectable/quantifiable at the spiked concentrations.                                                          |

**MDL/LOQ values for Rat Liver:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate Concentrations to be used for preparing the<br/>Standard Calibration Curve</b> |
|-----------------|------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 24.7             | 78.7             | 62 ppb – 1200 ppb                                                                                                                |
| PFOSA           | 20.7             | 65.8             | 20 ppb – 1200 ppb                                                                                                                |
| PFOSAA          | n/v              | n/v              | 62 ppb – 1200 ppb                                                                                                                |
| EtFOSE-OH       | n/v              | n/v              | 120 ppb – 1200 ppb                                                                                                               |
| POAA            | n/v              | n/v              | 62 ppb – 1200 ppb                                                                                                                |
| PFOSEA          | n/v              | n/v              | 120 ppb – 1200 ppb                                                                                                               |
| Monoester       | n/v              | n/v              | Monoester was not detectable/quantifiable at the spiked concentrations.                                                          |

**MDL/LOQ values for Monkey Liver:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate Concentrations to be used for preparing the<br/>Standard Calibration Curve</b> |
|-----------------|------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | n/v              | n/v              | 59 ppb – 1200 ppb                                                                                                                |
| PFOSA           | 27.4             | 87.1             | 28 ppb – 1200 ppb                                                                                                                |
| PFOSAA          | n/v              | n/v              | 120 ppb – 1200 ppb                                                                                                               |
| EtFOSE-OH       | n/v              | n/v              | 58 ppb – 1200 ppb                                                                                                                |
| POAA            | n/v              | n/v              | 120 ppb – 1200 ppb                                                                                                               |
| PFOSEA          | n/v              | n/v              | 120 ppb – 1200 ppb                                                                                                               |
| Monoester       | n/v              | n/v              | Monoester was not detectable/quantifiable at the spiked concentrations.                                                          |

n/v = Not valid. Upon analyzing the data, value did not pass the criteria set for this characterization.  
Until further analysis is completed, use the LCR to determine the range of standard concentrations for calibration curve preparation.

| Summary Table: Limits of Quantitation |        |                                   |          | Approximate Linear Range <sup>1</sup> |               |
|---------------------------------------|--------|-----------------------------------|----------|---------------------------------------|---------------|
| Compound                              | Matrix | MDL                               | LOQ      | Low Standard                          | High Standard |
|                                       |        |                                   |          |                                       |               |
| PFOS                                  | Rabbit | 11.8 ppb                          | 37.4 ppb | 38 ppb                                | 1000 ppb      |
|                                       | Bovine | n/d = not determined <sup>2</sup> |          | 60 ppb                                | 1200 ppb      |
|                                       | Rat    | 24.7 ppb                          | 78.7 ppb | 62 ppb                                | 1200 ppb      |
|                                       | Monkey | n/v = not valid <sup>3</sup>      |          | 59 ppb                                | 1200 ppb      |
| PFOSA                                 | Rabbit | 6.06 ppb                          | 19.3 ppb | 20 ppb                                | 1200 ppb      |
|                                       | Bovine | n/d                               | n/d      | 30 ppb                                | 1200 ppb      |
|                                       | Rat    | 20.7 ppb                          | 65.8 ppb | 6 ppb                                 | 1200 ppb      |
|                                       | Monkey | 27.4 ppb                          | 87.1 ppb | 6 ppb                                 | 1200 ppb      |
| PFOSAA                                | Rabbit | 55.7 ppb                          | 177 ppb  | 180 ppb                               | 1900 ppb      |
|                                       | Bovine | n/d                               | n/d      | 120 ppb                               | 1200 ppb      |
|                                       | Rat    | n/v                               | n/v      | 62 ppb                                | 1200 ppb      |
|                                       | Monkey | n/v                               | n/v      | 120 ppb                               | 1200 ppb      |
| EtFOSE-OH                             | Rabbit | 58.7 ppb                          | 187 ppb  | 190 ppb                               | 1800 ppb      |
|                                       | Bovine | n/d                               | n/d      | 120 ppb                               | 1200 ppb      |
|                                       | Rat    | n/v                               | n/v      | 120 ppb                               | 1200 ppb      |
|                                       | Monkey | n/v                               | n/v      | 58 ppb                                | 1200 ppb      |
| POAA                                  | Rabbit | 23.7 ppb                          | 75.5 ppb | 76 ppb                                | 1800 ppb      |
|                                       | Bovine | n/d                               | n/d      | 120 ppb                               | 1200 ppb      |
|                                       | Rat    | n/v                               | n/v      | 62 ppb                                | 1200 ppb      |
|                                       | Monkey | n/v                               | n/v      | 120 ppb                               | 1200 ppb      |
| PFOSEA                                | Rabbit | 16.2 ppb                          | 51.7 ppb | 62 ppb                                | 1200 ppb      |
|                                       | Bovine | n/d                               | n/d      | 30 ppb                                | 1200 ppb      |
|                                       | Rat    | n/v                               | n/v      | 120 ppb                               | 1200 ppb      |
|                                       | Monkey | n/v                               | n/v      | 120 ppb                               | 1200 ppb      |
| Monoester                             | Rabbit | n/d                               | n/d      | n/a                                   | n/a           |
|                                       | Bovine | n/d                               | n/d      | n/a                                   | n/a           |
|                                       | Rat    | n/d                               | n/d      | n/a                                   | n/a           |
|                                       | Monkey | n/d                               | n/d      | n/a                                   | n/a           |

1 - Upper Limit chosen where the value was within the Linear Calibration Range (LCR) but did not excessively weight the standard curve or affect Repeatability & Reproducibility values.

2 - Not determined refers to no sample was analyzed for this data.

3 - Not valid refers to data from the analysis failed to meet specific criteria for a valid MDL/LOQ determination.

| Compound     |                             | PFOS                   |                        |                         |                         |                          |                          |
|--------------|-----------------------------|------------------------|------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 5.95 - 1790                 | 5.95 - 1790            | 29.8 - 1190            | 5.95 - 298              | 11.9 - 298              | 119 - 1790               | 119 - 1790               |
| Bovine       | 6.00 - 1200                 | 6.00 - 1200            | 60.0 - 1200            | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.22 - 1240                 | 6.22 - 1240            | 62.2 - 1240            | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.93 - 1190                 | 5.93 - 1190            | 59.3 - 1190            | n/a                     | n/a                     | n/a                      | n/a                      |

| Compound     |                             | PFOSA                  |                        |                         |                         |                          |                          |
|--------------|-----------------------------|------------------------|------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 6.04 - 1810                 | 6.04 - 1210            | 60.4 - 1210            | 6.04 - 302              | 12.1 - 302              | 121 - 1210               | 302 - 1210               |
| Bovine       | 5.95 - 1190                 | 5.95 - 1190            | 29.8 - 1190            | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.17 - 1240                 | 6.17 - 1240            | 6.17 - 1240            | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.88 - 1180                 | 5.88 - 1180            | 5.88 - 1180            | n/a                     | n/a                     | n/a                      | n/a                      |
|              |                             |                        |                        |                         |                         |                          |                          |
| Compound     |                             | PFOSAA                 |                        |                         |                         |                          |                          |
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 6.33 - 1900                 | 127 - 1900             | 127 - 1900             | n/a                     | n/a                     | n/a                      | n/a                      |
| Bovine       | 5.99 - 1200                 | 120 - 1200             | 122 - 1200             | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.21 - 1240                 | 62.1 - 1240            | 62.1 - 1240            | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.92 - 1180                 | 59.2 - 1180            | 118 - 1180             | n/a                     | n/a                     | n/a                      | n/a                      |
|              |                             |                        |                        |                         |                         |                          |                          |
| Compound     |                             | EtFOSE-OH              |                        |                         |                         |                          |                          |
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 5.96 - 1790                 | 119 - 1790             | 119 - 1790             | n/a                     | n/a                     | n/a                      | n/a                      |
| Bovine       | 5.87 - 1170                 | 58.7 - 1170            | 117 - 1170             | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.09 - 1220                 | 122 - 1220             | 122 - 1220             | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.80 - 1160                 | 29.4 - 1160            | 58.0 - 1160            | n/a                     | n/a                     | n/a                      | n/a                      |
|              |                             |                        |                        |                         |                         |                          |                          |
| Compound     |                             | POAA                   |                        |                         |                         |                          |                          |
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 6.06 - 1820                 | 30.3 - 1820            | 121 - 1820             | 30.3 - 606              | 30.3 - 606              | 303 - 1820               | 303 - 1820               |
| Bovine       | 5.93 - 1190                 | 59.3 - 1190            | 119 - 1190             | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.15 - 1230                 | 61.5 - 1230            | 61.5 - 1230            | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.86 - 1170                 | 117 - 1170             | 117 - 1170             | n/a                     | n/a                     | n/a                      | n/a                      |
|              |                             |                        |                        |                         |                         |                          |                          |
| Compound     |                             | PFOSEA                 |                        |                         |                         |                          |                          |
| Liver Matrix | Prepared Range of Standards | Range of Average Curve | LCR from Average Curve | Range of Low Std. Curve | LCR from Low Std. Curve | Range of High Std. Curve | LCR from High Std. Curve |
|              | (ppb)(ng/g)                 | (ppb)(ng/g)            | (ppb)(ng/g)            | (ppb)(ng/g)             | (ppb)(ng/g)             | (ppb)(ng/g)              | (ppb)(ng/g)              |
| Rabbit       | 6.20 - 1860                 | 62.0 - 1240            | 62.0 - 1240            | n/a                     | n/a                     | n/a                      | n/a                      |
| Bovine       | 5.92 - 1180                 | 29.6 - 1180            | 29.6 - 1180            | n/a                     | n/a                     | n/a                      | n/a                      |
| Rat          | 6.14 - 1230                 | 123 - 1230             | 123 - 1230             | n/a                     | n/a                     | n/a                      | n/a                      |
| Monkey       | 5.85 - 1170                 | 58.5 - 1170            | 117 - 1170             | n/a                     | n/a                     | n/a                      | n/a                      |

Monoester was not detectable/quantifiable in liver matrix for the concentration range of 4.94 – 1450 ppb.

| Ion Pair Standard Curves--Tissues                            |               |               |                |                |                            |                |                  |             |
|--------------------------------------------------------------|---------------|---------------|----------------|----------------|----------------------------|----------------|------------------|-------------|
| Prep Date(s):                                                | 11/2/98       |               |                |                | Study Number:              |                | Cross Validation |             |
| Analyst(s):                                                  | RWW-IAS       |               |                |                | Equipment Number:          |                |                  |             |
| Sample Matrix:                                               | Monkey Liver  |               |                |                | Final Solvent & TN Number: | MeOH TN-A-2076 |                  |             |
| Method/Revision                                              | FACT-M-1.0    |               |                |                | Blank Tissue/Identifier:   | Liver          |                  |             |
| Target Analyte(s):                                           | FC-Mix        |               |                |                |                            |                |                  |             |
| FC Mix Std Approx. 0.500 ppm: W398-1004                      |               |               |                |                |                            |                |                  |             |
| FC Mix Std Approx. 5.00 ppm: W398-1003                       |               |               |                |                |                            |                |                  |             |
| FC Mix Std Approx. 50.0 ppm: W398-1002                       |               |               |                |                |                            |                |                  |             |
| Surrogate Std Approx. 16.5 ppm: W398-989                     |               |               |                |                |                            |                |                  |             |
| Actual Concentrations of Standards in the FC Mix             |               |               |                |                |                            |                |                  |             |
| PFOS                                                         | PFOSA         | PFOSAA        | EtFOSE         | POAA           | PFOSEA                     |                | All              | All         |
| Std Conc.                                                    | Std Conc.     | Std Conc.     | Std Conc.      | Std Conc.      | Std Conc.                  |                | Am't Spiked      | Liver conc. |
| ug/mL                                                        | ug/mL         | ug/mL         | ug/mL          | ug/mL          | ug/mL                      |                | mL               | g/ml        |
| 0.501                                                        | 0.497         | 0.500         | 0.490          | 0.495          | 0.494                      |                | 0.002            | 0.169       |
| 0.501                                                        | 0.497         | 0.500         | 0.490          | 0.495          | 0.494                      |                | 0.004            | 0.169       |
| 0.501                                                        | 0.497         | 0.500         | 0.490          | 0.495          | 0.494                      |                | 0.010            | 0.169       |
| 0.501                                                        | 0.497         | 0.500         | 0.490          | 0.495          | 0.494                      |                | 0.020            | 0.169       |
| 0.501                                                        | 0.497         | 0.500         | 0.490          | 0.495          | 0.494                      |                | 0.040            | 0.169       |
| 5.01                                                         | 4.97          | 5.00          | 4.90           | 4.95           | 4.94                       |                | 0.010            | 0.169       |
| 5.01                                                         | 4.97          | 5.00          | 4.90           | 4.95           | 4.94                       |                | 0.020            | 0.169       |
| 5.01                                                         | 4.97          | 5.00          | 4.90           | 4.95           | 4.94                       |                | 0.030            | 0.169       |
| 50.1                                                         | 49.7          | 50.0          | 49.0           | 49.5           | 49.4                       |                | 0.004            | 0.169       |
| Calculated Concentrations of Standards in the Sample Matrix. |               |               |                |                |                            |                |                  |             |
| PFOS                                                         | PFOSA         | PFOSAA        | EtFOSE         | POAA           | PFOSEA                     |                | Surrogate        | All         |
| Final Conc.                                                  | Final Conc.   | Final Conc.   | Final Conc.    | Final Conc.    | Final Conc.                |                | Std Conc.        | Am't Spiked |
| ng/g                                                         | ng/g          | ng/g          | ng/g           | ng/g           | ng/g                       |                | ug/mL            | mL          |
| 5.93                                                         | 5.88          | 5.92          | 5.80           | 5.86           | 5.85                       |                | 16.50            | 0.005       |
| 11.9                                                         | 11.8          | 11.8          | 11.6           | 11.7           | 11.7                       |                | Surrogate        |             |
| 29.6                                                         | 29.4          | 29.6          | 29.0           | 29.3           | 29.2                       |                | Final Conc.      |             |
| 59.3                                                         | 58.8          | 59.2          | 58.0           | 58.6           | 58.5                       |                | ng/g             |             |
| 119                                                          | 118           | 118           | 116            | 117            | 117                        |                | 488.2            |             |
| 296                                                          | 294           | 296           | 290            | 293            | 292                        |                |                  |             |
| 593                                                          | 588           | 592           | 580            | 586            | 585                        |                |                  |             |
| 889                                                          | 882           | 888           | 870            | 879            | 877                        |                |                  |             |
| 1186                                                         | 1176          | 1183          | 1160           | 1172           | 1169                       |                |                  |             |
| Validated Ranges -- Approximate Concentrations               |               |               |                |                |                            |                |                  |             |
| Liver                                                        | PFOS          | PFOSA         | PFOSAA         | EtFOSE-OH      | POAA                       | PFOSEA         |                  |             |
| Rabbit                                                       | 40 - 1000 ppb | 20 - 1200 ppb | 180 - 1900 ppb | 190 - 1800 ppb | 80 - 1800 ppb              | 60 - 1200 ppb  |                  |             |
| Bovine                                                       | 60 - 1200 ppb | 30 - 1200 ppb | 120 - 1200 ppb | 120 - 1200 ppb | 80 - 1200 ppb              | 30 - 1200 ppb  |                  |             |
| Rat                                                          | 60 - 1200 ppb | 70 - 1200 ppb | 60 - 1200 ppb  | 120 - 1200 ppb | 60 - 1200 ppb              | 120 - 1200 ppb |                  |             |
| Monkey                                                       | 60 - 1200 ppb | 90 - 1200 ppb | 120 - 1200 ppb | 60 - 1200 ppb  | 120 - 1200 ppb             | 120 - 1200 ppb |                  |             |

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### ANALYSIS OF FLUOROchemicals IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

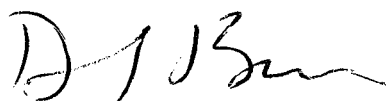
Method Number: FACT-M-2.1

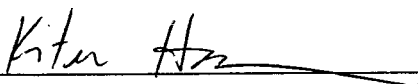
Adoption Date: 05/26/98

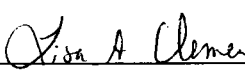
Revision Date: 06/03/99

Author: Lisa Clemen

Approved By:

 6/3/99  
Laboratory Manager Date

 6/1/99  
Group Leader Date

 6/01/99  
Technical Reviewer Date

Initial RSD  
Date 6/5/01  
Exact Copy of Original

#### SCOPE AND APPLICATION

**Scope:** This method is for the analysis of extracts of liver or other tissues for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

**Applicable Compounds:** Potassium perfluorooctanesulfonate, anionic fluorochemical surfactants, or other ionizable compounds.

**Matrices:** Rabbit, rat, bovine, and monkey livers or other livers as designated in the validation report.

## **2.0 SUMMARY OF METHOD**

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- 2.1** This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion,  $M/Z=499$ . Samples may also be screened to verify compound identification.

## **3.0 DEFINITIONS**

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- 3.1 Atmospheric Pressure Ionization (API):** The Micromass platform systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API platforms are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio ( $m/z$ ) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass platform systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these platform systems. Currently MassLynx has Windows 95 and WindowsNT 3.1 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Platform II or Quattro II MassLynx or MassLynx NT USER'S GUIDE).

## **4.0 WARNINGS AND CAUTIONS**

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### **4.1 Health and Safety Warnings:**

- 4.1.1** Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.
- 4.1.2** When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

## **4.2 Cautions:**

- 4.2.1 Do not run solvent pumps above capacity of 400 bar (5800 psi). If pressure goes over 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

## **5.0 INTERFERENCES**

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- 5.1 To minimize interferences when analyzing samples for perfluorooctanoate(POAA), teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

## **6.0 EQUIPMENT**

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- 6.1 Equipment listed below may be modified in order to optimize the system.

- 6.1.1 Micromass Electrospray Mass Spectrometer
- 6.1.2 HP1100 low pulse solvent pumping system and autosampler.

## **7.0 SUPPLIES AND MATERIALS**

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### **7.1 Supplies**

- 7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi
- 7.1.2 HPLC column, specifics to be determined by the analyst.
- 7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

## **8.0 REAGENTS AND STANDARDS**

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### **8.1 Reagents**

- 8.1.1 Methanol, HPLC grade or equivalent.
- 8.1.2 ASTM, Type I water, Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.
- 8.1.3 Ammonium acetate, reagent grade or equivalent.

### **8.2 Standards**

- 8.2.1 Typically one method blank, one matrix blank, and ten matrix standards are prepared during the extraction procedure. See **FACT-M-1.1**.

## **9.0 SAMPLE HANDLING**

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- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.
- 9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature or refrigerated at 4° C until analysis can be performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Matrix Blanks and Method Blanks**

**10.1.1** Analyze a method blank and matrix blank prior to each calibration curve.

### **10.2 Matrix Spikes**

**10.2.1** Analyze a matrix spike and matrix spike duplicate with each analysis. With a minimum of 2 spikes per batch.

**10.2.2** Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

**10.2.3** See section 13 to calculate percent recovery.

### **10.3 Continuing Calibration Checks**

**10.3.1** Analyze a mid-range calibration standard after every tenth sample. If a significant change ( $\pm 30\%$ ) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

**10.3.2** See section 13 to calculate percent difference.

## **11.0 CALIBRATION AND STANDARDIZATION**

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**11.1** Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ( $y = my + b$ ), not forced through zero, using MassLynx or other suitable software.

**11.2** If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

**11.3** For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

## **12.0 PROCEDURES**

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### **12.1 Acquisition Set up**

**12.1.1** Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

**12.1.2** To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass

MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

- 12.1.3 Typically the sample list begins with the first set of liver standards and ends with the second set of standards.
- 12.1.4 Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

## 12.2 Using the Autosampler

- 12.2.1 Set up sample tray according to the sample list prepared in section 12.1.1.
- 12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:
  - 12.2.2.1 Sample size = 10  $\mu$ L injection with a sample wash
  - 12.2.2.2 Inject/sample = 1
  - 12.2.2.3 Cycle time = 13.5 minutes
  - 12.2.2.4 Solvent ramp =

| Time      | MeOH | 2.0 mM<br>Ammonium acetate |
|-----------|------|----------------------------|
| 0.00 min. | 40%  | 60%                        |
| 8.0 min.  | 90%  | 10%                        |
| 11.0 min. | 90%  | 10%                        |
| 12.0 min. | 40%  | 60%                        |

- 12.2.2.5 Press the "Start" button.

## 12.3 Instrument Sep-up

- 12.3.1 Refer to ETS-9-24.0 for more details .
- 12.3.2 Check the solvent level in reservoirs and refill if necessary.
- 12.3.3 Check the stainless steel capillary at the end of the probe. Use an eye piece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500  $\mu$ L/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.
- 12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

- 12.3.6.1 Drying gas 250-400 liters/hour
- 12.3.6.2 ESI nebulizing gas 10-15 liters/hour
- 12.3.6.3 LC constant flow mode flow rate 10 – 500 uL/min
- 12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the instrument is operating correctly.)
- 12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10 Click on start button in the Acquisition Control Panel. Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

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#### **13.1 Calculations:**

- 13.1.1 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.2 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.3 Calculate actual concentration of PFOS anion in total liver (mg):

$$\frac{\left( \frac{\text{ug PFOS anion calc. from std curve}}{\text{g of liver used for analysis}} \right)}{1000 \text{ ug} / 1 \text{ mg}} \times \text{Total mass of liver (g)}$$

### **14.0 METHOD PERFORMANCE**

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- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-1.1, Attachment B, for a listing of current validated MDL and LOQ values.

#### **14.1 14.2 Solvent Blanks, Method Blanks, and Matrix Blanks**

- 14.1.1 Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve.

#### **14.2 Calibration Curves**

- 14.2.1 The r<sup>2</sup> value for the calibration curve must be 0.980 or better.

### **14.3 Matrix Spikes**

**14.3.1** Matrix spike percent recoveries are must be within  $\pm 30\%$  of the spiked concentration.

### **14.4 Continuing Calibration Verifications**

**14.4.1** Continuing calibration verification percent recoveries must be  $\pm 30\%$  of the spiked concentration.

**14.5** If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

**14.6** If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

## **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

**15.1** Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers. All containers are located in the laboratory.

## **16.0 RECORDS**

**16.1** Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

**16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.

**16.3** Plot the calibration curve by linear regression, weighted  $1/x$ , then print these graphs and store in the study folder.

**16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

**16.5** Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.

**16.6** Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

## **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

**17.1** Attachment A: FACT-M-2.1 Data reporting spreadsheet

## **18.0 REFERENCES**

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- 18.1** FACT-M-1.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2** ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3** The validation report associated with this method is **FACT-M-1.1-V & 2.1-V-1.**

## **19.0 AFFECTED DOCUMENTS**

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- 19.1** FACT-M-1.1, "Extraction of Potassium Perfluorooctanesulfonate from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

## **20.0 REVISIONS**

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| <u>Revision<br/>Number.</u> | <u>Reason For Revision</u>                                                                                                                                                                                                                                       | <u>Revision<br/>Date</u> |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 1                           | Section 6.1.2 Clarification of HP1100 system components.<br>Section 11.1 Average of two curves, not standard values, are used for plotting linear regression.<br>Section 12.2.2.4 Clarification of solvent ramp.<br>Section 17.1 Changed from attachment B to A. | 05/04/99                 |

## Laboratory Study #

Study:  
 Test Material:  
 Matrix/Final Solvent:  
 Method/Revision:  
 Analytical Equipment System Number:  
 Instrument Software/Version:  
 Filename:  
 R-Squared Value:  
 Slope:  
 Y Intercept:  
 Date of Extraction/Analyst:  
 Date of Analysis/Analyst:

| Group<br>Dose | Sample# | Concentration<br>ug/mL | Initial Vol.<br>mL | Dilution<br>Factor | Final Conc.<br>ug/mL |
|---------------|---------|------------------------|--------------------|--------------------|----------------------|
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |

**Slope:** Taken from linear regression equation.

**Group/Dose:** Taken from the study folder.

**Sample#:** Taken from the study folder.

**Concentration (ug/mL):** Taken from the MassLynx integration summary.

**Initial Volume (mL):** Taken from the study folder.

**Dilution Factor:** Taken from the study folder.

**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-6.0

Adoption Date: 07/22/99

Revision Date: NR

Author: Lisa Clemen, Robert Wynne

Approved By:

7/1 Bm 7/22/99  
Laboratory Manager Date

Kritu Ha 7/14/99  
Group Leader Date

Lisa A Clemen 07/14/99  
Technical Reviewer Date

#### 1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from liver.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

1.3 Matrices: Rabbit, rat, bovine, and monkey livers or other tissues as designated in the validation report.

Exact Copy of Original  
Initial SD  
Date 6/5/01

## **2.0 SUMMARY OF METHOD**

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- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver, or other tissues, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals can be extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2  $\mu$ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

## **3.0 DEFINITIONS**

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- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt)  $C_8F_{17}SO_3$
- 3.2 PFOSA: perfluorooctane sulfonylamide  $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol  
 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide  $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556:  $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

## **4.0 WARNINGS AND CAUTIONS**

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### **4.1 Health and Safety Warnings:**

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

## **5.0 INTERFERENCES**

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- 5.1 There are no interferences known at this time.

## **6.0 EQUIPMENT**

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- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples
- 6.1.2 Vortex mixer, VWR, Vortex Genie 2
- 6.1.3 Centrifuge, Mistral 1000 or IEC
- 6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

## 7.0 SUPPLIES AND MATERIALS

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- 7.1 Gloves
- 7.2 Dissecting scalpels
- 7.3 Eppendorf or disposable pipettes
- 7.4 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.5 Volumetric flasks, glass, type A
- 7.6 I-CHEM vials, 40 mL glass
- 7.7 Plastic sample vials, Wheaton, 6 mL (or appropriate size)
- 7.8 Centrifuge tubes, polypropylene, 15 mL
- 7.9 Labels
- 7.10 Oxford Dispensor – 3.0 to 10.0 ml
- 7.11 Syringes, capable of measuring 5 µL to 50 µL
- 7.12 Graduated pipettes
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2 µm, 25 mm
- 7.15 Timer
- 7.16 Crimp cap autovials and caps
- 7.17 Crimpers

Note: Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

## 8.0 REAGENTS AND STANDARDS

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- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T. Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO<sub>3</sub>), J.T. Baker or equivalent
- 8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Liver, frozen from supplier
- 8.9 Dry ice from supplier
- 8.10 Fluorochemical standards
  - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

- 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
- 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.10.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.10.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H  $C_8F_{13}SO_3H$ ) molecular weight = 428
- 8.10.8 Other fluorochemicals, as appropriate

### 8.11 Reagent preparation

**NOTE:** When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.
  - 8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ( $Na_2CO_3/NaHCO_3$ ): Weigh approximately 26.5 g of sodium carbonate ( $Na_2CO_3$ ) and 21.0 g of sodium bicarbonate ( $NaHCO_3$ ) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

### 8.12 Standards preparation

- 8.12.1 Prepare PFOS standards for the standard curve.
- 8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.12.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ( $\mu g/mL$ ).
- 8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

### **8.13 Surrogate stock standard preparation**

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H,  $C_8F_{13}SO_3H$  into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 1.0 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

## **9.0 SAMPLE HANDLING**

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9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

## **10.0 QUALITY CONTROL**

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### **10.1 Matrix blanks and method blanks**

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of liver homogenate following this procedure and use as matrix blanks. Refer to 11.1.6.

### **10.2 Matrix spikes**

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually a control liver received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

### **10.3 Continuing calibration verifications**

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification sample per group of 10 samples. For example, if a sample set = 34, four verifications are prepared and extracted.

- 10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.
- 10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

## **11.0 CALIBRATION AND STANDARDIZATION**

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### **11.1 Prepare matrix calibration standards**

- 11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.
  - 11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.
  - 11.1.3 Refer to 13.0 to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.
  - 11.1.5 Add 1 mL of homogenate to a 15 mL centrifuge tube. Re-suspend solution by shaking between aliquots while preparing a total of eighteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.
  - 11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.
  - 11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.
  - 11.1.8 Refer to validation reports ETS-8-6.0 and ETS-8-7.0-V-1 or Attachment B, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
  - 11.1.9 Use Attachment C as an aid in calculating the concentrations of the working standards. Refer to 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2 To each working standard, blank, or continuing verification, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb – 1000ppb.

- 11.3 Extract spiked liver homogenates following 12.14-12.25 of this method. Use these standards to establish each initial curve on the mass spectrometer.

| Table 1<br>Approximate Spiking Amounts for Calibration Standards |    |                                         |
|------------------------------------------------------------------|----|-----------------------------------------|
| Working Standard<br>(Approx. Conc.)                              | μl | Approx. final conc. of<br>PFOS in liver |
| -                                                                | -  | Blank                                   |
| 0.50 ppm                                                         | 2  | 0.005 ppm                               |
| 0.50 ppm                                                         | 4  | 0.010 ppm                               |
| 0.50 ppm                                                         | 10 | 0.025 ppm                               |
| 0.50 ppm                                                         | 20 | 0.050 ppm                               |
| 0.50 ppm                                                         | 40 | 0.100 ppm                               |
| 5.0 ppm                                                          | 10 | 0.250 ppm                               |
| 5.0 ppm                                                          | 20 | 0.500 ppm                               |
| 5.0 ppm                                                          | 30 | 0.750 ppm                               |
| 50 ppm                                                           | 4  | 1.00 ppm                                |

## 12.0 PROCEDURE

- 12.1 Obtain frozen liver samples.
- 12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.
- 12.3 Weigh the sample directly into a tared plastic sample vial.
- 12.4 Record the liver weight in the study notebook.
- 12.5 Return unused liver portions to freezer.
- 12.6 Add 2.5 mLs of water to sample vial.
- 12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.
- 12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.
- 12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.
- 12.10 Cap the sample and vortex for 15 seconds. Label the sample vial with the study number, weight, liver ID, date and analyst initials.

- 12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sample vial. Refer to attached worksheet for documenting the remaining steps.
- 12.12 Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as method blanks.
- 12.13 Spike all samples, including blanks and standards ready for extraction with surrogate standard as described in section 11.2.
- 12.14 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 of that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.15 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.16 Check to ensure 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.17 To each sample, add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.18 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.19 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.20 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.
- 12.21 Label a fresh 15 mL centrifuge tube with the same information as in 12.10.
- 12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.
- 12.23 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.
- 12.25 Vortex mix for 30 seconds.
- 12.26 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.27 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.28 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.29 Complete the extraction worksheet, attached to this document, and tape in study notebook or include in study binder, as appropriate.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

---

#### **13.1 Calculations:**

- 13.1.1 Calculate the average density of the liver homogenate by recording each mass of ten separate 1.0 mL aliquots of homogenate.

$$\text{Average density (mg/mL)} = \frac{\text{Average mass (mg) of the aliquots}}{1.0 \text{ mL aliquot}}$$

- 13.1.2 Calculate the amount of liver (mg) per 1.0 mL homogenate (or concentration of dispersed solid tissue per mL of homogenate suspension) using the following equation:

$$\frac{\text{g of Liver} \times \text{Average density* of homogenate (mg/mL)}}{(\text{g of Liver} + \text{g of Water})}$$

\* refer to 13.1.1 for details.

- 13.1.3 Calculate actual concentrations of PFOS and other fluorochemicals in calibration standards using the following equation:

$$\frac{\mu\text{L of Standard} \times \text{Concentration} (\mu\text{g / mL})}{\text{mg Liver / 1 mL homogenate*}} = \text{Final Concentration} (\mu\text{g/g or mg/kg}) \text{ of PFOS in Liver}$$

\*refer to 13.1.2 for details.

### **14.0 METHOD PERFORMANCE**

---

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (refer to **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
- 14.2.1 Method blanks and matrix blanks.
  - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
  - 14.2.3 Continuing calibration verification samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-7.0 for method performance criteria.

### **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

---

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

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- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

## **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

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- 17.1 Attachment A, Extraction worksheet
- 17.2 Attachment B, MDL/LOQ values and summary
- 17.3 Attachment C, Calibration standard calculation and concentration worksheet

## **18.0 REFERENCES**

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- 18.1 The validation report associated with this method is ETS-8-6.0 & 7.0-V-1.
- 18.2 AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"
- 18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

## **19.0 AFFECTED DOCUMENTS**

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- 19.1 ETS-8-7.0, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## **20.0 REVISIONS**

---

Revision  
Number.

Reason For Revision

Revision  
Date

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**MDL/LOQ values for rabbit liver**

| Compound  | MDL (ppb) | LOQ (ppb) | Linear Calibration Range (LCR)<br>Approximate concentrations to be used for preparing the Standard Calibration Curve |
|-----------|-----------|-----------|----------------------------------------------------------------------------------------------------------------------|
| PFOS      | 8.45      | 26.9      | 30 ppb – 1200 ppb                                                                                                    |
| PFOSA     | 3.50      | 11.1      | 12 ppb – 1200 ppb                                                                                                    |
| PFOSAA    | 24.6      | 78.3      | 30 ppb – 1200 ppb                                                                                                    |
| EtFOSE-OH | 108       | 345       | 60 ppb – 900 ppb*                                                                                                    |
| M556      | 82.3      | 262       | 60 ppb – 1200 ppb                                                                                                    |
| PFOSEA    | 33.9      | 108       | 30 ppb- 1200 ppb                                                                                                     |

MDL/LOQ values in rat, bovine, and monkey liver were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit liver curves to determine equivalence. Responses in the rat, bovine, and monkey liver curves were equivalent to the rabbit responses, therefore, their MDL and LOQ will be assumed to be equivalent to those values as determined for the rabbit liver.

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information

\* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

**Compound: PFOS**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.19 - 1237                               | 12 - 1200                            | 12 - 1200                        | 6 - 300                              | 12 - 300                             | 60 - 1200                             | 60 - 1200                             |

**Compound: PFOSA**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.19 - 1237                               | 12 - 1200                            | 12 - 1200                        | 12 - 300                             | 12 - 300                             | 60 - 1200                             | 60 - 1200                             |

**Compound: PFOSAA**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.16 - 1232                               | 12 - 1200                            | 30 - 1200                        | 30 - 900                             | 60 - 900                             | N/A                                   | N/A                                   |

**Compound: EtFOSE-OH**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.17 - 1235                               | 31 - 900                             | 31 - 900                         | N/A                                  | N/A                                  | N/A                                   | N/A                                   |

**Compound: PFOSEA**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.17 - 1235                               | 31 - 1200                            | 31 - 1200                        | N/A                                  | N/A                                  | N/A                                   | N/A                                   |

**Compound: M556**

| Liver matrix | Prepared range of standards (ppb) (ng/mL) | Range of average curve (ppb) (ng/mL) | LCR from ave curve (ppb) (ng/mL) | Range of low std curve (ppb) (ng/mL) | LCR from low std curve (ppb) (ng/mL) | Range of high std curve (ppb) (ng/mL) | LCR from high std curve (ppb) (ng/mL) |
|--------------|-------------------------------------------|--------------------------------------|----------------------------------|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------------|
| Rabbit       | 6.17 - 1235                               | 31 - 1200                            | 60 - 1200                        | N/A                                  | N/A                                  | N/A                                   | N/A                                   |

## Ion Pair Standard Curves – Tissue

Prep date(s):  
Analyte(s):  
Sample matrix:

Standard number:  
Equipment number:  
Final solvent and TN:  
Blank liver/identifier:

Method/revision:  
Target analyte(s):  
FC mix std approx. 0.500 ppm:  
FC mix std approx. 5.00 ppm:  
FC mix std approx. 50.0 ppm:  
Surrogate std approx. 100 ppm:

### Actual concentrations of standards in the FC mix

| PFOS<br>Std conc<br>ug/mL | PFOSA<br>Std conc<br>ug/mL | PFOSAA<br>Std conc<br>ug/mL | EtFOSE<br>Std conc<br>ug/mL | PFOSEA<br>Std conc<br>ug/mL | M556<br>Std conc<br>ug/mL | Std conc<br>ug/mL | All<br>Am't spiked<br>mL | All<br>Density<br>g |
|---------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------|-------------------|--------------------------|---------------------|
| 0.500                     | 0.500                      | 0.500                       | 0.500                       | 0.500                       | 0.500                     |                   | 0.002                    | 0.167               |
| 0.500                     | 0.500                      | 0.500                       | 0.500                       | 0.500                       | 0.500                     |                   | 0.004                    | 0.167               |
| 0.500                     | 0.500                      | 0.500                       | 0.500                       | 0.500                       | 0.500                     |                   | 0.010                    | 0.167               |
| 0.500                     | 0.500                      | 0.500                       | 0.500                       | 0.500                       | 0.500                     |                   | 0.020                    | 0.167               |
| 0.500                     | 0.500                      | 0.500                       | 0.500                       | 0.500                       | 0.500                     |                   | 0.040                    | 0.167               |
| 5.00                      | 5.00                       | 5.00                        | 5.00                        | 5.00                        | 5.00                      |                   | 0.010                    | 0.167               |
| 5.00                      | 5.00                       | 5.00                        | 5.00                        | 5.00                        | 5.00                      |                   | 0.020                    | 0.167               |
| 5.00                      | 5.00                       | 5.00                        | 5.00                        | 5.00                        | 5.00                      |                   | 0.030                    | 0.167               |
| 50.0                      | 50.0                       | 50.0                        | 50.0                        | 50.0                        | 50.0                      |                   | 0.004                    | 0.167               |

### Calculated concentrations of standards in the sample matrix

| PFOS<br>Final<br>conc<br>ng/g | PFOSA<br>Final<br>conc<br>ng/g | PFOSAA<br>Final conc<br>ng/g | EtFOSE<br>Final<br>conc<br>ng/g | PFOSEA<br>Final<br>conc<br>ng/g | M556<br>Final<br>conc<br>ng/g | Std conc<br>ng/g | Surrogate<br>Std conc<br>ng/mL   | All<br>Am't<br>spiked<br>mL |
|-------------------------------|--------------------------------|------------------------------|---------------------------------|---------------------------------|-------------------------------|------------------|----------------------------------|-----------------------------|
| 5.99                          | 5.99                           | 5.99                         | 5.99                            | 5.99                            | 5.99                          |                  | 100                              | 0.005                       |
| 12.0                          | 12.0                           | 12.0                         | 12.0                            | 12.0                            | 12.0                          |                  | Surrogate<br>Final conc<br>ng/mL |                             |
| 29.9                          | 29.9                           | 29.9                         | 29.9                            | 29.9                            | 29.9                          |                  |                                  |                             |
| 59.9                          | 59.9                           | 59.9                         | 59.9                            | 59.9                            | 59.9                          |                  |                                  |                             |
| 120                           | 120                            | 120                          | 120                             | 120                             | 120                           |                  |                                  |                             |
| 299                           | 299                            | 299                          | 299                             | 299                             | 299                           |                  | 0.500                            |                             |
| 599                           | 599                            | 599                          | 599                             | 599                             | 599                           |                  |                                  |                             |
| 898                           | 898                            | 898                          | 898                             | 898                             | 898                           |                  |                                  |                             |
| 1198                          | 1198                           | 1198                         | 1198                            | 1198                            | 1198                          |                  |                                  |                             |

### Validated ranges – approximate concentrations

| Liver  | PFOS                               | PFOSA      | PFOSAA     | EtFOSE-OH  | POAA       | PFOSEA     |
|--------|------------------------------------|------------|------------|------------|------------|------------|
| Rabbit | 5-1000 ppb                         | 5-1000 ppb | 5-1000 ppb | 5-1000 ppb | 5-1000 ppb | 5-1000 ppb |
| Bovine | Estimates only, use rabbit values. |            |            |            |            |            |
| Rat    | Estimates only, use rabbit values. |            |            |            |            |            |
| Monkey | Estimates only, use rabbit values. |            |            |            |            |            |

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN LIVER EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

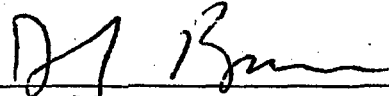
Method Number: ETS-8-7.0

Adoption Date: 07/22/99

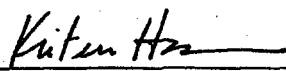
Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

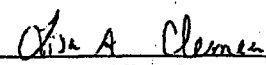
Approved By:

  
Laboratory Manager

7/22/99  
Date

  
Group Leader

7/14/99  
Date

  
Technical Reviewer

07/14/99  
Date

Exact Copy of Original  
RSD  
Initial Date 6/5/01

#### 1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of liver extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 Matrices: Rabbit, rat, bovine, monkey liver, or other tissues as designated in the validation report.

## **2.0 SUMMARY OF METHOD**

---

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from liver using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion,  $m/z = 499$ . Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the selected parent ion.

## **3.0 DEFINITIONS**

---

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio ( $m/z$ ) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

## **4.0 WARNINGS AND CAUTIONS**

---

### **4.1 Health and Safety Warnings:**

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

- 4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

**4.2 Cautions:**

- 4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

**5.0 INTERFERENCES**

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- 5.1 To minimize interferences when analyzing samples, Teflon shall not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

**6.0 EQUIPMENT**

---

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
- 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.
- 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

**7.0 SUPPLIES AND MATERIALS**

---

**7.1 Supplies**

- 7.1.1 High purity grade air regulated to approximately 100 psi (house air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data
- 7.1.3 Capped autovials or capped 15 ml centrifuge tubes

**8.0 REAGENTS AND STANDARDS**

---

**8.1 Reagents**

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent
- 8.1.3.1 When preparing different amounts than those listed, adjust accordingly.
- 8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

## **8.2 Standards**

- 8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. Refer to ETS-8-6.0.

## **9.0 SAMPLE HANDLING**

---

- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.
- 9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature, or refrigerated at approximately 4° C, until analysis can be performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Method Blanks and Matrix Blanks**

- 10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.
- 10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

### **10.2 Matrix Spikes**

- 10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.
- 10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.
- 10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.
- 10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

### **10.3 Continuing Calibration Checks**

- 10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.
- 10.3.2 Analyze a mid-range calibration standard every tenth sample, with a minimum of one per batch.

## **11.0 CALIBRATION AND STANDARDIZATION**

---

- 11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ( $y = mx + b$ ), weighted  $1/x$ , not forced through the origin, using MassLynx or other suitable software.
- 11.2 If the curve does not meet requirements perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

- 11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

## **12.0 PROCEDURES**

---

### **12.1 Acquisition Set up**

#### **12.1.1 Set up the sample list.**

12.1.1.1 Assign a sample list filename using MO-DAY-last digit of year-increasing letter of the alphabet starting with a

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

12.1.2 To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

12.1.3 Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

12.1.4 Samples are analyzed with a continuing calibration verification injected standard after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

### **12.2 Using the Autosampler**

12.2.1 Set up sample tray according to the sample list prepared in Section 12.1.1.

12.2.2 Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

12.2.2.1 Sample size = 10  $\mu$ L injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

#### 12.2.2.4 Solvent ramp conditions

| Time      | MeOH | 2.0 mM<br>Ammonium acetate |
|-----------|------|----------------------------|
| 0.00 min. | 40%  | 60%                        |
| 1.0 min.  | 40%  | 60%                        |
| 4.5 min.  | 95%  | 5%                         |
| 6.5 min.  | 95%  | 5%                         |
| 7.0 min.  | 40%  | 60%                        |
| 9.0 mi.   | 40%  | 60%                        |

#### 12.2.2.5 Press the "Start" button.

### 12.3 Instrument Set-up

12.3.1 Refer to ETS-9-24.0, "Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source," for more details.

12.3.2 Check the solvent level in reservoirs and refill if necessary.

12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

12.3.4 Turn on the nitrogen.

12.3.5 Open the tune page. Clicks on operate to initiate source block and desolvation heaters.

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500  $\mu$ L/min or as appropriate

12.3.6.3 Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

12.3.7 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

12.3.7.3 HPLC constant flow mode flow rate 10 – 500  $\mu$ L/min

12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

#### **13.1 Calculations:**

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6 Calculate actual concentrations in matrix (µg/g):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\frac{(\text{Initial Weight of Liver (g)}}{\text{Final Volume (mL)}}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

### **14.0 METHOD PERFORMANCE**

- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Refer to ETS-8-6.0, Attachment B for a listing of current validated MDL and LOQ values.

#### **14.2 Solvent Blanks, Method Blanks and Matrix Blanks**

- 14.2.1 Solvent blanks, method blanks, and matrix blanks must be below the lowest standard in the calibration curve.

#### **14.3 Calibration Curves**

- 14.3.1 The  $r^2$  value for the calibration must be 0.980 or better.

#### **14.4 Matrix Spikes**

- 14.4.1 Matrix spike percent recoveries must be within  $\pm 30\%$  of the spiked concentration.

#### **14.5 Continuing Calibration Verification**

- 14.5.1 Continuing calibration verification percent recoveries must be within  $\pm 30\%$  of the spiked concentration.

- 14.6 If criteria listed in the method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.

- 14.7 If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

#### **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

- 15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

#### **16.0 RECORDS**

- 16.1 Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2 Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3 Plot the calibration curve by linear regression, weighted 1/x, then print these graphs and store in the study folder.
- 16.4 Print data integration summary, integration method, and chromatograms from MassLynx and store in the study folder.
- 16.5 Summarize data using suitable software (Excel 5.0+) and store in the study folder, refer to **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

#### **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

- 17.1 Attachment A: ETS-8-7.0 Data summary spreadsheet

#### **18.0 REFERENCES**

- 18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**

#### **19.0 AFFECTED DOCUMENTS**

- 19.1 ETS-8-6.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

## 20.0 REVISIONS

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Revision  
Number

Reason For Revision

Revision  
Date

## Laboratory Study #

Study:  
 Test Material:  
 Matrix/Final Solvent:  
 Method/Revision:  
 Analytical Equipment System Number:  
 Instrument Software/Version:  
 Filename:  
 R-Squared Value:  
 Slope:  
 Y Intercept:  
 Date of Extraction/Analyst:  
 Date of Analysis/Analyst:

| Group<br>Dose | Sample# | Concentration<br>ng/g | Initial Wt.<br>g | Dilution<br>Factor | Final Conc.<br>ug/g |
|---------------|---------|-----------------------|------------------|--------------------|---------------------|
|               |         |                       |                  |                    |                     |
|               |         |                       |                  |                    |                     |
|               |         |                       |                  |                    |                     |
|               |         |                       |                  |                    |                     |
|               |         |                       |                  |                    |                     |

**Slope:** Taken from linear regression equation.

**Group/Dose:** Taken from the study folder.

**Sample#:** Taken from the study folder.

**Concentration (ng/g):** Taken from the MassLynx integration summary.

**Initial Wt. (g):** Taken from the study folder.

**Dilution Factor:** Taken from the study folder.

**Final Conc. (ug/g):** Calculated by dividing the initial volume from the concentration

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS FROM SERUM FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY

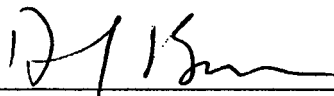
Method Number: ETS-8-4.1

Adoption Date: 03/01/99

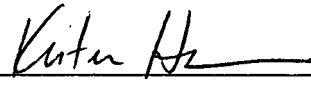
Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

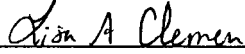
Approved By:

  
\_\_\_\_\_  
Laboratory Manager

4/27/99  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Group Leader

4/26/99  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Technical Reviewer

04/26/99  
\_\_\_\_\_  
Date

Initial  
RSD  
Exact Copy of Original  
Date  
6/5/01

#### 0 SCOPE AND APPLICATION

1 **Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum.

2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.

3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum or other fluids as designated in the validation report.

## 2.0 SUMMARY OF METHOD

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- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from serum, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, PFOSEA, M556, and surrogate standard (see 3.0 **Definitions**). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2  $\mu$ m nylon filter into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

## 3.0 DEFINITIONS

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- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt)  $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide  $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 PFOSEA: perfluorooctane sulfonyl ethylamide  $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.6 M556:  $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.7 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

## 4.0 WARNINGS AND CAUTIONS

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### 4.1 Health and safety warnings

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

## 5.0 INTERFERENCES

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- 5.1 There are no interferences known at this time.

## 6.0 EQUIPMENT

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- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Vortex mixer, VWR, Vortex Genie 2
- 6.1.2 Centrifuge, Mistral 1000 or IEC
- 6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance ( $\pm 0.100$  g)

## **7.0 SUPPLIES AND MATERIALS**

---

- 7.1 Gloves
- 7.2 Eppendorf or disposable pipettes
- 7.3 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.4 Volumetric flasks, glass, type A
- 7.5 I-CHEM vials, glass, 40 mL glass
- 7.6 Centrifuge tubes, polypropylene, 15 mL
- 7.7 Labels
- 7.8 Oxford Dispenser – 3.0 to 10.0 mL
- 7.9 Syringes, capable of measuring 5  $\mu$ L to 50  $\mu$ L
- 7.10 Graduated pipettes
- 7.11 Syringes, disposable plastic, 3 cc
- 7.12 Syringe filters, nylon, 0.2  $\mu$ m, 25 mm
- 7.13 Timer
- 7.14 Crimp cap autovials and caps
- 7.15 Crimpers

**Note:** Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

## **8.0 REAGENTS AND STANDARDS**

---

- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T. Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
- 8.4 Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate ( $\text{NaHCO}_3$ ), J.T. Baker or equivalent
- 8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Serum or blood, frozen from supplier
- 8.9 **Fluorochemical standards**
  - 8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
  - 8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

- 8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570
- 8.9.5 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557
- 8.9.7 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H  $C_8F_{13}SO_3H$ ) molecular weight = 428
- 8.9.8 Other fluorochemicals, as appropriate

## 8.10 Reagent preparation

**NOTE:** When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

- 8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.
  - 8.10.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer ( $Na_2CO_3/NaHCO_3$ ): Weigh approximately 26.5 g of sodium carbonate ( $Na_2CO_3$ ) and 21.0 g of sodium bicarbonate ( $NaHCO_3$ ) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

## 8.11 Standards preparation

- 8.11.1 Prepare PFOS standards for the standard curve.
- 8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.11.3 Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.
- 8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ( $\mu g/mL$ ).
- 8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.

#### **8.12 Surrogate stock standard preparation**

8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H,  $C_8F_{13}SO_3H$  into a 50 mL volumetric flask and record the actual weight.

8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.12.3 Prepare a surrogate working standard. Transfer approximately 1 mL of surrogate stock to a 10 mL volumetric flask and bring to volume with methanol for a working standard of 100 ppm. Record the actual volume transferred.

### **9.0 SAMPLE HANDLING**

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9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

9.2 Allow samples to thaw to room temperature prior to extraction.

### **10.0 QUALITY CONTROL**

---

#### **10.1 Solvent Blanks, Method blanks and matrix blanks**

10.1.1 An aliquot of 1.0 mL methanol is used as a solvent blank.

10.1.2 Extract two 1.0 mL aliquots of Milli-Q™ water following this procedure and use as method blanks.

10.1.3 Extract two 1.0 mL aliquots of the serum following this procedure and use as matrix blanks. See 11.1.4.

#### **10.2 Matrix spikes**

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

#### **10.3 Continuing calibration checks**

10.3.1 Prepare continuing calibration check samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing check per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration check from the same matrix used to prepare the initial curve.

- 10.3.4** The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

## **11.0 CALIBRATION AND STANDARDIZATION**

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### **11.1 Prepare matrix calibration standards**

- 11.1.1** Transfer 1 mL of serum to a 15 mL centrifuge tube.
- 11.1.2** If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.
- 11.1.3** While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.
- 11.1.4** Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.
- 11.1.5** Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1**, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.6** Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2** To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.
- 11.3** Extract spiked matrix standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

| <b>Table 1</b><br><b>Approximate spiking amounts for standards and spikes</b><br><b>Using 1.0 mL of matrix</b> |               |                                             |
|----------------------------------------------------------------------------------------------------------------|---------------|---------------------------------------------|
| Working standard<br>(approx. conc.)                                                                            | $\mu\text{L}$ | Approx. final conc. of<br>analyte in matrix |
| -                                                                                                              | -             | Blank                                       |
| 0.500 ppm                                                                                                      | 10            | 0.005 ppm                                   |
| 0.500 ppm                                                                                                      | 20            | 0.010 ppm                                   |
| 5.00 ppm                                                                                                       | 5             | 0.025 ppm                                   |
| 5.00 ppm                                                                                                       | 10            | 0.050 ppm                                   |
| 5.00 ppm                                                                                                       | 20            | 0.100 ppm                                   |
| 50.0 ppm                                                                                                       | 5             | 0.250 ppm                                   |
| 50.0 ppm                                                                                                       | 10            | 0.500 ppm                                   |
| 50.0 ppm                                                                                                       | 15            | 0.750 ppm                                   |
| 50.0 ppm                                                                                                       | 20            | 1.00 ppm                                    |

## 12.0 PROCEDURE

---

- 12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.
- 12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.
- 12.3 Return unused samples to freezer after extraction amounts have been removed.
- 12.4 Record the initial volume on the extraction worksheet.
- 12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in 11.2.
- 12.7 Spike each matrix with the appropriate amount of standard as described in 11.1, or Table 1 in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.
- 12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.
- 12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.
- 12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

- 12.14 Label a fresh 15 mL centrifuge tube with the same information as in 12.5.
- 12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.
- 12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.
- 12.17 Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.
- 12.18 Vortex mix for 30 seconds.
- 12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.
- 12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

---

#### **13.1 Calculations**

- 13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard} \times \text{concentration of standard } (\mu\text{g /mL})}{\text{mL of standard} + \text{mL of surrogate standard} + \text{initial matrix volume (mL)}} =$$

Final Concentration (µg/mL) of PFOS in matrix

### **14.0 METHOD PERFORMANCE**

---

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
  - 14.2.1 Method blanks and matrix blanks.
  - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
  - 14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

### **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

---

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

---

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

## **17.0 ATTACHMENTS**

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- 17.1 Attachment A, Extraction worksheet  
17.2 Attachment B, MDL/LOQ values and summary  
17.3 Attachment C, Calibration standard concentration worksheet

## **18.0 REFERENCES**

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- 18.1 The validation report associated with this method is ETS-8-4.0 & 5.0-V-1.  
18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## **19.0 AFFECTED DOCUMENTS**

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- 19.1 ETS-8-5.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## **20.0 REVISIONS**

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| <u>Revision<br/>Number</u> | <u>Reason For Revision</u>                                                                                                                                                                                | <u>Revision<br/>Date</u> |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 1                          | Section 12.21 Changed to include sample storage at room temperature.<br>Section 12.13 Added the shaker speed.<br>Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL. | 04/02/99                 |

## Extraction Worksheet ETS-8-4.1

[illegible]

**MDL/LOQ values for rabbit serum**

| <b>Compound</b> | <b>MDL<br/>(ppb)</b> | <b>LOQ<br/>(ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate concentrations to be used for preparing the<br/>Standard Calibration Curve</b> |
|-----------------|----------------------|----------------------|----------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 1.74                 | 5.55                 | 5 ppb - 1000 ppb                                                                                                                 |
| PFOSA           | 1.51                 | 4.79                 | 5 ppb - 1000 ppb                                                                                                                 |
| PFOSAA          | 3.46                 | 20.5                 | 5 ppb - 1000 ppb                                                                                                                 |
| EtFOSE-OH       | 11.4                 | 36.2                 | 5 ppb - 1000 ppb                                                                                                                 |
| M556            | 6.03                 | 19.2                 | 5 ppb - 1000 ppb                                                                                                                 |
| PFOSEA          | 5.71                 | 18.2                 | 5 ppb - 1000 ppb                                                                                                                 |

MDL/LOQ values in rat, bovine, monkey, and human serum, and monkey plasma were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the rabbit serum curves to determine equivalence. Responses in the rat, bovine, monkey, and human were equivalent to the rabbit responses, therefore, their MDL and LOQ will be the same values as determined in rabbit serum.

Please see LOQ Summary and MDL study in ETS-8-4.0 & 5.0-V-1 for further information.

**Compound: PFOS**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.995 - 978                                     | 24.8 - 978                            | 83-108              | 4.67-11.0    |
| Low Curve    | 4.94 - 248                                      | 4.94 - 248                            | 85-104              | 5.34-12.0    |
| High curve   | 97.8 - 978                                      | 97.8 - 978                            | 85-106              | 4.84-9.80    |
| 1/X          | 0.995 - 978                                     | 4.94 - 978                            | 94-111              | 4.60-10.5    |

**Compound: PFOSA**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 4.93 - 976                            | 88-103              | 5.10-14.7    |
| Low Curve    | 4.93 - 97.6                                     | 4.93 - 97.6                           | 87-105              | 9.85-14.7    |
| High curve   | 24.8 - 976                                      | 24.8 - 978                            | 93-102              | 5.08-13.9    |
| 1/X          | 0.993 - 976                                     | 4.93 - 976                            | 94-103              | 5.10-14.5    |

**Compound: PFOSAA**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.991 - 974                                     | 24.7 - 974                            | 81-111              | 4.18-10.6    |
| Low Curve    | 4.92 - 247                                      | 9.74 - 247                            | 97-107              | 6.38-21.8    |
| High curve   | 49.2 - 974                                      | 97.4 - 974                            | 85-108              | 4.33-12.5    |
| 1/X          | 0.991 - 974                                     | 9.74 - 974                            | 95-115              | 4.11-23.2    |

**Compound: EtFOSE-OH**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 49.3 - 976                            | 77-110              | 11.2-25.5    |
| Low Curve    | 4.93 - 97.6                                     | 9.76 - 97.6                           | 97-107              | 14.1-21.3    |
| High curve   | 49.3 - 976                                      | 97.6 - 976                            | 90-109              | 11.5-19.6    |
| 1/X          | 0.993 - 493                                     | 9.76 - 976                            | 86-111              | 11.1-21.2    |

**Compound: PFOSEA -**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 24.8 - 976                            | 96-106              | 10.1-16.2    |
| Low Curve    | 4.93 - 248                                      | 9.76 - 248                            | 91-110              | 11.8-19.5    |
| High curve   | 49.3 - 976                                      | 49.3 - 976                            | 86-106              | 10.2-18.2    |
| 1/X          | 0.993 - 976                                     | 9.76 - 976                            | 95-117              | 10.1-19.1    |

**Compound: M556**

| Rabbit Serum | Prepared range<br>of standards<br>(ppb) (ng/mL) | LCR from<br>curve<br>(ppb)<br>(ng/mL) | % Recovery<br>Range | RSD<br>Range |
|--------------|-------------------------------------------------|---------------------------------------|---------------------|--------------|
| Full Range   | 0.993 - 976                                     | 24.8 - 976                            | 88-106              | 4.82-17.9    |
| Low Curve    | 4.93 - 97.6                                     | 9.76 - 97.6                           | 100-105             | 5.95-18.2    |
| High curve   | 97.6 - 976                                      | 97.6 - 976                            | 81-111              | 5.11-9.74    |
| 1/X          | 0.993 - 976                                     | 9.76 - 976                            | 97-110              | 4.77-19.5    |

## Ion Pair Standard Curves – Fluids

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Blank fluid/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

### Actual concentrations of standards in the FC mix

| PFOS<br>Std conc<br>ug/mL | PFOSA<br>Std conc<br>ug/mL | PFOSAA<br>Std conc<br>ug/mL | EtFOSE<br>Std conc<br>ug/mL | PFOSEA<br>Std conc<br>ug/mL | M556<br>Std conc<br>ug/mL | All<br>Am't<br>spiked mL | All<br>Final vol<br>mL |
|---------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|---------------------------|--------------------------|------------------------|
| 0.500                     | 0.507                      | 0.532                       | 0.501                       | 0.521                       | 0.501                     | 0.010                    | 1.015                  |
| 0.500                     | 0.507                      | 0.532                       | 0.501                       | 0.521                       | 0.501                     | 0.020                    | 1.025                  |
| 5.00                      | 5.07                       | 5.32                        | 5.01                        | 5.21                        | 5.01                      | 0.005                    | 1.010                  |
| 5.00                      | 5.07                       | 5.32                        | 5.01                        | 5.21                        | 5.01                      | 0.010                    | 1.015                  |
| 5.00                      | 5.07                       | 5.32                        | 5.01                        | 5.21                        | 5.01                      | 0.020                    | 1.025                  |
| 50.0                      | 50.1                       | 53.2                        | 50.1                        | 52.1                        | 50.1                      | 0.005                    | 1.010                  |
| 50.0                      | 50.1                       | 53.2                        | 50.1                        | 52.1                        | 50.1                      | 0.010                    | 1.015                  |
| 50.0                      | 50.1                       | 53.2                        | 50.1                        | 52.1                        | 50.1                      | 0.015                    | 1.020                  |
| 50.0                      | 50.1                       | 53.2                        | 50.1                        | 52.1                        | 50.1                      | 0.020                    | 1.025                  |

### Calculated concentrations of standards in the sample matrix

| PFOS<br>Final conc<br>ng/mL | PFOSA<br>Final conc<br>ng/mL | PFOSAA<br>Final conc<br>ng/mL | EtFOSE<br>Final conc<br>ng/mL | PFOSEA<br>Final conc<br>ng/mL | M556<br>Final conc<br>ng/mL | Surrogate<br>Std conc<br>ng/mL          | All<br>Am't spiked<br>mL |
|-----------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------------|-----------------------------------------|--------------------------|
| 4.93                        | 5.00                         | 5.24                          | 4.94                          | 5.01                          | 5.13                        | 100                                     | 0.005                    |
| 9.76                        | 9.89                         | 10.4                          | 9.78                          | 9.93                          | 10.2                        | Surrogate<br>Final conc<br>ng/mL<br>500 |                          |
| 24.8                        | 25.1                         | 26.3                          | 24.8                          | 25.2                          | 25.8                        |                                         |                          |
| 49.3                        | 50.0                         | 52.4                          | 49.4                          | 50.1                          | 51.3                        |                                         |                          |
| 97.6                        | 98.9                         | 104                           | 97.8                          | 99.3                          | 102                         |                                         |                          |
| 248                         | 251                          | 263                           | 248                           | 252                           | 258                         |                                         |                          |
| 493                         | 500                          | 524                           | 494                           | 501                           | 513                         |                                         |                          |
| 735                         | 746                          | 782                           | 737                           | 749                           | 766                         |                                         |                          |
| 976                         | 989                          | 1038                          | 978                           | 993                           | 1017                        |                                         |                          |

### Validated ranges – approximate concentrations

| Serum           | PFOS                                   | PFOSA     | PFOSAA    | EtFOSE-OH | PFOSEA    | M556      |
|-----------------|----------------------------------------|-----------|-----------|-----------|-----------|-----------|
| Rabbit          | 5.00-1000                              | 5.00-1000 | 5.00-1000 | 5.00-1000 | 5.00-1000 | 5.00-1000 |
| Bovine          | Estimates only. Use values for rabbit. |           |           |           |           |           |
| Rat             | Estimates only. Use values for rabbit. |           |           |           |           |           |
| Monkey & Plasma | Estimates only. Use values for rabbit. |           |           |           |           |           |
| Human           | Estimates only. Use values for rabbit. |           |           |           |           |           |

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM SERUM OR OTHER FLUID FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

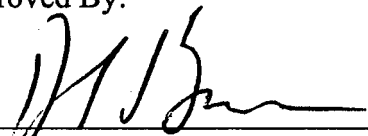
Method Number: FACT-M-3.1

Adoption Date: 04/22/98

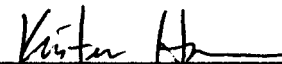
Revision Date: 10/01/98

Author: Lisa Clemen, Glenn Langenburg

Approved By:

  
Laboratory Manager

10/1/98  
Date

  
Group Leader

9/28/98  
Date

  
Technical Reviewer

9/28/98  
Date

Initial  
Date  
6/5/01  
Original

#### 1. SCOPE AND APPLICATION

- 1.1 **Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from serum or other fluid.
- 1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated compounds.
- 1.3 **Matrices:** Rabbit, rat, bovine, and monkey serum, rat whole blood, and rat milk curd.

## 2.0 SUMMARY OF METHOD

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- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemicals from serum, blood, or milk curd using an ion pairing reagent and 5.0 ml of ethyl acetate. In this method, seven fluorochemicals were extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, POAA, PFOSEA, and FC-807 monoester (see 3.0 *Definitions*). An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into ethyl acetate. Four ml of extract are removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 ml of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2  $\mu$ m nylon filter into glass autovials.

## 3.0 DEFINITIONS

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- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt)  $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide  $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonylamido)-ethyl alcohol  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 POAA: perfluorooctanoate (anion of ammonium salt)  $C_7F_{15}COO^-$
- 3.6 PFOSEA: perfluorooctane sulfonyl ethylamide  $C_8F_{17}SO_2N(CH_2CH_3)H$
- 3.7 FC-807 monoester  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O-PO_3H$
- 3.8 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

## 4.0 WARNINGS AND CAUTIONS

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### 4.1 Health and safety warnings

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

## 5.0 INTERFERENCES

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- 5.1 There are no known interferences at this time.

## 6.0 EQUIPMENT

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- 6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.
- 6.1.1 Vortex mixer, VWR, Vortex Genie 2
- 6.1.2 Centrifuge, Mistral 1000 or IEC
- 6.1.3 Shaker, Eberbach or VWR
- 6.1.4 Nitrogen evaporator, Organomation
- 6.1.5 Balance ( $\pm 0.100$  g)

## **7.0 SUPPLIES AND MATERIALS**

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- 7.1 Gloves
- 7.2 Eppendorf or disposable pipettes
- 7.3 Electronic pipettor, Eppendorf or equivalent
- 7.4 Graduated pipettes
- 7.5 Nalgene bottles, capable of holding 250 mL and 1 L
- 7.6 Volumetric flasks, glass, type A
- 7.7 Volumetric pipets, glass, type A
- 7.8 I-CHEM vials, glass, 40 mL glass
- 7.9 Crimp cap autovials
- 7.10 Centrifuge tubes, polypropylene, 15 mL
- 7.11 Labels
- 7.12 Syringes, capable of measuring 5 µL to 50 µL
- 7.13 Syringes, disposable plastic, 3 cc
- 7.14 Syringe filters, nylon, 0.2 µm, 25 mm
- 7.15 Timer

**Note:** Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

## **8.0 REAGENTS AND STANDARDS**

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- 8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system
- 8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent
- 8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
- 8.4 Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), J.T. Baker or equivalent
- 8.5 Sodium bicarbonate (NaHCO<sub>3</sub>), J.T. Baker or equivalent
- 8.6 Ethyl acetate, Omnisolv, glass distilled or HPLC grade
- 8.7 Methanol, Omnisolv, glass distilled or HPLC grade
- 8.8 Serum or blood, frozen from supplier
- 8.9 Control matrix or blank matrix for purpose of standards, QC checks, blanks, etc.
- 8.10 **Fluorochemical standards**
  - 8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
  - 8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

- 8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585
- 8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 571
- 8.10.5 POAA (3M Specialty Chemical Division), molecular weight = 431
- 8.10.6 PFOSEA (3M Specialty Chemical Division), molecular weight = 527
- 8.10.7 FC-807 monoester (3M Specialty Chemical Division). FC-807 is a mixture of triester, diester, and monoester fluorochemical components. The monoester molecular weight = 650
- 8.10.8 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H  $C_8F_{13}SO_3H$ ) molecular weight = 428
- 8.10.9 Other fluorochemicals, as appropriate

## 8.11 Reagent preparation

- 8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.
- 8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.
- 8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH and dilute to volume with Milli-Q™ water. While adding the last mL of NaOH, add slowly because the pH changes abruptly. Store in a 1 L Nalgene bottle.
  - 8.11.3.1 TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1 N NaOH solution.
- 8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer ( $Na_2CO_3/NaHCO_3$ ): Weigh approximately 26.5 g of sodium carbonate ( $Na_2CO_3$ ) and 21.0 g of sodium bicarbonate ( $NaHCO_3$ ) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

## 8.12 Standards preparation

- 8.12.1 Prepare PFOS standards for the standard curve.
- 8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)
- 8.12.3 Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight.
- 8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ( $\mu g/ml$ ).
- 8.12.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

8.12.6 Dilute the stock solution with methanol for a working standard 2 solution of approx. 5.0 ppm.

8.12.7 Dilute the stock solution with methanol for a working standard 3 solution of approx. 0.50 ppm.

### **8.13 Surrogate stock standard preparation**

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, C<sub>8</sub>F<sub>17</sub>SO<sub>3</sub>H into a 50 ml volumetric flask and record the actual weight.

8.13.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.

8.13.3 Prepare a surrogate working standard. Transfer approximately 0.5 ml of surrogate stock to a 50 ml volumetric flask and bring to volume with methanol for a working standard of 10-20 ppm. Record the actual volume transferred.

## **9.0 SAMPLE HANDLING**

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9.1 All samples are received frozen and must be kept frozen until the extraction is performed.

## **10.0 QUALITY CONTROL**

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### **10.1 Matrix blanks and method blanks**

10.1.1 Extract two 1.0 mL aliquots of the appropriate matrix (serum or blood, with blood samples diluted 1:1 with Milli-Q™ water) following this procedure and use as matrix blanks. See 11.1.4.

10.1.2 Extract two 1.0 ml aliquots of Milli-Q™ water following this procedure and use as method blanks.

### **10.2 Matrix spikes**

10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.

10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.

10.2.3 Expected concentrations will fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

### **10.3 Continuing calibration checks**

10.3.1 Prepare and analyze continuing calibration check samples to ensure the accuracy of the initial calibration curve. If the percent difference between the initial curve and the continuing check differ by >30%, re-analyze samples analyzed after the last acceptable check.

10.3.2 Prepare one check per group of ten samples. For example, if a sample set = 34, prepare and extract four checks.

- 10.3.3** Prepare each continuing calibration check from the same matrix used to prepare the initial curve.
- 10.3.4** The expected concentration will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

## **11.0 CALIBRATION AND STANDARDIZATION**

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### **11.1 Prepare matrix calibration standards**

**Note:** Blood coagulates in air; therefore, minimize air contact until dilution. At this point, add TBA and buffer to each centrifuge tube as in step 12.9, then add 1.0 mL of the diluted matrix sample to each tube.

- 11.1.1** Transfer 1 mL of serum or 1 mL of blood (blood is diluted 1:1 with Milli-Q™ water) to a 15 mL centrifuge tube. The blood is similar in composition to milk curd and can be used in place of milk curd for standard curves when extracting that matrix.
- 11.1.2** If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract below 0.50 mL of matrix. Record the sample volume on the extraction sheet.
- 11.1.3** While preparing a total of twenty aliquots in 15 mL centrifuge tubes, mix or shake between aliquots.
- 11.1.4** Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards and two matrix blanks.
- 11.1.5** Refer to validation reports **FACT-M-3.1-V-1** and **FACT-M-4.1-V-1**, which list the working ranges and the Linear Calibration Range (LCR) for calibration curves.
- 11.1.6** Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.
- 11.2** To each standard, blank, or QC check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb -1000 ppb.
- 11.3** Extract spiked matrix standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

| <b>Table 1</b><br><b>Approximate spiking amounts for standards and spikes</b><br><b>using 1.0 ml of matrix</b> |    |                                             |
|----------------------------------------------------------------------------------------------------------------|----|---------------------------------------------|
| Working standard<br>(approx. conc.)                                                                            | μL | Approx. final conc. of<br>analyte in matrix |
| -                                                                                                              | -  | Blank                                       |
| 0.500 ppm                                                                                                      | 10 | 0.005 ppm                                   |
| 0.500 ppm                                                                                                      | 20 | 0.010 ppm                                   |
| 5.00 ppm                                                                                                       | 5  | 0.025 ppm                                   |
| 5.00 ppm                                                                                                       | 10 | 0.050 ppm                                   |
| 5.00 ppm                                                                                                       | 20 | 0.100 ppm                                   |
| 50.0 ppm                                                                                                       | 5  | 0.250 ppm                                   |
| 50.0 ppm                                                                                                       | 10 | 0.500 ppm                                   |
| 50.0 ppm                                                                                                       | 15 | 0.750 ppm                                   |
| 50.0 ppm                                                                                                       | 20 | 1.00 ppm                                    |

| <b>Table 2</b><br><b>Approximate spiking amounts for standards and spikes</b><br><b>using 0.5 ml of matrix</b> |     |                                             |
|----------------------------------------------------------------------------------------------------------------|-----|---------------------------------------------|
| Working standard<br>(approx. conc.)                                                                            | μL  | Approx. final conc. of<br>analyte in matrix |
| -                                                                                                              | -   | Blank                                       |
| 0.500 ppm                                                                                                      | 5   | 0.005 ppm                                   |
| 0.500 ppm                                                                                                      | 10  | 0.010 ppm                                   |
| 5.00 ppm                                                                                                       | 2.5 | 0.025 ppm                                   |
| 5.00 ppm                                                                                                       | 5   | 0.050 ppm                                   |
| 5.00 ppm                                                                                                       | 10  | 0.100 ppm                                   |
| 50.0 ppm                                                                                                       | 2.5 | 0.250 ppm                                   |
| 50.0 ppm                                                                                                       | 5   | 0.500 ppm                                   |
| 50.0 ppm                                                                                                       | 7.5 | 0.750 ppm                                   |
| 50.0 ppm                                                                                                       | 10  | 1.00 ppm                                    |

## 12.0 PROCEDURE

- 12.1 Obtain frozen samples and allow to thaw.
- 12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube. For blood samples, remove 0.5 mL and dilute to 1.0 mL with Milli-Q™ water. As soon after diluting as possible, pipet diluted blood into TBA-buffer mixture shown in step 12.9 and mix well.
- 12.3 Return samples to freezer after extraction amount has been removed.

- 12.4 Record the volume on the extraction worksheet. The final methanol volume equals the volume transferred from the sample. For example, if 0.5 mL is removed for a blood sample, the final methanol volume will equal 0.5 mL.
- 12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike each matrix with the appropriate amount of standard as described in 11.1 or Table 1 or 2 in that section for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.7 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in 11.2.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer.
- 12.10 Using a volumetric pipette, add 5 mL ethyl acetate.
- 12.11 Cap each sample and put on the shaker for 20 minutes.
- 12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.
- 12.13 Transfer 4 mL of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in 12.5.
- 12.14 Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.
- 12.15 Add 1.0 mL or other appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume to add equals the initial volume of sample used for the extraction.
- 12.16 Vortex mix for 30 seconds.
- 12.17 Attach a 0.2  $\mu$ m nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.
- 12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.
- 12.19 Cap and store extracts at approximately 4 °C until analysis.
- 12.20 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

#### **13.1 Calculations**

- 13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{mL of standard} \times \text{concentration of standard } (\mu\text{g/mL})}{\text{mL of standard} + \text{mL of surrogate standard} + \text{initial matrix volume (mL)}} =$$

Final Concentration ( $\mu\text{g/mL}$ ) of PFOS in matrix

### **14.0 METHOD PERFORMANCE**

- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).
- 14.2 The following quality control samples are extracted with each batch of samples to ensure the quality of the extraction and analysis.
- 14.2.1 Method blanks and matrix blanks
  - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction
  - 14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve

### **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

- 15.1 Sample waste is disposed in biohazard containers, flammable solvent waste is disposed in high BTU containers, and used glass pipette waste is disposed in broken glass containers located in the laboratory.

### **16.0 RECORDS**

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in study 3-ring binder, as appropriate.

### **17.0 ATTACHMENTS**

- 17.1 Attachment A, Extraction worksheet
- 17.2 Attachment B, MDL/LOQ values
- 17.3 Attachment C, LOQ Summary
- 17.4 Attachment D, Calibration standard concentration worksheet

### **18.0 REFERENCES**

- 18.1 The validation reports associated with this method are **FACT-M-3.1 & 4.1-V-1**.

## **19.0 AFFECTED DOCUMENTS**

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**19.1** FACT-M-4.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## **20.0 REVISIONS**

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| <u>Revision<br/>Number</u> | <u>Reason For Revision</u>                                                                                                                                                                                                              | <u>Revision<br/>Date</u> |
|----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 1                          | Validation of method to include 7 fluorochemicals, an additional matrix, new API/MS(MS) systems, monkey serum cross validation, improvements to ion pairing extraction, MDL study, updates in record keeping and storing policies, etc. | 07/01/98                 |

|                                                                                       |                   |              |                 |
|---------------------------------------------------------------------------------------|-------------------|--------------|-----------------|
| Study number where the original worksheet is located.                                 |                   |              |                 |
| Blank                                                                                 | Std #             | amount =     | mL              |
| Serum Extraction Method                                                               |                   | :            | Date & Initials |
| Vortex 15 sec.                                                                        |                   |              |                 |
| Pipette Matrix                                                                        | Volume            | ml           |                 |
| Pipette 1 ml of 0.5 M TBA, pH 10.                                                     | Std. #            |              |                 |
| Pipette 2 ml of 0.25 Na <sub>2</sub> CO <sub>3</sub> /0.25M NaHCO <sub>3</sub> buffer | Std. #            |              |                 |
| Pipette 5 ml of ethyl acetate                                                         | TN-A-             |              |                 |
| Shake 20 min.                                                                         |                   |              |                 |
| Centrifuge 20-25 min.                                                                 | Centrifuge speed: |              |                 |
| Remove a 4 ml aliquot of organic layer                                                |                   |              |                 |
| Put on Nitrogen Evaporator to dryness                                                 | Evaporator #:     | Temperature: |                 |
| Add methanol                                                                          | Volume            | ml           | TN-A-           |
| Vortex 30 sec.                                                                        |                   |              |                 |
| Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 ml autosample vial  |                   |              |                 |

Attachment A: Extraction worksheet      FACT-M-3.1      Page 11 of 17  
Extraction of PFOS from Serum or Other Fluid

**MDL/LOQ values for Rabbit Serum:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate concentrations to be used for preparing the<br/>Standard Calibration Curve</b>                                                                     |
|-----------------|------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 1.38             | 4.39             | 5 ppb – 1000 ppb                                                                                                                                                                                     |
| PFOSA           | 2.23             | 7.09             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSAA          | 2.84             | 9.04             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| EtFOSE-OH       | 3.90             | 12.4             | 15 ppb – 1000 ppb                                                                                                                                                                                    |
| POAA            | 4.31             | 13.7             | 15 ppb – 750 ppb                                                                                                                                                                                     |
| PFOSEA          | 1.09             | 3.48             | 25 ppb – 1000 ppb                                                                                                                                                                                    |
| Monoester       | 149              | 248              | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |

**MDL/LOQ values for Rat Serum:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate concentrations to be used for preparing the<br/>Standard Calibration Curve</b>                                                                     |
|-----------------|------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 1.27             | 4.04             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSA           | 2.14             | 6.81             | 25 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSAA          | 2.32             | 7.38             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| EtFOSE-OH       | 3.25             | 10.3             | 50 ppb – 1000 ppb                                                                                                                                                                                    |
| POAA            | 1.20             | 3.81             | 5 ppb – 1000 ppb                                                                                                                                                                                     |
| PFOSEA          | 1.84             | 5.86             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| Monoester       | 149              | 248              | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |

**MDL/LOQ values for Bovine Serum:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate concentrations to be used for preparing the<br/>Standard Calibration Curve</b>                                                                     |
|-----------------|------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 2.11             | 6.70             | 25 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSA           | 5.04             | 16.0             | 25 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSAA          | 2.34             | 7.45             | 260 ppb – 1000 ppb                                                                                                                                                                                   |
| EtFOSE-OH       | 11.3             | 35.8             | 50 ppb – 1000 ppb                                                                                                                                                                                    |
| POAA            | 4.64             | 14.8             | 15 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSEA          | 3.71             | 11.8             | 15 ppb – 1000 ppb                                                                                                                                                                                    |
| Monoester       | 149              | 248              | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |

No data is available for MDL or LOQ in Monkey Serum. Use validated Linear Calibration Range instead.

Please see Attachment C (LOQ Summary) and MDL study in FACT-M-3.1 & 4.1-V-1 for specifics.

**MDL/LOQ values for Monkey Serum:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate concentrations to be used for preparing the<br/>Standard Calibration Curve</b>                                                                     |
|-----------------|------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 1.38             | 4.39             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOS will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.      |
| PFOSA           | 2.23             | 7.09             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.     |
| PFOSAA          | 2.84             | 9.04             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSAA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.    |
| EtFOSE-OH       | 3.90             | 12.4             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |
| POAA            | 4.31             | 13.7             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for POAA will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics.      |
| PFOSEA          | 1.09             | 3.48             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for PFOSEA-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |
| Monoester       | 149              | 248              | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |

**MDL/LOQ values for Rat Whole Blood:**

| <b>Compound</b> | <b>MDL (ppb)</b> | <b>LOQ (ppb)</b> | <b>Linear Calibration Range (LCR)<br/>Approximate Concentrations to be used for preparing the<br/>Standard Calibration Curve</b>                                                                     |
|-----------------|------------------|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| PFOS            | 1.25             | 3.96             | 5 ppb – 1000 ppb                                                                                                                                                                                     |
| PFOSA           | 1.77             | 5.65             | 10 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSAA          | 17.3             | 55.0             | 55 ppb – 1000 ppb                                                                                                                                                                                    |
| EtFOSE-OH       | 7.89             | 25.1             | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for EtFOSE-OH will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |
| POAA            | 4.73             | 15.1             | 15 ppb – 1000 ppb                                                                                                                                                                                    |
| PFOSEA          | 24.2             | 77.1             | 80 ppb – 1000 ppb                                                                                                                                                                                    |
| Monoester       | 58.0             | 185              | MDL and LOQ are estimates only. No valid MDL was determinable from MDL study. Any quantitation performed for monoester will be an estimate only. Please refer to FACT-M-3.1 & 4.1-V-1 for specifics. |

Please see Attachment C (LOQ Summary) and MDL study in FACT-M-3.1 & 4.1-V-1 for specifics.

Ion Pairing Extraction of Fluorochemicals from Serum and Analysis by API/MS(MS)  
Summary Table: Limits of Quantitation

| Compound               | Matrix | MDL      | LOQ       | Approximate linear range <sup>2</sup> |          |
|------------------------|--------|----------|-----------|---------------------------------------|----------|
|                        |        |          |           | Low std                               | High std |
| PFOS                   | Rabbit | 1.38 ppb | 4.39 ppb  | 5 ppb                                 | 1000 ppb |
|                        | Bovine | 2.11 ppb | 6.70 ppb  | 25 ppb                                | 1000 ppb |
|                        | Rat    | 1.27 ppb | 4.04 ppb  | 10 ppb                                | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 25 ppb                                | 1000 ppb |
| PFOSA                  | Rabbit | 2.23 ppb | 7.09 ppb  | 10 ppb                                | 1000 ppb |
|                        | Bovine | 5.04 ppb | 16.0 ppb  | 25 ppb                                | 1000 ppb |
|                        | Rat    | 2.14 ppb | 6.81 ppb  | 25 ppb                                | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 25 ppb                                | 1000 ppb |
| PFOSAA                 | Rabbit | 2.84 ppb | 9.04 ppb  | 10 ppb                                | 1000 ppb |
|                        | Bovine | 2.34 ppb | 7.45 ppb  | 263 ppb                               | 1000 ppb |
|                        | Rat    | 2.32 ppb | 7.38 ppb  | 10 ppb                                | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 25 ppb                                | 1000 ppb |
| EtFOSE-OH              | Rabbit | 3.90 ppb | 12.4 ppb  | 15 ppb                                | 1000 ppb |
|                        | Bovine | 11.3 ppb | 35.8 ppb  | 50 ppb                                | 1000 ppb |
|                        | Rat    | 3.25 ppb | 10.3 ppb  | 50 ppb                                | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 10 ppb                                | 1000 ppb |
| POAA                   | Rabbit | 4.31 ppb | 113.7 ppb | 15 ppb                                | 750 ppb  |
|                        | Bovine | 4.64 ppb | 14.8 ppb  | 5 ppb                                 | 1000 ppb |
|                        | Rat    | 1.20 ppb | 3.81 ppb  | 5 ppb                                 | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 5 ppb                                 | 1000 ppb |
| PFOSEA                 | Rabbit | 1.03 ppb | 3.48 ppb  | 25 ppb                                | 1000 ppb |
|                        | Bovine | 3.71 ppb | 11.8 ppb  | 5 ppb                                 | 1000 ppb |
|                        | Rat    | 1.84 ppb | 5.86 ppb  | 10 ppb                                | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 5 ppb                                 | 1000 ppb |
| Monoester <sup>1</sup> | Rabbit | 149 ppb  | 474.0 ppb | 250 ppb                               | 1000 ppb |
|                        | Bovine | 149 ppb  | 474.0 ppb | 250 ppb                               | 1000 ppb |
|                        | Rat    | 149 ppb  | 474.0 ppb | 250 ppb                               | 1000 ppb |
|                        | Monkey | n/d      | n/d       | 100 ppb                               | 1000 ppb |

1. Values for monoester are estimates only.

2. Highest standard (approx. 1500 ppb) was excluded from final LCR and upper LOQ values due to poor R & R values and excessive weighting of the calibration curve.

Compound: PFOS

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 4.93 - 1450                              | 4.93 - 1450                         | 49.3 - 1000                     | 49.3 - 97.6                         | 4.93 - 97.6                         | 97.6 - 1450                          | 97.6 - 1000                          |
| Bovine       | 4.93 - 1450                              | 4.93 - 1450                         | 97.6 - 1000                     | 4.93 - 248                          | 24.8 - 248                          | 97.6 - 1450                          | 97.6 - 1000                          |
| Rat          | 4.93 - 1450                              | 4.93 - 976                          | 24.8 - 976                      | 4.93 - 248                          | 9.76 - 248                          | 97.6 - 1450                          | 248 - 1000                           |
| Monkey       | 4.93 - 1450                              | 4.93 - 1450                         | 24.8 - 1000                     | 24.8 - 493                          | 24.8 - 493                          | 97.6 - 1450                          | 97.6 - 1000                          |

Compound: PFOSA

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 5.00 - 1470                              | 5.00 - 1470                         | 9.89 - 1000                     | 5.00 - 251                          | n/a                                 | 98.9 - 1470                          | 98.9 - 1000                          |
| Bovine       | 5.00 - 1470                              | 5.00 - 1470                         | 25.1 - 1000                     | 5.00 - 98.9                         | n/a                                 | 98.9 - 1470                          | 98.9 - 1000                          |
| Rat          | 5.00 - 1470                              | 5.00 - 1470                         | 50.0 - 1000                     | 9.89 - 500                          | 25.1 - 500                          | 98.9 - 1470                          | 98.9 - 1000                          |
| Monkey       | 5.00 - 1470                              | 5.00 - 1470                         | 98.9 - 1000                     | 25.1 - 500                          | 25.1 - 500                          | 98.9 - 1470                          | n/a                                  |

Compound: PFOSAA

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 5.20 - 1540                              | 5.20 - 1540                         | 104 - 1000                      | 5.20 - 263                          | 10.4 - 263                          | 104 - 1540                           | 263 - 1000                           |
| Bovine       | 5.20 - 1540                              | 5.20 - 1540                         | 263 - 1000                      | 10.4 - 521                          | n/a (1)                             | 104 - 1540                           | 263 - 1000                           |
| Rat          | 5.20 - 1540                              | 5.20 - 1540                         | 104 - 1000                      | 5.20 - 263                          | 10.4 - 263                          | 104 - 1540                           | 263 - 1000                           |
| Monkey       | 5.20 - 1540                              | 5.20 - 1540                         | 52.4 - 1000                     | 5.20 - 263                          | 26.3 - 263                          | 104 - 1540                           | 263 - 1000                           |

Compound: EtFOSE-OH

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 4.94 - 1450                              | 4.94 - 1450                         | 49.4 - 1000                     | 4.94 - 248                          | 9.78 - 248                          | 97.8 - 1450                          | n/a                                  |
| Bovine       | 4.94 - 1450                              | 4.94 - 1450                         | 97.8 - 1000                     | 4.94 - 248                          | 4.94 - 248                          | 97.8 - 1450                          | 248 - 1000                           |
| Rat          | 4.94 - 1450                              | 4.94 - 1450                         | 494 - 1000                      | 4.94 - 248                          | n/a                                 | 97.8 - 1450                          | 97.8 - 1000                          |
| Monkey       | 4.94 - 1450                              | 4.94 - 1450                         | 97.8 - 1000                     | 4.94 - 248                          | 9.78 - 248                          | 97.8 - 1450                          | n/a                                  |

Compound: POAA

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 5.01 – 1480                              | 5.13 – 1510                         | 25.8 – 1000                     | 5.13 – 258                          | n/a                                 | 102 – 1510                           | n/a                                  |
| Bovine       | 5.01 – 1480                              | 5.13 – 1510                         | 102 – 1000                      | 5.13 – 258                          | 5.13 – 258                          | 102 – 1510                           | 258 – 1000                           |
| Rat          | 5.01 – 1480                              | 5.13 – 1510                         | 51.3 – 1000                     | 5.13 – 102                          | 5.13 – 102                          | 102 – 1510                           | 102 – 1000                           |
| Monkey       | 5.01 – 1480                              | 5.13 – 1510                         | 102 - 1000                      | 5.13 – 102                          | 5.13 - 102                          | 102 – 1510                           | 258 – 1000                           |

Compound: PFOSEA

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) | Range of low std curve (ppb)(ng/mL) | LCR from low std curve (ppb)(ng/mL) | Range of high std curve (ppb)(ng/mL) | LCR from high std curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|--------------------------------------|--------------------------------------|
| Rabbit       | 5.13 - 1510                              | 5.13 - 1510                         | 25.8 - 1000                     | 5.13 – 258                          | n/a                                 | 102 - 1510                           | n/a                                  |
| Bovine       | 5.13 - 1510                              | 5.13 - 1510                         | 102 - 1000                      | 5.13 – 258                          | 5.13 – 258                          | 102 - 1510                           | 258 - 1000                           |
| Rat          | 5.13 - 1510                              | 5.13 - 1510                         | 51.3 – 1000                     | 5.13 – 102                          | 5.13 – 102                          | 102 - 1510                           | 102 - 1000                           |
| Monkey       | 5.13 - 1510                              | 5.13 - 1510                         | 102 - 1000                      | 5.13 – 102                          | 5.13 – 102                          | 102 - 1510                           | 258 - 1000                           |

Compound: Monoester

| Serum matrix | Prepared range of standards (ppb)(ng/mL) | Range of average curve (ppb)(ng/mL) | LCR from ave curve (ppb)(ng/mL) |
|--------------|------------------------------------------|-------------------------------------|---------------------------------|
| Rabbit       | 4.94 - 1450                              | 9.78 – 978                          | n/a                             |
| Bovine       | 4.94 - 1450                              | 97.8 – 1450                         | n/a                             |
| Rat          | 4.94 - 1450                              | 248 – 1450                          | 248 – 1000                      |
| Monkey       | 4.94 - 1450                              | 49.4 - 1450                         | 97.8 - 1000                     |

In general, the chromatography for the monoester was very poor (broad peaks, high baseline).

Curves for monoester in rabbit and bovine were unacceptable. Any quantitation performed with the monoester is only an estimate and should not be used for reliable, accurate data reporting.

## Ion Pair Standard Curves – Fluids

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Blank fluid/identifier:

Method/revision:

Target analyte(s):

FC mix std approx. 0.500 ppm: W398-641

FC mix std approx. 5.00 ppm: W398-640

FC mix std approx. 50.0 ppm: W398-639

Surrogate std approx. 17.71 ppm: W398-605

### Actual concentrations of standards in the FC mix

| PFOS           | PFOSA          | PFOSAA         | EtFOSE-OH      | POAA           | PFOSEA         | Monoester      | All            | All             |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
| Std conc ug/mL | Std conc ug/mL | Std conc ug/mL | Std conc ug/mL | Std conc ug/mL | Std conc ug/mL | Std conc ug/mL | Am't spiked mL | Final Volume mL |
| 0.500          | 0.507          | 0.532          | 0.501          | 0.509          | 0.521          | 0.501          | 0.010          | 1.015           |
| 0.500          | 0.507          | 0.532          | 0.501          | 0.509          | 0.521          | 0.501          | 0.020          | 1.025           |
| 5.00           | 5.07           | 5.32           | 5.01           | 5.09           | 5.21           | 5.01           | 0.005          | 1.010           |
| 5.00           | 5.07           | 5.32           | 5.01           | 5.09           | 5.21           | 5.01           | 0.010          | 1.015           |
| 5.00           | 5.07           | 5.32           | 5.01           | 5.09           | 5.21           | 5.01           | 0.020          | 1.025           |
| 50.0           | 50.1           | 53.2           | 50.1           | 50.9           | 52.1           | 50.1           | 0.005          | 1.010           |
| 50.0           | 50.1           | 53.2           | 50.1           | 50.9           | 52.1           | 50.1           | 0.010          | 1.015           |
| 50.0           | 50.1           | 53.2           | 50.1           | 50.9           | 52.1           | 50.1           | 0.015          | 1.020           |
| 50.0           | 50.1           | 53.2           | 50.1           | 50.9           | 52.1           | 50.1           | 0.020          | 1.025           |

### Calculated concentrations of standards in the sample matrix

| PFOS             | PFOSA            | PFOSAA           | EtFOSE           | POAA             | PFOSEA           | Monoester      | Surrogate                                | All              |
|------------------|------------------|------------------|------------------|------------------|------------------|----------------|------------------------------------------|------------------|
| Final conc ng/mL | Final conc ng/mL | Final conc ng/mL | Final conc ng/mL | Final conc ng/mL | Final conc ng/mL | Std conc ng/mL | Std conc ng/mL                           | Am't spiked (mL) |
| 4.93             | 5.00             | 5.24             | 4.94             | 5.01             | 5.13             | 4.94           | 2.64                                     | 0.005            |
| 9.76             | 9.89             | 10.4             | 9.78             | 9.93             | 10.2             | 9.78           | Surrogate<br>Final conc<br>ng/mL<br>81.0 |                  |
| 24.8             | 25.1             | 26.3             | 24.8             | 25.2             | 25.8             | 24.8           |                                          |                  |
| 49.3             | 50.0             | 52.4             | 49.4             | 50.1             | 51.3             | 49.4           |                                          |                  |
| 97.6             | 98.9             | 104              | 97.8             | 99.3             | 102              | 97.8           |                                          |                  |
| 248              | 251              | 263              | 248              | 252              | 258              | 248            |                                          |                  |
| 493              | 500              | 524              | 494              | 501              | 513              | 494            |                                          |                  |
| 735              | 746              | 782              | 737              | 749              | 766              | 737            |                                          |                  |
| 976              | 989              | 1038             | 978              | 993              | 1017             | 978            |                                          |                  |

### Validated ranges – approximate concentrations

| Sera   | PFOS            | PFOSA          | PFOSAA       | EtFOSE-OH   | POAA       | PFOSEA      |
|--------|-----------------|----------------|--------------|-------------|------------|-------------|
| Rabbit | 5-1000 ppb      | 10-1000 ppb    | 10-1000 ppb  | 10-1000 ppb | 10-750 ppb | 25-1000 ppb |
| Bovine | 25-1000 ppb     | 25-1000 ppb    | 263-1000 ppb | 5-1000 ppb  | 5-1000 ppb | 5-1000 ppb  |
| Rat    | 10-1000 ppb     | 25-1000 ppb    | 10-1000 ppb  | 50-500 ppb  | 5-1000 ppb | 5-1000 ppb  |
| Monkey | Estimates only. | Use values for | Rabbit       |             |            |             |

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

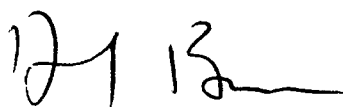
Method Number: ETS-8-5.1

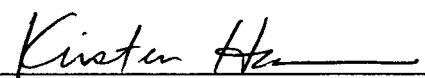
Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

  
\_\_\_\_\_  
Laboratory Manager Date 4/26/99

  
\_\_\_\_\_  
Group Leader Date 4/26/99

  
\_\_\_\_\_  
Technical Reviewer Date 04/26/99

Initial  
Date  
6/5/01  
Exact Copy of Original

#### SCOPE AND APPLICATION

1.1 **Scope:** This method describes the analysis of serum extracts for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 **Applicable Compounds:** Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 **Matrices:** Rabbit, rat, bovine, monkey, and human serum, or other fluids as designated in the validation report.

## 2.0 SUMMARY OF METHOD

---

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from serum or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion,  $m/z = 499$ . Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

## 3.0 DEFINITIONS

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- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole systems are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio ( $m/z$ ) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

## 4.0 WARNINGS AND CAUTIONS

---

### 4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.
- 4.1.2** When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

## **4.2 Cautions:**

- 4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

## **5.0 INTERFERENCES**

---

- 5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

## **6.0 EQUIPMENT**

---

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
  - 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source
  - 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

## **7.0 SUPPLIES AND MATERIALS**

---

### **7.1 Supplies**

- 7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)
- 7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.
- 7.1.3 Capped autovials or capped 15 mL centrifuge tubes

## **8.0 REAGENTS AND STANDARDS**

---

### **8.1 Reagents**

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent

### **8.2 Standards**

- 8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See ETS-8-4.1.

## **9.0 SAMPLE HANDLING**

---

- 9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

- 9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Solvent Blanks, Method Blanks and Matrix Blanks**

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

10.1.2 Analyze a method blank and a matrix blank prior to each calibration curve.

### **10.2 Matrix Spikes**

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

### **10.3 Continuing Calibration Verifications**

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

## **11.0 CALIBRATION AND STANDARDIZATION**

---

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ( $y = my + b$ ), weighted  $1/x$ , not forced through zero, using MassLynx or other suitable software.

11.2 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.3 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

## 12.0 PROCEDURES

---

### 12.1 Acquisition Set up

- 12.1.1** Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.
- 12.1.2** To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording) or MRM. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).
- 12.1.3** Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.
- 12.1.4** Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

### 12.2 Using the Autosampler

- 12.2.1** Set up sample tray according to the sample list prepared in Section 12.1.1.
- 12.2.2** Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:
- 12.2.2.1** Sample size = 10  $\mu$ L injection
- 12.2.2.2** Inject/sample = 1
- 12.2.2.3** Cycle time = 13.5 minutes
- 12.2.2.4** Solvent ramp =

| Time      | MeOH | 2.0 mM<br>Ammonium acetate |
|-----------|------|----------------------------|
| 0.00 min. | 40%  | 60%                        |
| 8.50 min. | 90%  | 10%                        |
| 11.0 min. | 90%  | 10%                        |
| 12.0 min. | 40%  | 60%                        |

- 12.2.2.5** Press the "Start" button.

### 12.3 Instrument Set-up

- 12.3.1** Refer to ETS-9-24.0 for more details.
- 12.3.2** Check the solvent level in reservoirs and refill if necessary.

- 12.3.3 Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.
- 12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.
- 12.3.6 The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
  - 12.3.6.1 Drying gas 250-400 liters/hour
  - 12.3.6.2 ESI nebulizing gas 10-15 liters/hour
  - 12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min
  - 12.3.6.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

## 13.0 DATA ANALYSIS AND CALCULATIONS

---

### 13.1 Calculations:

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{(\text{Initial Volume of matrix (mL)} + \text{mL of Surrogate Standard})} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \text{Final Volume (mL)}$$

## **14.0 METHOD PERFORMANCE**

---

- 14.1** Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-4.1, Attachment B, for a listing of current validated MDL and LOQ values.
- 14.2 Solvent Blanks, Method Blanks, and Matrix Blanks**
- 14.2.1** Solvent blanks, method blanks, and matrix blanks values are must be below the lowest standard in the calibration curve
- 14.3 Calibration Curves**
- 14.3.1** The  $r^2$  value for the calibration curve must be 0.980 or better.
- 14.4 Matrix Spikes**
- 14.4.1** Matrix spike percent recoveries are must be within  $\pm 30\%$  of the spiked concentration.
- 14.5 Continuing Calibration Verifications**
- 14.5.1** Continuing calibration verification percent recoveries must be  $\pm 30\%$  of the spiked concentration.
- 14.6** If criteria listed in this method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.
- 14.7** If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

## **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

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- 15.1** Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

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- 16.1** Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3** Plot the calibration curve by linear regression, weighted  $1/x$ , then print these graphs and store in the study folder.
- 16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.

- 16.5 Summarize data using suitable software (Excel 5.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.
- 16.6 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

#### **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

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- 17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

#### **18.0 REFERENCES**

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- 18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- 18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

#### **19.0 AFFECTED DOCUMENTS**

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- 19.1 ETS-8-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

#### **20.0 REVISIONS**

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| <u>Revision Number</u> | <u>Reason For Revision</u>                                                                                                                                                                                                                                                                                | <u>Revision Date</u> |
|------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| 1                      | Section 6.1.2 Clarification of HP1100 system components.<br>Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve.<br>Section 12.2.2.4 Clarification of solvent ramp.<br>Section 17.1 Changed from attachment B to A. | 04/02/99             |

## Laboratory Study #

Study:

Test Material:

Matrix/Final Solvent:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Filename:

R-Squared Value:

Slope:

Y Intercept:

Date of Extraction/Analyst:

Date of Analysis/Analyst:

| Group<br>Dose | Sample# | Concentration<br>ug/mL | Initial Vol.<br>mL | Dilution<br>Factor | Final Conc.<br>ug/mL |
|---------------|---------|------------------------|--------------------|--------------------|----------------------|
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |

**Slope:** Taken from linear regression equation.

**Group/Dose:** Taken from the study folder.

**Sample#:** Taken from the study folder.

**Concentration (ug/mL):** Taken from the MassLynx integration summary.

**Initial Volume (mL):** Taken from the study folder.

**Dilution Factor:** Taken from the study folder.

**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROchemicals IN SERUM OR OTHER FLUID EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: FACT-M-4.1

Adoption Date: 4/22/98

Revision Date: 10-1-98

Author: Lisa Clemen, Glenn Langenburg

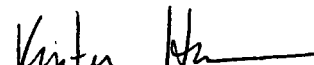
Approved By:



Laboratory Manager

10/1/98

Date



Group Leader

9/29/98

Date



Technical Reviewer

9/29/98

Date

Initial

ASD

Date

4/5/01

#### 1.0 SCOPE AND APPLICATION

1.1 **Scope:** This method is for the analysis of extracts from serum or blood for fluorochemical surfactants using HPLC-electrospray/mass spectrometry.

1.2 **Applicable Compounds:** Fluorochemical surfactants or other fluorinated compounds, or other ionizable compounds.

1.3 **Matrices:** Rabbit, rat, bovine, or monkey serum and rat whole blood or milk curd.

## 2.0 SUMMARY OF METHOD

---

- 2.1** This method describes the analysis of fluorochemical surfactants extracted from serum, whole blood, or milk curd using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion,  $M/Z = 499$ . Samples may also be analyzed using an API/MS/MS system to further verify compound identification.

## 3.0 DEFINITIONS

---

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass platform systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API platforms are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio ( $m/z$ ) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass platform systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these platform systems. Currently MassLynx has Windows 95 and WindowsNT 3.1 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Platform II or Quattro II MassLynx or MassLynx NT USER'S GUIDE).

## 4.0 WARNINGS AND CAUTIONS

---

### 4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.
- 4.1.2** When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

## **4.2 Cautions:**

- 4.2.1** Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2** Do not run solvent pumps to dryness.

## **5.0 INTERFERENCES**

---

- 5.1** To minimize interferences when analyzing samples for perfluorooctanoate(POAA), teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

## **6.0 EQUIPMENT**

---

- 6.1** Equipment listed below may be modified in order to optimize the system.
  - 6.1.1** Micromass Electrospray Mass Spectrometer
  - 6.1.2** HP1100 low pulse solvent pumping system and autosampler

## **7.0 SUPPLIES AND MATERIALS**

---

- 7.1** Supplies
  - 7.1.1** High purity grade nitrogen gas regulated to approximately 100 psi
  - 7.1.2** HPLC analytical column, specifics to be determined by the analyst
  - 7.1.3** Capped autovials or capped 15 ml centrifuge tubes

## **8.0 REAGENTS AND STANDARDS**

---

- 8.1** Reagents
  - 8.1.1** Methanol, HPLC grade or equivalent
  - 8.1.2** Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system
  - 8.1.3** Ammonium acetate, reagent grade or equivalent
- 8.2** Standards
  - 8.2.1** Typically one method blank, one matrix blank, and ten matrix standards are prepared during the extraction procedure. See **FACT-M-3.1**.

## **9.0 SAMPLE HANDLING**

---

- 9.1** Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.
- 9.2** If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C until analysis can be performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Method Blanks and Matrix Blanks**

10.1.1 Analyze a method blank and a matrix blank prior to each calibration curve.

### **10.2 Matrix Spikes**

10.2.1 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

10.2.2 Expected spike concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.2.3 See Section 13 to calculate percent recovery.

### **10.3 Continuing Calibration Checks**

10.3.1 Analyze a mid-range calibration standard after every tenth sample. If a significant change ( $\pm 30\%$ ) in peak area occurs, relative to the initial standard curve, stop the run. Only those samples analyzed before the last acceptable calibration standard will be used. The remaining samples must be reanalyzed.

10.3.2 See Section 13 to calculate percent difference.

## **11.0 CALIBRATION AND STANDARDIZATION**

---

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression ( $r^2$ ) for the calibration curve using MassLynx or other suitable software.

11.2 The  $r^2$  value for the data should be 0.980 or greater. Lower values may be acceptable at the discretion of the analyst and documented approval of the Project Lead.

11.3 If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

11.4 For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 10 ppb of analyte, generate a calibration curve consisting of the standards from 5 ppb to 100 ppb rather than the full range of the curve (5 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

## **12.0 PROCEDURES**

---

### **12.1 Acquisition Set up**

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using letter-MO-DAY-last digit of year-sample number, assign a method (MS) for acquiring, and type in sample descriptions.

**12.1.2** To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. See Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM (Multiple Reaction Monitoring).

**12.1.3** Typically the analytical batch run sequence begins with a set of extracted matrix standards and ends with a set of extracted matrix standards.

**12.1.4** Samples are analyzed with a continuing calibration check injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

## **12.2 Using the Autosampler**

**12.2.1** Set up sample tray according to the sample list prepared in Section 12.1.1.

**12.2.2** Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

**12.2.2.1** Sample size = 10  $\mu$ L injection with a sample wash

**12.2.2.2** Inject/sample = 1

**12.2.2.3** Cycle time = 15 minutes

**12.2.2.4** Solvent ramp =

| Time      | MeOH | 2.0 mM<br>Ammonium acetate |
|-----------|------|----------------------------|
| 0.00 min. | 45%  | 55%                        |
| 7.5 min.  | 90%  | 10%                        |
| 11.0 min. | 90%  | 10%                        |
| 11.5 min. | 45%  | 55%                        |

**Note:** In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

**12.2.2.5** Press the "Start" button.

## **12.3 Instrument Set-up**

**12.3.1** Refer to **FACT-EP-3.0** for more details.

**12.3.2** Check the solvent level in reservoirs and refill if necessary.

**12.3.3** Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

- 12.3.4** Set HPLC pump to “On”. Set the flow to 10 - 500  $\mu\text{L}/\text{min}$  or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.
- 12.3.5** Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.
- 12.3.6** The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:
- 12.3.6.1** Drying gas 250-400 liters/hour
  - 12.3.6.2** ESI nebulizing gas 10-15 liters/hour
  - 12.3.6.3** HPLC constant flow mode flow rate 10 – 500  $\mu\text{L}/\text{min}$
  - 12.3.6.4** Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7** Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.
- 12.3.8** Record tune parameters in the instrument log.
- 12.3.9** Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.
- 12.3.10** Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER’S GUIDE). Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

## **13.0 DATA ANALYSIS AND CALCULATIONS**

---

### **13.1 Calculations:**

- 13.1.4** Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5** Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6** Calculate actual concentration of PFOS, or other fluorochemical, in matrix ( $\mu\text{g}/\text{ml}$ ):

$$\frac{(\text{ng of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{(\text{Initial Volume of matrix (ml)} + \text{ml of Surrogate Standard})} \times \frac{1 \mu\text{g}}{1000 \text{ ng}} \times \text{Final Volume (mL)}$$

## **14.0 METHOD PERFORMANCE**

---

- 14.1** Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see FACT-M-3.1, Attachment A for a listing of current validated MDL and LOQ values.

## **14.2 Method Blanks and Matrix Blanks**

14.2.1 Method blanks and matrix blanks will be analyzed with each sample set for possible contamination or carryover. Values are expected to fall below the lowest standard in the calibration curve.

## **14.3 Matrix Spikes**

14.3.1 Matrix spikes are analyzed with each sample set and the percent recoveries are expected to fall within  $\pm 30\%$  of the spiked concentration.

## **14.4 Continuing Calibration Checks**

14.4.1 Continuing calibration checks are analyzed at a minimum of after every 10 samples with each sample set. The percent recoveries are expected to fall within  $\pm 30\%$  of the spiked concentration.

14.5 If any criteria listed in the method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. All actions will be documented in the instrument runlog, the maintenance log, or on the summary sheet with the sample results.

## **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

15.1 Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

16.1 Store chromatograms in the study or project folder. Each chromatogram must have the following information included either in the header or hand written on the chromatogram: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and store in appropriate study folder.

16.6 Summarize data using suitable software and store in the study folder.

16.7 Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

## **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

17.1 Attachment A: FACT-M-4.1 Data reporting spreadsheet

17.2 The validation report associated with this method is FACT-M-3.1 & 4.1-V-1.

## **18.0 REFERENCES**

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- 18.1 FACT-EP-3.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Platform Systems"

## **19.0 AFFECTED DOCUMENTS**

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- 19.1 FACT-M-3.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

## **20.0 REVISIONS**

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| <u>Revision<br/>Number.</u> | <u>Reason For Revision</u>                                                                                                                                                                                                         | <u>Revision<br/>Date</u> |
|-----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 1                           | <i>Validation of method to include 7 fluorochemicals addition of whole blood matrix, surrogate standard, new API/MS(MS) systems, monkey sera cross validation, MDL study, updates in record keeping and storing policies, etc.</i> | 07/01/98                 |

## Laboratory Study #

Study:

Test Material:

Matrix/Final Solvent:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Filename:

R-Squared Value:

Slope:

Y Intercept:

Date of Extraction/Analyst:

Date of Analysis/Analyst:

| Group<br>Dose | Sample# | Concentration<br>ug/mL | Initial Vol.<br>mL | Dilution<br>Factor | Final Conc.<br>ug/mL |
|---------------|---------|------------------------|--------------------|--------------------|----------------------|
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |

**Slope:** Taken from linear regression equation.**Group/Dose:** Taken from the study folder.**Sample#:** Taken from the study folder.**Concentration (ug/mL):** Taken from the MassLynx integration summary.**Initial Volume (mL):** Taken from the study folder.**Dilution Factor:** Taken from the study folder.**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

# 3M ENVIRONMENTAL LABORATORY

## METHOD

### EXTRACTION-OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUORO-CHEMICAL COMPOUNDS FROM URINE FOR ANALYSIS USING HPLC- ELECTROSPRAY/MASS SPECTROMETRY/MASS SPECTROMETRY

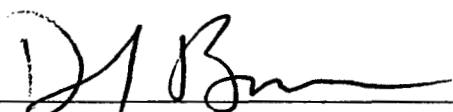
Method Number: ETS-8-96.0

Adoption Date: 7-28-99

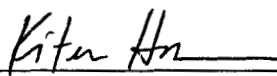
Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

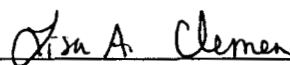
Approved By:

  
Laboratory Manager

7/28/99  
Date

  
Group Leader

7/28/99  
Date

  
Technical Reviewer

7/27/99  
Date

Initial  
Date  
Exact Copy of Original

#### 1. SCOPE AND APPLICATION

**Scope:** This method is for the extraction of potassium perfluorooctanesulfonate (PFOS) or other fluorochemical compounds from urine.

**Applicable compounds:** Fluorochemicals or other fluorinated compounds.

**Matrices:** Human, rat, and monkey urine or other fluids as designated in the validation report.

## 2.0 SUMMARY OF METHOD

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- 2.1 This method describes the procedure for extracting potassium perfluorooctanesulfonate (PFOS) or other fluorochemicals from urine, or other fluids, using an ion pairing reagent and methyl-*tert*-butyl ether (MtBE). In this method, eight fluorochemicals are extracted: PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, M570, perfluorooctanoate (POAA), and surrogate standard (see 3.0 *Definitions*). An ion pairing reagent is added to two ml of sample and the analyte ion pair is partitioned into MtBE. The MtBE extract is removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 0.5 ml of methanol, then filtered through a 0.2  $\mu$ m nylon filter attached to 3 cc plastic syringe into glass autovials.
- 2.2 These sample extracts are analyzed following method ETS-8-97.0 or other appropriate method.

## 3.0 DEFINITIONS

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- 3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt)  $C_8F_{17}SO_3^-$
- 3.2 PFOSA: perfluorooctane sulfonylamide  $C_8F_{17}SO_2NH_2$
- 3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate  $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$
- 3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol  
 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$
- 3.5 M556:  $C_8F_{17}SO_2N(H)(CH_2COOH)$
- 3.6 M570:  $C_8F_{17}SO_2N(CH_3)CH_2COOH$
- 3.7 POAA: perfluorooctanoate  $C_7F_{15}COO^-$
- 3.8 Surrogate standard THPFOS: 1H-1H-2H-2H perfluorooctane sulfonic acid, used as an internal standard in this method.

## 4.0 WARNINGS AND CAUTIONS

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### 4.1 Health and safety warnings

- 4.1.1 Use universal precautions, especially laboratory coats, goggles, and gloves when handling animal tissue, which may contain pathogens.

## 5.0 INTERFERENCES

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- 5.1 At this time, it is unknown how the extraction method is affected by potential interferences that may be present such as conjugated fluorochemicals (eg. Glucuronides). Conjugates may become deconjugated during extraction or analysis resulting in a high bias for reported results of target analytes.

## **6.0 EQUIPMENT**

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**6.1** The following equipment is used while performing this method. Equivalent equipment is acceptable.

**6.1.1** Vortex mixer, VWR, Vortex Genie 2

**6.1.2** Centrifuge, Mistral 1000 or IEC

**6.1.3** Shaker, Eberbach or VWR

**6.1.4** Nitrogen evaporator, Organomation

**6.1.5** Balance ( $\pm 0.100$  g)

## **7.0 SUPPLIES AND MATERIALS**

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**7.1** Gloves

**7.2** Eppendorf or disposable pipettes

**7.3** Nalgene bottles, capable of holding 250 ml and 1 L

**7.4** Volumetric flasks, glass, type A

**7.5** I-CHEM vials, glass, 40 ml glass

**7.6** Centrifuge tubes, polypropylene, 15 ml

**7.7** Labels

**7.8** Oxford Dispenser – 3.0 to 10.0 ml

**7.9** Syringes, capable of measuring 2.5  $\mu$ L to 50  $\mu$ L

**7.10** Graduated pipettes

**7.11** Syringes, disposable plastic, 3 cc

**7.12** Syringe filters, nylon, 0.2  $\mu$ m, 25 mm

**7.13** Timer

**7.14** Crimp cap autovials and caps

**7.15** Crimpers

**Note:** Prior to using glassware and bottles, rinse 3 times with methanol and 3 times with Milli-Q™ water. Rinse glass syringes a minimum of 9 times with methanol, 3 rinses from 3 separate vials.

## **8.0 REAGENTS AND STANDARDS**

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**8.1** Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

**8.2** Sodium hydroxide (NaOH), J.T Baker or equivalent

**8.3** Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

**8.4** Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), J.T. Baker or equivalent

**8.5** Sodium bicarbonate (NaHCO<sub>3</sub>), J.T. Baker or equivalent

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Urine frozen from supplier

**8.9 Fluorochemical standards**

8.9.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.9.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.9.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

8.9.5 M556 (3M Specialty Chemical Division), molecular weight = 557

8.9.6 M570 (3M Specialty Chemical Division), molecular weight = 571

8.9.7 POAA (3M Specialty Chemical Division), molecular weight = 452

8.9.8 THPFOS (1-H,1-H, 2-H, 2-H C<sub>8</sub>F<sub>13</sub>SO<sub>3</sub>H) molecular weight = 428

8.9.9 Other fluorochemicals, as appropriate

**8.10 Reagent preparation**

**NOTE:** When preparing larger volumes than listed in reagent, standard, or surrogate preparation, adjust accordingly.

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 ml beaker containing 500 ml Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 ml of 10 N NaOH solution into a 100 ml volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 ml Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 ml Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 ml of 10 N NaOH (While adding the last ml of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.10.3.1 TBA requires a check prior to each use to ensure pH = 10.0. Adjust as needed using 1 N NaOH solution.

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>): Weigh approximately 26.5 g of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and 21.0 g of sodium bicarbonate (NaHCO<sub>3</sub>) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

**8.11 Standards preparation**

8.11.1 Prepare PFOS standards for the standard curve.

8.11.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

- 8.11.3 Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight in the Standard Logbook.
- 8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm ( $\mu\text{g/ml}$ ).
- 8.11.5 Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.
- 8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.
- 8.11.7 Dilute working standard 1 with methanol for a working standard 3 solution of approx. 0.50 ppm.
- 8.12 Surrogate stock standard preparation**
  - 8.12.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H,  $\text{C}_8\text{F}_{13}\text{SO}_3\text{H}$  into a 50 ml volumetric flask and record the actual weight.
  - 8.12.2 Bring to volume with methanol for a surrogate stock of approximately 1000-1200 ppm.
  - 8.12.3 Prepare a surrogate working standard. Transfer approximately 1 ml of surrogate stock to a 10 ml volumetric flask and bring to volume with methanol for a working standard of 100-120 ppm. Record the actual volume transferred in the Standard Logbook.

## **9.0 SAMPLE HANDLING**

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- 9.1 All samples are received frozen and must be kept frozen until the extraction is performed.
- 9.2 Allow samples to thaw to room temperature prior to extraction.

## **10.0 QUALITY CONTROL**

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### **10.1 Solvent Blanks, Method blanks and matrix blanks**

- 10.1.1 An aliquot of 2.0 ml methanol is used as a solvent blank.
- 10.1.2 Extract two 2.0 ml aliquots of Milli-Q<sup>TM</sup> water following this procedure and use as method blanks.
- 10.1.3 Extract two 2.0 ml aliquots of the urine following this procedure and use as matrix blanks. See 11.1.4.

### **10.2 Matrix spikes**

- 10.2.1 Prepare and analyze matrix spike and matrix spike duplicate samples to determine the accuracy of the extraction.
- 10.2.2 Prepare each spike using a sample chosen by the analyst, usually the control matrix received with each sample set.
- 10.2.3 Expected concentrations should fall in the mid-range of the initial calibration curve. Additional spikes may be included and may fall in the low-range of the initial calibration curve.

10.2.4 Prepare one matrix spike and matrix spike duplicate per 40 samples, with a minimum of 2 matrix spikes per batch.

### 10.3 Continuing calibration verifications

10.3.1 Prepare continuing calibration verification samples to ensure the accuracy of the initial calibration curve.

10.3.2 Prepare, at a minimum, one continuing calibration verification per group of 10 samples. For example, if a sample set = 34, four checks are prepared and extracted.

10.3.3 Prepare each continuing calibration verification from the same matrix used to prepare the initial curve.

10.3.4 The expected concentrations will fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

## **11.0 CALIBRATION AND STANDARDIZATION**

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### 11.1 Prepare matrix calibration standards

11.1.1 Transfer 2.0 ml of urine to a 15 ml centrifuge tube.

11.1.2 Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty-two aliquots in 15 ml centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 2.0 ml aliquots serve as matrix blanks.

11.1.5 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of twenty standards, two matrix blanks, and two method blanks.

11.1.6 Refer to validation report **FACT-TOX-131, W2067**, which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.7 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 10 ppb - 1500 ppb.

11.3 Extract spiked matrix standards following 12.6-12.16 of this method. Use these standards to establish each initial curve on the mass spectrometer.

| <b>Table 1</b><br><b>Approximate spiking amounts for standards and spikes</b><br><b>Using 2.0 ml of matrix</b> |     |                                             |                                              |
|----------------------------------------------------------------------------------------------------------------|-----|---------------------------------------------|----------------------------------------------|
| Working standard<br>(approx. conc.)                                                                            | μL  | Approx. final conc.<br>of analyte in matrix | Approx. final conc.<br>Of analyte in solvent |
| -                                                                                                              | -   | Blank                                       | Blank                                        |
| 0.500 ppm                                                                                                      | 5   | 1.25 ppb                                    | 5.00 ppb                                     |
| 0.500 ppm                                                                                                      | 10  | 2.50 ppb                                    | 10.0 ppb                                     |
| 0.500 ppm                                                                                                      | 25  | 6.00 ppb                                    | 25.0 ppb                                     |
| 5.00 ppm                                                                                                       | 5   | 12.5 ppb                                    | 50.0 ppb                                     |
| 5.00 ppm                                                                                                       | 10  | 25.0 ppb                                    | 100 ppb                                      |
| 5.00 ppm                                                                                                       | 25  | 62.5 ppb                                    | 250 ppb                                      |
| 50.0 ppm                                                                                                       | 5   | 125 ppb                                     | 500 ppb                                      |
| 50.0 ppm                                                                                                       | 7.5 | 200 ppb                                     | 750 ppb                                      |
| 50.0 ppm                                                                                                       | 10  | 250 ppb                                     | 1000 ppb                                     |
| 50.0 ppm                                                                                                       | 15  | 375 ppb                                     | 1500 ppb                                     |

## 12.0 PROCEDURE

- 12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.
- 12.2 Vortex mix for 15 seconds, then transfer 2.0 ml or other appropriate volume to a 15 ml polypropylene centrifuge tube.
- 12.3 Return unused samples to freezer after extraction amounts have been removed.
- 12.4 Record the initial volume on the sample weight/volume worksheet. . See **Attachment D**. The original weight/volume worksheet is included in the study binder.
- 12.5 Label the tube with the study number, sample ID, date and analyst initials. See attached worksheet for documenting the remaining steps.
- 12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in 11.2.
- 12.7 Spike each matrix with the appropriate amount of standard as described in 11.1, or **Table 1** in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.
- 12.8 Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.
- 12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.
- 12.10 To each sample, add 1 ml 0.5 M TBA and 2 ml of 0.25M sodium carbonate/sodium bicarbonate buffer.
- 12.11 Using an Oxford Dispenser, add 5 ml methyl-*tert*-butyl ether.
- 12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

- 12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are separated.
- 12.14 Label a fresh 15 ml centrifuge tube with the same information as in 12.5.
- 12.15 Remove 4.0 ml of the organic layer to this clean 15 ml centrifuge tube.
- 12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 30 to 60 minutes.
- 12.11 Add 0.5 ml of methanol to each centrifuge tube using a graduated pipette. If excessive residue is present, add the methanol and allow the extract to sit for 30 minutes prior to vortexing.
- 12.17 Vortex mix for 30 seconds.
- 12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, fluorochemical components, extraction type, vial file archive number, and analyst(s) performing the extraction.
- 12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 ml glass autovial or low-volume autovial when necessary.
- 12.20 Cap and store extracts at room temperature or refrigerated at approximately 4 °C until analysis.
- 12.21 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

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#### **13.1 Calculations**

- 13.1.1 Calculate actual concentrations of PFOS, or other applicable fluorochemical, in calibration standards using the following equation:

$$\frac{\text{ml of standard} \times \text{concentration of standard } (\mu\text{g/ml})}{\text{ml of standard} + \text{ml of surrogate standard} + \text{initial matrix volume (ml)}} =$$

Final Concentration (µg/ml) of PFOS in matrix

### **14.0 METHOD PERFORMANCE**

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- 14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachment B**).
- 14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.
  - 14.2.1 Method blanks and matrix blanks.
  - 14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.
  - 14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.
- 14.3 Refer to section 14 of ETS-8-97.0 for method performance criteria.

## **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

- 15.1 Human and monkey sample waste is disposed in infectious biohazard waste containers, all other sample waste is disposed in noninfectious biohazard waste containers. Flammable solvent waste is disposed in high BTU containers. Used glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

- 16.1 Complete the extraction worksheet attached to this method, and tape in the study notebook or include in the 3-ring study binder, as appropriate.

## **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATE**

- 17.1 Attachment A, Extraction worksheet  
17.2 Attachment B, MDL/LOQ values and summary  
17.3 Attachment C, Calibration standard concentration worksheet  
17.4 Attachment D, Sample weight/volume worksheet

## **18.0 REFERENCES**

- 18.1 The validation report associated with this method is **FACT-TOX-131, W2067**.

## **19.0 AFFECTED DOCUMENTS**

- 19.1 ETS-8-97.0, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray Mass Spectrometry/Mass Spectrometry"

## **20.0 REVISIONS**

| <u>Revision<br/>Number</u> | <u>Reason For Revision</u> | <u>Revision<br/>Date</u> |
|----------------------------|----------------------------|--------------------------|
|----------------------------|----------------------------|--------------------------|

## Extraction Worksheet ETS-8-96.0

[illegible]

**MDL/LOQ values for human urine**

| Compound  | MDL (ppb) | LOQ (ppb) | Linear Calibration Range (LCR)            |
|-----------|-----------|-----------|-------------------------------------------|
| PFOS      | 4.6       | 14.7      | 15 ppb - 1500 ppb (in final MeOH extract) |
| POAA      | 22.9      | 72.9      | 50 ppb - 1500 ppb (in final MeOH extract) |
| PFOSA     | n/d       | n/d       | 25 ppb - 1000 ppb (in final MeOH extract) |
| PFOSAA    | n/d       | n/d       | 25 ppb - 1000 ppb (in final MeOH extract) |
| EtFOSE-OH | n/d       | n/d       | 25 ppb - 1000 ppb (in final MeOH extract) |
| M556      | n/d       | n/d       | 25 ppb - 1000 ppb (in final MeOH extract) |
| M570      | n/d       | n/d       | 25 ppb - 1000 ppb (in final MeOH extract) |

n/d = not determined

**NOTE:** to calculate MDL, LOQ, and LCR values in ug/ml of urine divide the above values by 4.

MDL/LOQ values in rat and monkey urine were not statistically determined. Two curves in each of these matrices were extracted and analyzed with the human urine curves to determine equivalence. Responses in the rat and monkey were similar to the human responses, therefore, their MDL and LOQ are assumed to be similar to the values determined for human urine.

If a suitable amount of clean, control matrix is available, samples will be evaluated versus a curve extracted from urine originating from the same species as the specimens.

Please see LOQ Summary and MDL study in **FACT-TOX-131, W2067** for further information.

**Compound: PFOS**

| Human Urine | Prepared range of standards (ppb) (ng/ml) | LCR from curve (ppb) (ng/ml) | % Recovery Range | RSD Range |
|-------------|-------------------------------------------|------------------------------|------------------|-----------|
| Full Range  | n/d                                       | n/d                          | n/d              | n/d       |
| Low Curve   | n/d                                       | n/d                          | n/d              | n/d       |
| High curve  | n/d                                       | n/d                          | n/d              | n/d       |
| 1/X         | 2.5ppb – 1500 ppb                         | 15 ppb – 1500 ppb            | 75-116           | +/- 30%   |

**Compound: POAA**

| Human Urine    | Prepared range of standards (ppb) (ng/ml) | LCR from curve (ppb) (ng/ml) | % Recovery Range | RSD Range |
|----------------|-------------------------------------------|------------------------------|------------------|-----------|
| Full Range     | n/d                                       | n/d                          | n/d              | n/d       |
| Low Curve      | n/d                                       | n/d                          | n/d              | n/d       |
| High curve     | n/d                                       | n/d                          | n/d              | n/d       |
| 1/X, quadratic | 2.5ppb – 1500 ppb                         | 50 ppb – 1500 ppb            | 86-105           | +/- 30%   |

### Ion Pair Standard Curves – Urine

Prep date(s):

Standard number:

Analyte(s):

Equipment number:

Sample matrix:

Final solvent and TN:

Blank fluid/identifier:

Method/revision:

Box Number:

Target analyte(s):

FC mix std approx. 0.500 ppm:

FC mix std approx. 5.00 ppm:

FC mix std approx. 50.0 ppm:

Surrogate std approx. 100 ppm:

#### Actual concentrations of standards in the FC mix

| PFOS     | PFOSA    | PFOSAA   | EtFOSE   | POAA     | M556     | M570     | All       | All       |
|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|
| Std conc | Std conc | Std conc | Std conc | Std conc | Std conc | Std conc | Am't      | Final vol |
| ug/ml    | ug/ml    | ug/ml    | ug/ml    | ug/ml    | ug/ml    | ug/ml    | spiked ml | ml        |
| 0.500    | 0.501    | 0.500    | 0.501    | 0.499    | 0.500    | 0.501    | 0.005     | 2.0075    |
| 0.500    | 0.501    | 0.500    | 0.501    | 0.499    | 0.500    | 0.501    | 0.010     | 2.0125    |
| 0.500    | 0.501    | 0.500    | 0.501    | 0.499    | 0.500    | 0.501    | 0.025     | 2.0275    |
| 5.00     | 5.01     | 5.00     | 5.01     | 4.99     | 5.00     | 5.01     | 0.005     | 2.0075    |
| 5.00     | 5.01     | 5.00     | 5.01     | 4.99     | 5.00     | 5.01     | 0.010     | 2.0125    |
| 5.00     | 5.01     | 5.00     | 5.01     | 4.99     | 5.00     | 5.01     | 0.025     | 2.0275    |
| 50.0     | 50.1     | 50.0     | 50.1     | 49.9     | 50.0     | 50.1     | 0.005     | 2.0075    |
| 50.0     | 50.1     | 50.0     | 50.1     | 49.9     | 50.0     | 50.1     | 0.0075    | 2.0100    |
| 50.0     | 50.1     | 50.0     | 50.1     | 49.9     | 50.0     | 50.1     | 0.010     | 2.0125    |
| 50.0     | 50.1     | 50.0     | 50.1     | 49.9     | 50.0     | 50.1     | 0.015     | 2.0130    |

#### Calculated concentrations of standards in the sample matrix

| PFOS       | PFOSA      | PFOSAA     | EtFOSE     | POAA       | M556       | M570       | Surrogate                        | All       |
|------------|------------|------------|------------|------------|------------|------------|----------------------------------|-----------|
| Final conc | Final conc | Final conc | Final conc | Final conc | Final conc | Final conc | Std conc                         | Am't      |
| ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml                            | spiked ml |
| 1.25       | 1.25       | 1.25       | 1.25       | 1.24       | 1.25       | 1.25       | 100                              | 0.0025    |
| 2.48       | 2.49       | 2.48       | 2.49       | 2.48       | 2.48       | 2.49       | Surrogate<br>Final conc<br>ng/ml |           |
| 6.17       | 6.18       | 6.17       | 6.18       | 6.15       | 6.17       | 6.18       |                                  |           |
| 12.5       | 12.5       | 12.5       | 12.5       | 12.4       | 12.5       | 12.5       |                                  |           |
| 24.8       | 24.9       | 24.8       | 24.9       | 24.8       | 24.8       | 24.9       |                                  |           |
| 61.7       | 61.8       | 61.7       | 61.8       | 61.5       | 61.7       | 61.8       |                                  |           |
| 125        | 125        | 125        | 125        | 124        | 125        | 125        | 125                              |           |
| 187        | 187        | 187        | 187        | 186        | 187        | 187        |                                  |           |
| 248        | 249        | 248        | 249        | 248        | 248        | 249        |                                  |           |
| 372        | 372        | 372        | 372        | 371        | 372        | 372        |                                  |           |

#### Calculated concentrations of standards in methanol extract (0.5 ml final volume)

| PFOS       | PFOSA      | PFOSAA     | EtFOSE     | POAA       | M556       | M570       | Surrogate                        | All       |
|------------|------------|------------|------------|------------|------------|------------|----------------------------------|-----------|
| Final conc | Final conc | Final conc | Final conc | Final conc | Final conc | Final conc | Std conc                         | Am't      |
| ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml      | ng/ml                            | spiked ml |
| 5.00       | 5.01       | 5.00       | 5.01       | 4.99       | 5.00       | 5.01       | 100                              | 0.0025    |
| 10.0       | 10.0       | 10.0       | 10.0       | 10.0       | 10.0       | 10.0       | Surrogate<br>Final conc<br>ng/ml |           |
| 25.0       | 25.1       | 25.0       | 25.1       | 25.0       | 25.0       | 25.1       |                                  |           |
| 50.0       | 50.1       | 50.0       | 50.1       | 49.9       | 50.0       | 50.1       |                                  |           |
| 100        | 100        | 100        | 100        | 100        | 100        | 100        |                                  |           |
| 250        | 251        | 250        | 251        | 250        | 250        | 251        |                                  |           |
| 500        | 501        | 500        | 501        | 499        | 500        | 501        | 500                              |           |
| 750        | 752        | 750        | 752        | 749        | 750        | 752        |                                  |           |
| 1000       | 1002       | 1000       | 1002       | 998        | 1000       | 1002       |                                  |           |
| 1500       | 1503       | 1500       | 1503       | 1497       | 1500       | 1503       |                                  |           |



# 3M ENVIRONMENTAL LABORATORY

## METHOD

### ANALYSIS OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER FLUOROCHEMICAL COMPOUNDS IN URINE EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY/MASS SPECTROMETRY

Method Number: ETS-8-97.0

Adoption Date: 7-28-99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne, Glenn Langenburg

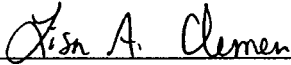
Approved By:

  
Laboratory Manager

7/28/99  
Date

  
Group Leader

7/28/99  
Date

  
Technical Reviewer

7/27/99  
Date

Initial  
RSD

Date  
6/5/01

Exact Copy of Original

#### 1.0 SCOPE AND APPLICATION

1.1 Scope: This method describes the analysis of urine extracts for fluorochemicals using HPLC-electrospray/mass spectrometry.

1.2 Applicable Compounds: Fluorochemicals or other ionizable compounds.

1.3 Matrices: Human, rat, and monkey urine, or other fluids as designated in the validation report.

## 2.0 SUMMARY OF METHOD

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- 2.1** This method describes the analysis of fluorochemicals extracted from urine or other fluids, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the perfluorooctanesulfonate (PFOS) anion,  $m/z = 499$ . Additionally, samples may be analyzed using a tandem mass spectrometer to further verify the identity of a compound by detecting daughter ions of the parent ion.

## 3.0 DEFINITIONS

---

- 3.1 Atmospheric Pressure Ionization (API):** The Micromass Quattro II triple quadrupole systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APCI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e., not under a vacuum).
- 3.2 Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.
- 3.3 Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio ( $m/z$ ) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.
- 3.4 Conventional vs. Z-spray probe interface:** The latest models of Micromass Quattro II triple quadrupole systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e., Z-spray components are compatible with some other Z-spray systems, etc.)
- 3.5 Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has WindowsNT 4.0 versions. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx NT User's Guide).

## 4.0 WARNINGS AND CAUTIONS

---

### 4.1 Health and Safety Warnings:

- 4.1.1** Use caution with the voltage cables for the probe. When engaged, the probe employs a voltage of approximately 5000 Volts.

- 4.1.2 When handling samples or solvents wear appropriate protective gloves, eyewear, and clothing.

#### **4.2 Cautions:**

- 4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.
- 4.2.2 Do not run solvent pumps to dryness.

### **5.0 INTERFERENCES**

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- 5.1 To minimize interferences when analyzing samples, teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

### **6.0 EQUIPMENT**

---

- 6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.
  - 6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source
  - 6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

### **7.0 SUPPLIES AND MATERIALS**

---

#### **7.1 Supplies**

- 7.1.1 High purity grade nitrogen regulated to approximately 100 psi. (House air system)
- 7.1.2 High purity grade argon regulated to approximately 6 psi.
- 7.1.3 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.
- 7.1.4 Capped autovials or capped 15 mL centrifuge tubes

### **8.0 REAGENTS AND STANDARDS**

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#### **8.1 Reagents**

- 8.1.1 Methanol, HPLC grade or equivalent
- 8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ASTM type I, or equivalent, and may be provided by a Milli-Q TOC Plus system or other vendor
- 8.1.3 Ammonium acetate, reagent grade or equivalent
  - 8.1.3.1 When preparing different amounts than those listed, adjust accordingly.
  - 8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

## **8.2 Standards**

- 8.2.1** Typically two method blanks, two matrix blanks, and twenty matrix standards are prepared during the extraction procedure. See ETS-8-96.0.

## **9.0 SAMPLE HANDLING**

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- 9.1** Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.
- 9.2** If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C, or at room temperature, until analysis can be performed.

## **10.0 QUALITY CONTROL**

---

### **10.1 Solvent Blanks, Method Blanks and Matrix Blanks**

- 10.1.1** Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.
- 10.1.2** Analyze a solvent blank, method blank, and matrix blank prior to each calibration curve.

### **10.2 Matrix Spikes**

- 10.2.1** Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.
- 10.2.2** Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.
- 10.2.3** Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.
- 10.2.4** Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

### **10.3 Continuing Calibration Verifications**

- 10.3.1** Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.
- 10.3.2** Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

## **11.0 CALIBRATION AND STANDARDIZATION**

---

- 11.1** Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by regression (linear or otherwise, see 11.2), weighted 1/x, not forced through zero, with IS reference (surrogate is used as an internal standard) using MassLynx or other suitable software.

**11.2** Use the following parameters for determining a calibration curve for each compound:

| Compound | Weighting | Regression Fit | Response type |
|----------|-----------|----------------|---------------|
| PFOS     | 1/X       | Linear         | IS reference  |
| POAA     | 1/X       | Quadratic      | IS reference  |

Suitability of curve fitting parameters for other fluorochemical compounds will be addressed by the analyst.

**11.3** If the curve does not meet requirements, perform routine maintenance or reextract the standard curve (if necessary) and reanalyze.

**11.4** For purposes of accuracy when quantitating low levels of analyte, it may be necessary to use the low end of the calibration curve rather than the full range of the standard curve. Example: when attempting to quantitate approximately 50 ppb of analyte, generate a calibration curve consisting of the standards from 25 ppb to 250 ppb rather than the full range of the curve (25 ppb to 1000 ppb). This will reduce inaccuracy attributed to linear regression weighting of high concentration standards.

## **12.0 PROCEDURES**

---

### **12.1 Acquisition Set up**

#### **12.1.1 Set up the sample list.**

**12.1.1.1** Assign a sample list filename using MO-DAY-last two digits of year-increasing letter of the alphabet starting with a

**12.1.1.2** Assign a method (MS file) for acquiring

**12.1.1.3** Assign an HPLC program (Inlet file)

**12.1.1.4** Type in sample descriptions and vial position numbers

**12.1.2** To create a method click on method in the Acquisition control panel then mass spectrometer headings and select SIR (Single Ion Recording) or MRM (Multiple Reaction Monitoring). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A full scan is usually collected along with the SIRs. Save acquisition method. If MS/MS instruments are employed, additional product ion fragmentation information may be collected. Refer to Micromass MassLynx GUIDE TO DATA ACQUISITION for additional information and MRM.

**12.1.3** Typically the analytical batch run sequence begins and ends with a set of extracted matrix standards.

**12.1.4** Samples are analyzed with a continuing calibration verification injected after every tenth sample. Solvent blanks should be analyzed periodically to monitor possible analyte carryover and are not considered samples but may be included as such.

### **12.2 Using the Autosampler**

**12.2.1** Set up sample tray according to the sample list prepared in Section 12.1.1.

**12.2.2** Set-up the HP1100/autosampler at the following conditions or at conditions the analyst considers appropriate for optimal response. Record actual conditions in the instrument logbook:

**12.2.2.1** Sample size = 10  $\mu$ L injection

**12.2.2.2** Inject/sample = 1

**12.2.2.3** Cycle time = 9.0 minutes

**12.2.2.4** Solvent ramp =

| Time     | MeOH | 2.0 mM<br>Ammonium acetate |
|----------|------|----------------------------|
| 0.00 min | 40%  | 60%                        |
| 1.0 min. | 40%  | 60%                        |
| 4.5 min. | 95%  | 5%                         |
| 6.5 min. | 95%  | 5%                         |
| 7.0 min. | 40%  | 60%                        |
| 9.0 min. | 40%  | 60%                        |

**12.2.2.5** Press the “Start” button.

### **12.3 Instrument Set-up**

**12.3.1** Refer to ETS-9-24.0, “Operation and Maintenance of the Micromass Quattro II Triple Quadrupole Mass Spectrometer Fitted with an Atmospheric Pressure Ionization Source,” for more details.

**12.3.2** Check the solvent level in reservoirs and refill if necessary.

**12.3.3** Check the stainless steel capillary at the end of the probe. Use an eyepiece to check the tip. The tip should be flat with no jagged edges. If the tip is found to be unsatisfactory, disassemble the probe and replace the stainless steel capillary.

**12.3.4** Turn on the nitrogen.

**12.3.5** Open the tune page. Click on operate to initiate source block and desolvation heaters.

**12.3.6** Open the Inlet Editor.

**12.3.6.1** Set HPLC pump to “On”

**12.3.6.2** Set the flow to 10 - 500  $\mu$ L/min or as appropriate

**12.3.6.3** Observe droplets coming out of the tip of the probe. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed

**12.3.6.4** Allow to equilibrate for approximately 10 minutes.

**12.3.7** The instrument uses these parameters at the following settings. These settings may change in order to optimize the response:

**12.3.7.1** Drying gas 250-400 liters/hour

- 12.3.7.2 ESI nebulizing gas 10-15 liters/hour
- 12.3.7.3 HPLC constant flow mode, flow rate 10 – 500 µL/min
- 12.3.7.4 Pressure <400 bar (This parameter is not set, it is a guide to ensure the HPLC is operating correctly.)
- 12.3.7.5 Source block temperature 150°
- 12.3.7.6 Desolvation temperature 250°
- 12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.
- 12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

### **13.0 DATA ANALYSIS AND CALCULATIONS**

---

#### **13.1 Calculations:**

- 13.1.4 Calculate matrix spike percent recoveries using the following equation:

$$\% \text{ Recovery} = \frac{\text{Observed Result} - \text{Background Result}}{\text{Expected Result}} \times 100$$

- 13.1.5 Calculate percent difference using the following equation:

$$\% \text{ Difference} = \frac{\text{Expected Conc.} - \text{Calculated Conc.}}{\text{Expected Conc.}} \times 100$$

- 13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{(\text{ng/mL of PFOS calc. from std. Curve} \times \text{Dilution Factor})}{\frac{\text{Initial Volume of matrix (mL)}}{\text{Final Volume (mL)}}} \times \frac{1 \mu\text{g}}{1000 \text{ ng}}$$

### **14.0 METHOD PERFORMANCE**

---

- 14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see ETS-8-96.0, Attachment B, for a listing of current validated MDL and LOQ values.

#### **14.2 Solvent Blanks, Method Blanks, and Matrix Blanks**

- 14.2.1 Solvent blanks, method blanks, and matrix blanks values must be below the lowest standard in the calibration curve

#### **14.3 Calibration Curves**

- 14.3.1 The r<sup>2</sup> value for the calibration curve must be 0.980 or better.

#### **14.4 Matrix Spikes**

- 14.4.1 Matrix spike percent recoveries must be within ± 30% of the spiked concentration.

## **14.5 Continuing Calibration Verifications**

- 14.5.1** Continuing calibration verification percent recoveries must be within  $\pm 30\%$  of the spiked concentration.
- 14.6** If criteria listed in this method performance section are not met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. Document all actions in the appropriate logbook.
- 14.7** If data are to be reported when performance criteria have not been met, the data must be footnoted on tables and discussed in the text of the report.

## **15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT**

---

- 15.1** Sample extract waste and flammable solvent is disposed in high BTU containers, and glass pipette waste is disposed in broken glass containers located in the laboratory.

## **16.0 RECORDS**

---

- 16.1** Each page generated for a study must have the following information included either in the header or hand written on the page: study or project number, acquisition method, integration method, sample name, extraction date, dilution factor (if applicable), and analyst.
- 16.2** Print the tune page, sample list, and acquisition method from MassLynx to include in the appropriate study folder. Copy these pages and tape into the instrument runlog.
- 16.3** Plot the calibration curve by a linear or quadratic fit, referenced to the internal standard (surrogate), weighted  $1/x$ , then print these graphs and store in the study folder.
- 16.4** Print data integration summary, integration method, and chromatograms, from MassLynx, and store in the study folder.
- 16.5** Summarize data using suitable software (Excel 7.0) and store in the study folder, see **Attachment A** for an example of a summary spreadsheet.
- 16.6** Back up electronic data to appropriate medium. Record in study notebook the file name and location of backup electronic data.

## **17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA**

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- 17.1** Attachment A: ETS-8-97.0 Data summary spreadsheet.

## **18.0 REFERENCES**

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- 18.1** ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"
- 18.2** The validation report associated with this method is **FACT-TOX-131, W2067**

## **19.0 AFFECTED DOCUMENTS**

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**19.1 ETS-8-96.0, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry"**

## **20.0 REVISIONS**

---

| <u>Revision<br/>Number.</u> | <u>Reason For Revision</u> | <u>Revision<br/>Date</u> |
|-----------------------------|----------------------------|--------------------------|
|-----------------------------|----------------------------|--------------------------|

## Laboratory Study #

Study:

Test Material:

Matrix/Final Solvent:

Method/Revision:

Analytical Equipment System Number:

Instrument Software/Version:

Filename:

R-Squared Value:

Slope:

Y Intercept:

Date of Extraction/Analyst:

Date of Analysis/Analyst:

| Group<br>Dose | Sample# | Concentration<br>ug/mL | Initial Vol.<br>mL | Dilution<br>Factor | Final Conc.<br>ug/mL |
|---------------|---------|------------------------|--------------------|--------------------|----------------------|
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |
|               |         |                        |                    |                    |                      |

**Slope:** Taken from linear regression equation.

**Group/Dose:** Taken from the study folder.

**Sample#:** Taken from the study folder.

**Concentration (ug/mL):** Taken from the MassLynx integration summary.

**Initial Volume (mL):** Taken from the study folder.

**Dilution Factor:** Taken from the study folder.

**Final Conc. (ug/mL):** Calculated by dividing the initial volume from the concentration

---

**Appendix D: Method Validation Summary (TOX134)****Table 5. TOX134 Method Validation Results Summary**

| Parameters              | Results                                                                       |
|-------------------------|-------------------------------------------------------------------------------|
| Linear Range:           | ~ 5-1500 µg/mL (based on method, some run-to-run variability can be expected) |
| LOQ:                    | ~ 10 µg/mL (based on method, some run-to-run variability can be expected)     |
| Precision:              |                                                                               |
| <i>Inter-assay</i>      | <5.4%                                                                         |
| <i>Intra-assay</i>      | <21%                                                                          |
| <i>System Precision</i> | <3%                                                                           |
| Accuracy (Monkey sera): | 88-94%                                                                        |
| Reproducibility:        | <8.5%                                                                         |

## Appendix E: Data Summary Tables

**Table 6. LOQ Values Used in FACT TOX-026 Analyses by Method and Usage Dates**

| Method            | Effective Date | LOQ          | Usage Dates                                     |
|-------------------|----------------|--------------|-------------------------------------------------|
| <b>Sera</b>       |                | <b>µg/mL</b> |                                                 |
| <b>FACT-M-4.1</b> | 10/11/98       | 0.0137       | 11/16/98 to 1/27/99 (Day 9, Wk 4, Wk 12, Wk 14) |
| <b>ETS-8-5.1</b>  | 4/26/99        | 0.0137       | 4/30/99 to 6/23/99 (Wk 6–Wk 10, Wk 16–Wk 34)    |
| <b>ETS-8-5.1</b>  | 4/26/99        | 0.0137       | 7/14/99 to 7/21/99 (Wk 36–40)                   |
| <b>Urine</b>      |                | <b>µg/mL</b> |                                                 |
| <b>ETS-8-97.0</b> | 7/28/99        | 0.0182       | 8/19/99 to 9/28/99 (Wk 2–Wk 40)                 |
| <b>Liver</b>      |                | <b>µg/g</b>  |                                                 |
| <b>FACT-M-2.1</b> | 6/03/99        | 0.0755       | 6/23/99 to 10/5/99 (Wk 20, Wk 27, Wk 40)        |

**Table 7. Recovery Summaries of Fortified Samples in FACT TOX-026**

| <b>Serum</b>              |                   |
|---------------------------|-------------------|
| <b>Sample Name</b>        | <b>% Recovery</b> |
| MKS05149-MS-3             | 84                |
| MKS05149-MSD-3            | 91                |
| I05709M-MS                | 79                |
| I05714M-MS                | 108               |
| MKS06119-MS-1             | 106               |
| MKS06119-MSD-1            | 104               |
| MKS07139-MS-1             | 92                |
| MKS07139-MSD-1            | 84                |
| <b>Average</b>            | 94                |
| <b>Standard Deviation</b> | 11                |

**Table 8. Recovery Summaries of Fortified Samples in FACT TOX-026**

| <b>Urine</b>              |                   |
|---------------------------|-------------------|
| <b>Sample Name</b>        | <b>% Recovery</b> |
| MKU08049 MS-1             | 133               |
| MKU08049 MSD-1            | 92                |
| MKU08049 MS-2             | 95                |
| MKU08049 MSD-2            | 70                |
| MKU08059 MS               | 91                |
| MKU08059 MSD              | 87                |
| MKU08179 MS 1-1           | 92                |
| MKU08179 MSD 1-1          | 104               |
| MKU08179 MS 1-2           | 105               |
| MKU08179 MSD 1-2          | 101               |
| MKU08179 MS 1-3           | 74                |
| MKU08179 MSD 1-3          | 74                |
| MKU08179 MS 1-4           | 73                |
| MKU08179 MSD 1-4          | 73                |
| MKU08209 MS 1-1           | 66                |
| MKU08209 MSD 1-1          | 63                |
| MKU08209 MS 1-2           | 90                |
| MKU08209 MSD 1-2          | 89                |
| MKU09219 MS-1             | 92                |
| MKU09219 MSD-1            | 95                |
| <b>Average</b>            | 88                |
| <b>Standard Deviation</b> | 17                |

**Table 9. Recovery Summaries of Fortified Samples in FACT TOX-026**

| <b>Liver</b>              |                       |
|---------------------------|-----------------------|
| <b>Sample Name</b>        | <b>%<br/>Recovery</b> |
| I05709M-MS-3              | 74                    |
| I05709-MSD-3              | 62                    |
| I05718M-MS-11             | 113                   |
| I05718M-MSD-12            | 111                   |
| <b>Average</b>            | 90                    |
| <b>Standard Deviation</b> | 26                    |

**Table 10. TOX 026 Data Summary of POAA Concentration—Serum (µg/mL)**

|                  | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|------------------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| <b>Timepoint</b> |                                                               |                                                             |                                                              |                                                              |
| Day 9            | 0.0613 ± 0.0472<br>(n=6)                                      | 126 ± 36.1<br>(n=5)                                         | 189 ± 48.9<br>(n=6)                                          | 1597 ± 2392<br>(n=6)                                         |
| Week 4           | 0.206 ± 0.105<br>(n=6)                                        | 98.4 ± 42.7 <sup>a</sup><br>(n=3)                           | 172 ± 71.9<br>(n=5)                                          | 1084 ± 1839<br>(n=6)                                         |
| Week 6           | 0.103 ± 0.0113<br>(n=6)                                       | 102 ± 33.6<br>(n=4)                                         | 95.8 ± 20.6<br>(n=6)                                         | 145 ± 21.6<br>(n=5)                                          |
| Week 8           | <LOQ<br>(n=6)                                                 | 94.7 ± 27.3<br>(n=4)                                        | 97.6 ± 23.5<br>(n=6)                                         | 166 ± 98.9<br>(n=5)                                          |
| Week 10          | 0.126 ± 0.0348<br>(n=6)                                       | 105 ± 37.7<br>(n=4)                                         | 93.6 ± 13.7<br>(n=6)                                         | 237 ± 158<br>(n=5)                                           |
| Week 12          | 0.123 ± 0.0507<br>(n=6)                                       | 79.6 ± 25.9<br>(n=4)                                        | 90.6 ± 24.5<br>(n=6)                                         | 140 ± 77.5<br>(n=5)                                          |
| Week 14          | 0.162 ± 0.0643<br>(n=6)                                       | 90.0 ± 28.9<br>(n=4)                                        | 92.2 ± 27.6<br>(n=6)                                         | 79.4 ± 28.3<br>(n=5)                                         |
| Week 16          | 0.128 ± 0.0721<br>(n=6)                                       | 68.6 ± 26.0<br>(n=4)                                        | 98.5 ± 42.3<br>(n=6)                                         | 83.9 ± 58.5<br>(n=5)                                         |
| Week 18          | 0.183 ± 0.0637<br>(n=6)                                       | 29.4 ± 25.4<br>(n=4)                                        | 17.4 ± 14.3<br>(n=6)                                         | 36.2 ± 21.2<br>(n=5)                                         |
| Week 20          | 0.224 ± 0.0730<br>(n=6)                                       | 33.0 ± 26.0<br>(n=4)                                        | 96.6 ± 26.6<br>(n=6)                                         | 97.7 ± 129<br>(n=5)                                          |
| Week 22          | 0.232 ± 0.131<br>(n=6)                                        | 77.6 ± 24.9<br>(n=3)                                        | 105 ± 36.3<br>(n=6)                                          | 58.6 ± 45.6<br>(n=5)                                         |
| Week 24          | <LOQ<br>(n=4)                                                 | 72.2 ± 68.8<br>(n=3)                                        | 90.9 ± 21.2<br>(n=6)                                         | 62.7 ± 74.3<br>(n=5)                                         |
| Week 26          | 0.209 ± 0.156<br>(n=6)                                        | 118 ± 27.5<br>(n=3)                                         | 77.4 ± 16.9<br>(n=4)                                         | 77.8 ± 126<br>(n=5)                                          |
| Week 26/27       | 0.223 ± 0.105<br>(n=6)                                        | 52.5 ± 9.14<br>(n=5)                                        | 74.1 ± 33.1<br>(n=6)                                         | 51.5 ± 77.6<br>(n=6)                                         |
| Week 28          | 0.181 ± 0.0391<br>(n=2)                                       | NS                                                          | 25.9 ± 7.07<br>(n=2)                                         | NS                                                           |
| Week 30          | 0.144 ± 0.0238<br>(n=2)                                       | NS                                                          | 11.3 ± 5.11<br>(n=2)                                         | NS                                                           |
| Week 32          | 0.110 ± 0.0216<br>(n=2)                                       | NS                                                          | 7.60 ± 3.90<br>(n=2)                                         | NS                                                           |
| Week 34          | 0.0861 ± 0.0256<br>(n=2)                                      | NS                                                          | 3.97 ± 2.09<br>(n=2)                                         | NS                                                           |
| Week 36          | 0.111 ± 0.0445<br>(n=2)                                       | NS                                                          | 2.75 ± 1.88<br>(n=2)                                         | NS                                                           |

**Table 10. TOX 026 Data Summary of POAA Concentration—Serum (µg/mL)**

|         | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|---------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| Week 38 | 0.0941 ± 0.0324<br>(n=2)                                      | NS                                                          | 1.84 ± 1.40<br>(n=2)                                         | NS                                                           |
| Week 40 | 0.0738 ± 0.00256<br>(n=2)                                     | NS                                                          | 1.18 ± 0.827<br>(n=2)                                        | NS                                                           |

NS—No sample

&lt;LOQ—Below limit of quantitation

<sup>a</sup>Group 2, Week 4, Animal I05706M result was an anomaly and not included in the mean value. See individual results in Table 14.

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 94% with a standard deviation of 11%.

**Table 11. TOX 026 Data Summary of POAA Concentration—Urine (µg/mL)**

|                  | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|------------------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| <b>Timepoint</b> |                                                               |                                                             |                                                              |                                                              |
| Week 2           | <LOQ<br>(n=6)                                                 | 73.5 ± 38.1<br>(n=4)                                        | 221 ± 124<br>(n=6)                                           | 909 ± 269<br>(n=6)                                           |
| Week 4           | 0.152 ± 0.337<br>(n=6)                                        | 54.9 ± 4.62<br>(n=4)                                        | 190 ± 91.6<br>(n=6)                                          | 240 ± 161<br>(n=6)                                           |
| Week 6           | 0.0587 ± 0.0716<br>(n=6)                                      | 65.7 ± 46.9<br>(n=4)                                        | 128 ± 50.0<br>(n=6)                                          | 272 ± 140<br>(n=5)                                           |
| Week 8           | 0.0161 ± 0.00940<br>(n=6)                                     | 47.6 ± 20.6<br>(n=4)                                        | 206 ± 73.1<br>(n=6)                                          | 180 ± 109<br>(n=5)                                           |
| Week 10          | 0.0177 ± 0.0114<br>(n=6)                                      | 39.9 ± 18.7<br>(n=4)                                        | 175 ± 92.3<br>(n=6)                                          | 359 ± 449<br>(n=5)                                           |
| Week 12          | 0.0141 ± 0.00648<br>(n=6)                                     | 48.4 ± 18.8<br>(n=4)                                        | 201 ± 92.7<br>(n=6)                                          | 118 ± 111<br>(n=5)                                           |
| Week 14          | 7.96 ± 19.5<br>(n=6)                                          | 63.1 ± 47.2<br>(n=4)                                        | 139 ± 52.2<br>(n=6)                                          | 72.9 ± 84.0<br>(n=5)                                         |
| Week 16          | 0.0299 ± 0.0339<br>(n=6)                                      | 50.2 ± 21.0<br>(n=4)                                        | 139 ± 57.0<br>(n=6)                                          | 50.2 ± 67.9<br>(n=5)                                         |
| Week 18          | 0.0256 ± 0.0248<br>(n=6)                                      | 37.7 ± 19.3<br>(n=4)                                        | 186 ± 63.9<br>(n=6)                                          | 43.1 ± 84.6<br>(n=5)                                         |
| Week 20          | 0.0211 ± 0.0130<br>(n=6)                                      | 52.1 ± 9.63<br>(n=4)                                        | 144 ± 135<br>(n=6)                                           | 44.0 ± 59.9<br>(n=5)                                         |
| Week 22          | 0.0231 ± 0.00688<br>(n=6)                                     | 95.8 ± 80.8<br>(n=3)                                        | 158 ± 78.4<br>(n=6)                                          | 98.5 ± 134<br>(n=5)                                          |
| Week 24          | 0.0125 ± 0.00749<br>(n=6)                                     | 46.3 ± 8.52<br>(n=3)                                        | 157 ± 63.3<br>(n=6)                                          | 56.0 ± 77.2<br>(n=5)                                         |
| Week 26          | 0.0268 ± 0.0265<br>(n=6)                                      | 51.6 ± 13.7<br>(n=3)                                        | 109 ± 75.2<br>(n=6)                                          | 19.2 ± 27.0<br>(n=5)                                         |
| Week 28          | 0.118 ± 0.142<br>(n=2)                                        | NS                                                          | 0.327 ± 0.0182<br>(n=2)                                      | NS                                                           |
| Week 30          | <LOQ<br>(n=2)                                                 | NS                                                          | 0.361 ± 0.118<br>(n=2)                                       | NS                                                           |
| Week 32          | <LOQ<br>(n=2)                                                 | NS                                                          | 0.114 ± 0.0608<br>(n=2)                                      | NS                                                           |
| Week 34          | <LOQ<br>(n=2)                                                 | NS                                                          | 0.121 ± 0.0305<br>(n=2)                                      | NS                                                           |
| Week 36          | 0.0117 ± 0.00841<br>(n=2)                                     | NS                                                          | 0.0502 ± 0.0166<br>(n=2)                                     | NS                                                           |
| Week 38          | <LOQ<br>(n=2)                                                 | NS                                                          | 0.0284 ± 0.0102<br>(n=2)                                     | NS                                                           |

**Table 11. TOX 026 Data Summary of POAA Concentration—Urine (µg/mL)**

|         | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|---------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| Week 40 | <LOQ<br>(n=2)                                                 | NS                                                          | 0.0254 ± 0.0101<br>(n=2)                                     | NS                                                           |

&lt;LOQ—Below limit of quantitation

NS—No sample

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the urine data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 88% with a standard deviation of 17%.

**Table 12. TOX 026 Data Summary of POAA Concentration—Liver (µg/g)**

|                  | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|------------------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| <b>Timepoint</b> |                                                               |                                                             |                                                              |                                                              |
| Week 20          | NS                                                            | 18.3<br>(n=1)                                               | NS                                                           | NS                                                           |
| Week 27          | 0.117 ± 0.0730<br>(n=4)                                       | 15.3 ± 3.02<br>(n=4)                                        | 14.0 ± 7.55<br>(n=4)                                         | 42.8 ± 63.3<br>(n=6)                                         |
| Week 40          | <LOQ<br>(n=2)                                                 | NS                                                          | 0.114 ± 0.0441<br>(n=2)                                      | NS                                                           |

NS—No sample

&lt;LOQ—Below limit of quantitation

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the liver data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 90% with a standard deviation of 26%.

**Table 13. TOX 026 Data Summary of POAA Concentration from Centre Analytical Laboratory—Feces (µg/g)**

|                  | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|------------------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| <b>Timepoint</b> |                                                               |                                                             |                                                              |                                                              |
| Week 2           | <LOQ<br>(n=6)                                                 | 7.43 ± 6.54<br>(n=4)                                        | 15.4 ± 10.2<br>(n=6)                                         | 56.6 ± 73.7<br>(n=6)                                         |
| Week 4           | 0.0214 ± 0.0178<br>(n=6)                                      | 10.4 ± 12.0<br>(n=4)                                        | 23.4 ± 10.6<br>(n=6)                                         | 22.0 ± 6.23<br>(n=6)                                         |
| Week 6           | 0.0108 ± 0.00192<br>(n=6)                                     | 12.1 ± 14.1<br>(n=4)                                        | 23.3 ± 8.46<br>(n=6)                                         | 101 ± 86.7<br>(n=5)                                          |
| Week 8           | 0.0782 ± 0.103<br>(n=6)                                       | 9.46 ± 9.21<br>(n=4)                                        | 41.0 ± 25.0<br>(n=6)                                         | 36.7 ± 34.2<br>(n=5)                                         |
| Week 10          | <LOQ<br>(n=6)                                                 | 3.96 ± 3.68<br>(n=4)                                        | 26.0 ± 17.4<br>(n=6)                                         | 48.0 ± 34.0<br>(n=5)                                         |
| Week 12          | 0.0498 ± 0.0894<br>(n=6)                                      | 7.15 ± 5.65<br>(n=4)                                        | 10.3 ± 6.07<br>(n=6)                                         | 32.0 ± 42.9<br>(n=5)                                         |
| Week 14          | 0.139 ± 0.308<br>(n=6)                                        | 7.50 ± 2.43<br>(n=4)                                        | 27.2 ± 29.4<br>(n=6)                                         | 19.9 ± 25.2<br>(n=5)                                         |
| Week 16          | 0.0572 ± 0.0762<br>(n=6)                                      | 6.88 ± 2.62<br>(n=4)                                        | 31.4 ± 23.3<br>(n=6)                                         | 18.2 ± 28.8<br>(n=5)                                         |
| Week 18          | 0.258 ± 0.604<br>(n=6)                                        | 5.72 ± 7.15<br>(n=4)                                        | 17.3 ± 13.3<br>(n=6)                                         | 22.1 ± 31.7<br>(n=5)                                         |
| Week 20          | 0.450 ± 1.08<br>(n=6)                                         | 6.81 ± 4.89<br>(n=4)                                        | 52.4 ± 39.5<br>(n=6)                                         | 37.8 ± 58.1<br>(n=5)                                         |

**Table 13. TOX 026 Data Summary of POAA Concentration from Centre Analytical Laboratory—Feces (µg/g)**

|            | <b>Group 1</b><br><b>0.0 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 2</b><br><b>3 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 3</b><br><b>10 mg/kg/day</b><br><b>Average ± SD</b> | <b>Group 4</b><br><b>30 mg/kg/day</b><br><b>Average ± SD</b> |
|------------|---------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------|--------------------------------------------------------------|
| Week 22    | 15.5 ± 36.9<br>(n=6)                                          | 13.8 ± 5.22<br>(n=3)                                        | 39.5 ± 21.0<br>(n=6)                                         | 25.2 ± 36.0<br>(n=5)                                         |
| Week 24    | 0.517 ± 1.13<br>(n=6)                                         | 6.22 ± 5.45<br>(n=3)                                        | 40.5 ± 21.8<br>(n=6)                                         | 34.6 ± 47.7<br>(n=5)                                         |
| Week 26    | 0.0172 ± 0.00892<br>(n=6)                                     | 2.92 ± 1.35<br>(n=3)                                        | 43.0 ± 36.9<br>(n=6)                                         | 10.3 ± 20.8<br>(n=5)                                         |
| Week 28–34 | 0.279 ± 0.732<br>(n=8)                                        | NS                                                          | 0.387 ± 0.372<br>(n=8)                                       | NS                                                           |
| Week 36–40 | 0.0103 ± 0.00684<br>(n=6)                                     | NS                                                          | 0.0336 ± 0.0313<br>(n=6)                                     | NS                                                           |

NS—No sample

&lt;LOQ—Below limit of quantitation

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the feces data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 117% with a standard deviation of 22%.

**Table 14. FACT TOX-026 Individual POAA Results per Sample—Serum (µg/mL)**

|                                                                        | Animal ID | Day 9  | Week 4            | Week 6 | Week 8 | Week 10 | Week 12 | Week 14 | Week 16 | Week 18 | Week 20 | Week 22 | Week 24 |
|------------------------------------------------------------------------|-----------|--------|-------------------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|
| <b>Group 1</b><br>0.0 mg/kg/day<br>Control                             | I05709M   | 0.0645 | 0.299             | <LOQ   | <LOQ   | 0.191   | 0.207   | 0.275   | 0.275   | 0.263   | 0.342   | 0.393   | E       |
|                                                                        | I05714M   | 0.137  | 0.230             | 0.126  | <LOQ   | 0.105   | 0.0994  | 0.156   | <LOQ    | 0.244   | 0.212   | 0.157   | E       |
|                                                                        | I05715M   | <LOQ   | 0.0785            | <LOQ   | <LOQ   | 0.0919  | 0.064   | 0.0981  | <LOQ    | 0.0950  | 0.115   | 0.106   | <LOQ    |
|                                                                        | I05718M   | 0.0889 | 0.347             | <LOQ   | <LOQ   | 0.129   | 0.139   | 0.153   | <LOQ    | 0.165   | 0.224   | 0.269   | <LOQ    |
|                                                                        | I05720M   | <LOQ   | 0.167             | <LOQ   | <LOQ   | 0.128   | 0.140   | 0.182   | <LOQ    | 0.193   | 0.242   | 0.372   | <LOQ    |
|                                                                        | I05725M   | 0.0499 | 0.114             | <LOQ   | <LOQ   | 0.110   | 0.0869  | 0.105   | <LOQ    | 0.138   | 0.207   | <LOQ    | <LOQ    |
| <b>Group 2</b><br>3.0 mg/kg/day<br>Low Dose<br><br>I05721M replacement | I05702M   | 91.6   | 96.8              | 67.5   | 76.4   | 75.9    | 64.0    | 72.7    | 55.3    | 11.1    | 17.8    | 69.8    | 10.2    |
|                                                                        | I05706M   | 177    | 3611 <sup>a</sup> | 115    | 101    | 114     | 85.3    | 112     | 72.6    | 16.0    | 11.4    | 105     | 146     |
|                                                                        | I05717M   | 116    | 142               | 83.7   | 70.5   | 74.8    | 55.5    | 58.4    | 43.4    | 66.8    | 33.3    | 57.5    | 60.2    |
|                                                                        | I05721M   |        | 56.5              | 143    | 130    | 154     | 114     | 117     | 103     | 23.8    | 69.6    |         |         |
|                                                                        | I05723M   | 119    |                   |        |        |         |         |         |         |         |         |         |         |
| <b>Group 3</b><br>10 mg/kg/day<br>Mid-Dose                             | I05707M   | 230    | 197               | 84.4   | 97.2   | 97.4    | 94.0    | 68.0    | 99.5    | 13.4    | 107     | 122     | 89.1    |
|                                                                        | I05708M   | 103    | 84.6              | 78.8   | 67.8   | 72.8    | 56.9    | 103     | 60.2    | 9.67    | 61.9    | 63.7    | 61.4    |
|                                                                        | I05710M   | 173    | 268               | 103    | 88.5   | 87.9    | 79.0    | 61.3    | 73.2    | 46.4    | 75.2    | 87.7    | 81.8    |
|                                                                        | I05712M   | 178    | E                 | 87.9   | 82.3   | 103     | 131     | 79.2    | 180     | 13.7    | 137     | 168     | 116     |
|                                                                        | I05716M   | 228    | 119               | 86.2   | 102    | 102     | 84.4    | 134     | 91.3    | 10.1    | 91.4    | 106     | 82.3    |
|                                                                        | I05719M   | 220    | 194               | 135    | 133    | 108     | 98.4    | 108     | 86.5    | 11.3    | 107     | 85.2    | 115     |
| <b>Group 4</b><br>30 mg/kg/day<br>High Dose                            | I05703M   | 6338   | 286               | 162    | 175    | 313     | 235     | 84.4    | 68.2    | 68.8    | 38.9    | 16.4    | 20.4    |
|                                                                        | I05704M   | 313    | 251               | 111    | 117    | 128     | 103     | 109     | 113     | 20.0    | 92.5    | 92.7    | 90.4    |
|                                                                        | I05711M   | 1676   | 4802              | 161    | 102    | 67.4    | 32.2    | 68.2    | 13.3    | 19.0    | 2.39    | 1.83    | 4.29    |
|                                                                        | I05713M   | 229    | 66.7              | 154    | 100    | 208     | 185     | 36.9    | 167     | 27.3    | 322     | 96.3    | 181     |
|                                                                        | I05722M   | 151*   | 273               | 137    | 334    | 467     | 143     | 98.8    | 57.7    | 45.9    | 32.9    | 86.0    | 17.0    |
|                                                                        | I05724M   | 876    | 822               |        |        |         |         |         |         |         |         |         |         |

E—No extract remaining

\*Sample evaporated to dryness, tentative value

Shaded areas—No sample

<sup>a</sup>Animal I05706M, Group 2, Week 4 result is an anomaly.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 94% with a standard deviation of 11%.

**Table 14 (continued). FACT TOX-026 Individual POAA Results per Sample—Serum (µg/mL)**

|                                                                        | Animal ID | Week 26 | Week 26&27 | Week 28 | Week 30 | Week 32 | Week 34 | Week 36 | Week 38 | Week 40 |
|------------------------------------------------------------------------|-----------|---------|------------|---------|---------|---------|---------|---------|---------|---------|
| <b>Group 1</b><br>0.0 mg/kg/day<br>Control                             | I05709M   | 0.471   | 0.377      |         |         |         |         |         |         |         |
|                                                                        | I05714M   | 0.108   | 0.219      |         |         |         |         |         |         |         |
|                                                                        | I05715M   | 0.101   | 0.0945     |         |         |         |         |         |         |         |
|                                                                        | I05718M   | 0.206   | 0.206      | 0.153   | 0.127   | 0.0943  | 0.0680  | 0.0795  | 0.0711  | 0.0719  |
|                                                                        | I05720M   | 0.308   | 0.308      | 0.209   | 0.161   | 0.125   | 0.104   | 0.142   | 0.117   | 0.0756  |
|                                                                        | I05725M   | 0.0623  | 0.136      |         |         |         |         |         |         |         |
| <b>Group 2</b><br>3.0 mg/kg/day<br>Low Dose<br><br>I05721M replacement | I05702M   | 125     | 47.9       |         |         |         |         |         |         |         |
|                                                                        | I05706M   | 141     | 64.5       |         |         |         |         |         |         |         |
|                                                                        | I05717M   | 87.6    | 44.1       |         |         |         |         |         |         |         |
|                                                                        | I05721M   |         | 46.0       |         |         |         |         |         |         |         |
|                                                                        | I05723M   |         | 60.0       |         |         |         |         |         |         |         |
| <b>Group 3</b><br>10 mg/kg/day<br>Mid-Dose                             | I05707M   | 77.9    | 121        |         |         |         |         |         |         |         |
|                                                                        | I05708M   | 55.4    | 32.1       |         |         |         |         |         |         |         |
|                                                                        | I05710M   | 96.5    | 47.6       |         |         |         |         |         |         |         |
|                                                                        | I05712M   |         | 101        | 30.9    | 14.9    | 10.4    | 5.45    | 4.08    | 2.83    | 1.77    |
|                                                                        | I05716M   |         | 78.1       | 21.0    | 7.70    | 4.85    | 2.49    | 1.42    | 0.842   | 0.600   |
|                                                                        | I05719M   | 79.6    | 65.2       |         |         |         |         |         |         |         |
| <b>Group 4</b><br>30 mg/kg/day<br>High Dose                            | I05703M   | 11.1    | 7.48       |         |         |         |         |         |         |         |
|                                                                        | I05704M   | 61.7    | 58.6       |         |         |         |         |         |         |         |
|                                                                        | I05711M   | 1.50    | 0.877      |         |         |         |         |         |         |         |
|                                                                        | I05713M   | 299     | 184        |         |         |         |         |         |         |         |
|                                                                        | I05722M   | 15.8    | 6.65       |         |         |         |         |         |         |         |
|                                                                        | I05724M   |         | 489        |         |         |         |         |         |         |         |

Shaded areas—No sample

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 94% with a standard deviation of 11%.

**Table 15. FACT TOX-026 Individual POAA Results per Sample—Urine (µg/mL)**

|                                                 | Animal ID | Week 2 | Week 4 | Week 6 | Week 8 | Week 10 | Week 12 | Week 14 | Week 16 | Week 18 | Week 20 | Week 22 | Week 24 | Week 26 |
|-------------------------------------------------|-----------|--------|--------|--------|--------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| <b>Group 1</b><br>0.0 mg/kg/day<br>Control      | I05709M   | <LOQ   | <LOQ   | 0.172  | <LOQ   | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | 0.0344  | <LOQ    | 0.0208  |
|                                                 | I05714M   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | 0.0287  | <LOQ    | <LOQ    |
|                                                 | I05715M   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    |
|                                                 | I05718M   | <LOQ   | 0.0270 | 0.0254 | 0.0307 | 0.0225  | 0.0181  | 0.0224  | 0.0952  | <LOQ    | 0.0213  | <LOQ    | <LOQ    | <LOQ    |
|                                                 | I05720M   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ    | 0.0184  | 47.7    | 0.0366  | 0.0752  | 0.0451  | 0.0208  | 0.0213  | 0.0797  |
|                                                 | I05725M   | <LOQ   | 0.839  | 0.126  | <LOQ   | 0.0359  | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    |
| <b>Group 2</b><br>3.0 mg/kg/day<br>Mid Dose     | I05702M   | 54.7   | 55.3   | 132    | 49.2   | 33.3    | 65.3    | 58.7    | 37.4    | 36.7    | 48.9    | 44.7    | 36.8    | 37.0    |
|                                                 | I05706M   | 60.1   | 55.5   | 55.6   | 75.4   | 63.7    | 62.8    | 131     | 62.4    | 58.3    | 66.1    | 53.7    | 53.3    | 53.6    |
|                                                 | I05717M   | 48.9   | 60.0   | 53.2   | 38.3   | 43.6    | 38.8    | 40.4    | 73.1    | 43.7    | 44.3    | 189     | 48.7    | 64.2    |
|                                                 | I05721M   | 130    | 48.7   | 21.8   | 27.5   | 19.2    | 26.6    | 22.9    | 28.1    | 12.0    | 48.9    |         |         |         |
| <b>Group 3</b><br>10 mg/kg/day<br>Mid-high Dose | I05707M   | 412    | 195    | 157    | 230    | 272     | 170     | 127     | 165     | 157     | 1648*   | 58.0    | 184     | 256     |
|                                                 | I05708M   | 199    | 271    | 195    | 221    | 252     | 144     | 171     | 187     | 228     | 362     | 168     | 216     | 66.6    |
|                                                 | I05710M   | 123    | 264    | 150    | 298    | 233     | 365     | 185     | 162     | 194     | 177     | 235     | 212     | 50.8    |
|                                                 | I05712M   | 60.9   | 35.8   | 54.4   | 75.2   | 45.1    | 259     | 50.3    | 42.7    | 75.6    | 59.3    | 86.9    | 54.0    | 72.3    |
|                                                 | I05716M   | 242    | 133    | 117    | 192    | 94.3    | 142     | 115     | 98.1    | 204     | 104     | 144     | 112     | 103     |
|                                                 | I05719M   | 288    | 242    | 94.3   | 220    | 154     | 130     | 183     | 181     | 258     | 18.0    | 255     | 163     | 107     |
| <b>Group 4</b><br>30 mg/kg/day<br>High Dose     | I05703M   | 525    | 383    | 354    | 167    | 1131    | 175     | 0.181   | 0.179   | <LOQ    | 0.363   | 0.352   | 0.306   | 0.223   |
|                                                 | I05704M   | 811    | 47.6   | 193    | 282    | 328     | 169     | 156     | 136     | 170     | 124     | 255     | 155     | 57.7    |
|                                                 | I05711M   | 941    | 186    | 427    | 3.62   | 1.41    | 0.725   | <LOQ    | 0.380   | 0.201   | 0.134   | 0.334   | 0.107   | 0.0682  |
|                                                 | I05713M   | 846    | 444    | 72.3   | 257    | 109     | 243     | 134     | 112     | 0.406   | 93.3    | 237     | 124     | 37.8    |
|                                                 | I05722M   | 983    | 295    | 314    | 193    | 225     | 1.35    | 1.11    | 2.34    | 1.67    | 2.33    | 0.868   | 0.248   | 0.215   |
|                                                 | I05724M   | 1350   | 83.0   |        |        |         |         |         |         |         |         |         |         |         |

&lt;LOQ—Below limit of quantitation

Shaded area—No sample

\*This sample was not diluted 1/5000; a 2µL injection of D1000 was analyzed instead. All other injections were 10µL. This provides a 1/5 dilution of D1000 for a total of D5000. A 1/4000 dilution of I05707M was also analyzed, but was just out of calibration range.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the urine data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 88% with a standard deviation of 17%.

Table 15 (continued). FACT TOX-026 Individual POAA Results per Sample—Urine ( $\mu\text{g/mL}$ )

|                                                 | Animal ID | Week 28 | Week 30 | Week 32 | Week 34 | Week 36 | Week 38 | Week 40 |
|-------------------------------------------------|-----------|---------|---------|---------|---------|---------|---------|---------|
| <b>Group 1</b><br>0.0 mg/kg/day<br>Control      | I05709M   |         |         |         |         |         |         |         |
|                                                 | I05714M   |         |         |         |         |         |         |         |
|                                                 | I05715M   |         |         |         |         |         |         |         |
|                                                 | I05718M   | 0.218   | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    | <LOQ    |
|                                                 | I05720M   | <LOQ    | <LOQ    | <LOQ    | <LOQ    | 0.0176  | <LOQ    | <LOQ    |
|                                                 | I05725M   |         |         |         |         |         |         |         |
| <b>Group 2</b><br>3.0 mg/kg/day<br>Mid Dose     | I05702M   |         |         |         |         |         |         |         |
|                                                 | I05706M   |         |         |         |         |         |         |         |
|                                                 | I05717M   |         |         |         |         |         |         |         |
|                                                 | I05721M   |         |         |         |         |         |         |         |
| <b>Group 3</b><br>10 mg/kg/day<br>Mid-high Dose | I05707M   |         |         |         |         |         |         |         |
|                                                 | I05708M   |         |         |         |         |         |         |         |
|                                                 | I05710M   |         |         |         |         |         |         |         |
|                                                 | I05712M   | 0.314   | 0.278   | 0.157   | 0.0993  | 0.0384  | 0.0357  | 0.0325  |
|                                                 | I05716M   | 0.340   | 0.445   | 0.0712  | 0.142   | 0.0619  | 0.0212  | <LOQ    |
|                                                 | I05719M   |         |         |         |         |         |         |         |
| <b>Group 4</b><br>30 mg/kg/day<br>High Dose     | I05703M   |         |         |         |         |         |         |         |
|                                                 | I05704M   |         |         |         |         |         |         |         |
|                                                 | I05711M   |         |         |         |         |         |         |         |
|                                                 | I05713M   |         |         |         |         |         |         |         |
|                                                 | I05722M   |         |         |         |         |         |         |         |
|                                                 | I05724M   |         |         |         |         |         |         |         |

&lt;LOQ—Below limit of quantitation

Shaded area—No sample

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the urine data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 88% with a standard deviation of 17%.

| Table 16. FACT TOX-026 Individual POAA Results per Sample—Liver (µg/g) |           |         |         |         |
|------------------------------------------------------------------------|-----------|---------|---------|---------|
|                                                                        | Animal ID | Week 20 | Week 27 | Week 40 |
| <b>Group 1</b><br>0.0 mg/kg/day<br>Control                             | I05709M   |         | 0.0913  |         |
|                                                                        | I05714M   |         | <LOQ    |         |
|                                                                        | I05715M   |         | 0.226   |         |
|                                                                        | I05725M   |         | <LOQ    |         |
|                                                                        | I05718M   |         |         | <LOQ    |
|                                                                        | I05720M   |         |         | <LOQ    |
| <b>Group 2</b><br>3.0 mg/kg/day<br>Mid Dose                            | I05702M   |         | 15.2    |         |
|                                                                        | I05706M   |         | 18.5    |         |
|                                                                        | I05717M   |         | 11.3    |         |
|                                                                        | I05723M   |         | 16.3    |         |
|                                                                        | I05721M   | 18.3    |         |         |
| <b>Group 3</b><br>10 mg/kg/day<br>Mid-high Dose                        | I05707M   |         | 21.9    |         |
|                                                                        | I05708M   |         | 6.29    |         |
|                                                                        | I05710M   |         | 8.86    |         |
|                                                                        | I05719M   |         | 18.8    |         |
|                                                                        | I05712M   |         |         | 0.146   |
|                                                                        | I05716M   |         |         | 0.0833  |
| <b>Group 4</b><br>30 mg/kg/day<br>High Dose                            | I05703M   |         | 1.31    |         |
|                                                                        | I05704M   |         | 16.0    |         |
|                                                                        | I05711M   |         | 0.216   |         |
|                                                                        | I05713M   |         | 83.3    |         |
|                                                                        | I05722M   |         | 1.41    |         |
|                                                                        | I05724M   |         | 154     |         |

\*Shaded areas—No sample. Samples were taken at sacrifice or time of death, therefore there is only one sample per animal.

NOTE: It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the liver data are accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 90% with a standard deviation of 26%.

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## **Appendix F: Data Spreadsheets**

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
 Product Number(Test Substance): T-6889.2 (POAA)  
 Matrix: Monkey Sera  
 Method/Revision: FACT-M-3.1 & FACT-M-4.1  
 Analytical Equipment System Number: Amelia 062498  
 Instrument Software/Version: MassLynx 3.1  
 Filename: See Attachments  
 R-Squared Value: See Attachments  
 Slope: See Attachments  
 Y-Intercept: See Attachments  
 Date of Extraction/Analyst: 11/13/98 IAS  
 Date of Analysis/Analyst: 11/16/98, 11/24/98, 12/03/98 HOJ  
 Date of Data Reduction/Analyst: 11/17/98, 11/30/98, 12/07/98, 09/29/00 HOJ/KJH

**FileNames**

| <b>POAA</b>            | <b>Dilutions</b>    |
|------------------------|---------------------|
| A111698003, 4 & 79, 80 | 1/1                 |
| 111698015-20           | 1/1                 |
| 112498018, 12039845-47 | 1/75, 1/750         |
| 12039851-56            | 1/1000              |
| 111698041, 12039859-63 | 1/1, 1/5000, 1/2000 |
| 111698075-76           | 1/1                 |

**Blks**  
**Grp 1**  
**Grp 2**  
**Grp 3**  
**Grp 4**  
**MS, MSD**

**Sample Data**

**Day 9 MONKEY SERA**

| Group Dose                                      | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-1         | NA                    | 0.9582                     | 1                    | <LOQ             | <LOQ                                  | <LOQ            | NA                       |
|                                                 | H2O Blk-2         | NA                    | 0.9582                     | 1                    | <LOQ             | <LOQ                                  |                 |                          |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                  | 0.9582                     | 1                    | <LOQ             | <LOQ                                  | <LOQ            | NA                       |
|                                                 | Rabbit Sera Blk-2 | 1.00                  | 0.9582                     | 1                    | 14.4             | 0.0138                                |                 |                          |
| QC - 500 ppb                                    | RBS11138-MS       | 1.00                  | NA                         | 1                    | 530              | 109% **                               | 104%            | 8%                       |
|                                                 | RBS11138-MSD      | 1.00                  | NA                         | 1                    | 489              | 100% **                               |                 |                          |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.50                  | 0.9582                     | 1                    | 33.7             | 0.0645                                | 0.0613          | 77.1<br>0.0472           |
|                                                 | I05714M           | 0.50                  | 0.9582                     | 1                    | 71.4             | 0.137                                 |                 |                          |
|                                                 | I05715M           | 0.60                  | 0.9582                     | 1                    | 4.68             | <LOQ                                  |                 |                          |
|                                                 | I05718M           | 0.50                  | 0.9582                     | 1                    | 46.4             | 0.0889                                |                 |                          |
|                                                 | I05720M           | 0.30                  | 0.9582                     | 1                    | 8.23             | <LOQ                                  |                 |                          |
|                                                 | I05725M           | 0.50                  | 0.9582                     | 1                    | 26.0             | 0.0499                                |                 |                          |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.70                  | 0.9582                     | 750                  | 89.2             | 91.6                                  | 126             | 28.7<br>36.1             |
|                                                 | I05706M           | 0.50                  | 0.9582                     | 750                  | 123              | 177                                   |                 |                          |
|                                                 | I05717M           | 0.60                  | 0.9582                     | 750                  | 96.8             | 116                                   |                 |                          |
|                                                 | I05721M           | -                     | 0.9582                     | NR                   | NR               | NR                                    |                 |                          |
|                                                 | I05723M           | 0.30                  | 0.9582                     | 75                   | 497              | 119                                   |                 |                          |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.50                  | 0.9582                     | 1000                 | 120              | 230                                   | 189             | 25.9<br>48.9             |
|                                                 | I05708M           | 0.60                  | 0.9582                     | 1000                 | 64.5             | 103                                   |                 |                          |
|                                                 | I05710M           | 0.50                  | 0.9582                     | 1000                 | 90.1             | 173                                   |                 |                          |
|                                                 | I05712M           | 0.60                  | 0.9582                     | 1000                 | 112              | 178                                   |                 |                          |
|                                                 | I05716M           | 0.50                  | 0.9582                     | 1000                 | 119              | 228                                   |                 |                          |
|                                                 | I05719M           | 0.50                  | 0.9582                     | 1000                 | 115              | 220                                   |                 |                          |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.40                  | 0.9582                     | 5000                 | 529              | 6338                                  | 1597            | 150<br>2392              |
|                                                 | I05704M           | 0.50                  | 0.9582                     | 2000                 | 81.8             | 313                                   |                 |                          |
|                                                 | I05711M           | 0.40                  | 0.9582                     | 2000                 | 350              | 1676                                  |                 |                          |
|                                                 | I05713M           | 0.60                  | 0.9582                     | 2000                 | 71.8             | 229                                   |                 |                          |
|                                                 | I05722M           | 0.30                  | 0.9582                     | 1                    | 47257            | 151 *                                 |                 |                          |
|                                                 | I05724M           | 0.40                  | 0.9582                     | 2000                 | 183              | 876                                   |                 |                          |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\*Tentative value, sample evaporated to dryness.

\*\*Bracketed by a CCV not within method criteria (31%).

POAA = Perfluorooctanoate

Date Entered/By: 11/21/98, 12/07/98, 10/10/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | FACT-M-3.1 & FACT-M-4.1                                        |
| Analytical Equipment System Number: | Amelia 062498                                                  |
| Instrument Software/Version:        | MassLynx 3.1                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 11/13/98 IAS                                                   |
| Date of Analysis/Analyst:           | 11/16/98, 11/24/98, 12/03/98 HOJ                               |
| Date of Data Reduction/Analyst:     | 11/17/98, 11/30/98, 12/07/98, 09/29/00 HOJ/KJH                 |

**Sample Data**

**Day 9 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | 0.0138                                      | <LOQ                  | NA                             |
| QC - 500 ppb                                    | RBS11138-MS       | 109% **                                     |                       |                                |
|                                                 | RBS11138-MSD      | 100% **                                     | 104%                  | 8%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.0645                                      |                       |                                |
|                                                 | I05714M           | 0.137                                       |                       |                                |
|                                                 | I05715M           | <LOQ                                        |                       |                                |
|                                                 | I05718M           | 0.0889                                      |                       |                                |
|                                                 | I05720M           | <LOQ                                        |                       | 77.1                           |
|                                                 | I05725M           | 0.0499                                      | 0.0613                | 0.0472                         |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 91.6                                        |                       |                                |
|                                                 | I05706M           | 177                                         |                       |                                |
|                                                 | I05717M           | 116                                         |                       |                                |
|                                                 | I05721M           | NR                                          |                       | 28.7                           |
|                                                 | I05723M           | 119                                         | 126                   | 36.1                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 230                                         |                       |                                |
|                                                 | I05708M           | 102.9                                       |                       |                                |
|                                                 | I05710M           | 173                                         |                       |                                |
|                                                 | I05712M           | 178                                         |                       |                                |
|                                                 | I05716M           | 228                                         |                       | 25.9                           |
|                                                 | I05719M           | 220                                         | 189                   | 48.9                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 6338                                        |                       |                                |
|                                                 | I05704M           | 313                                         |                       |                                |
|                                                 | I05711M           | 1676                                        |                       |                                |
|                                                 | I05713M           | 229                                         |                       |                                |
|                                                 | I05722M           | 151 *                                       |                       | 150                            |
|                                                 | I05724M           | 876                                         | 1597                  | 2392                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\*Tentative value, sample evaporated to dryness.

\*\*Bracketed by a CCV not within method criteria (31%).

POAA = Perfluorooctanoate

Date Entered/By: 11/21/98, 12/07/98, 10/10/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: FACT-M-3.1 & FACT-M-4.1  
Analytical Equipment System Number: Amelia 062498  
Instrument Software/Version: MassLynx 3.1  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 11/13/98 IAS  
Date of Analysis/Analyst: 11/16/98, 11/24/98, 12/03/98, 01/04/99 HOJ  
Date of Data Reduction/Analyst: 11/17/98, 11/30/98, 12/07/98, 01/07/99, 09/29/00 HOJ/KJH

**FileNames**

|                |                        |                  |
|----------------|------------------------|------------------|
|                | <b>POAA</b>            | <b>Dilutions</b> |
| <b>Blks</b>    | A111698003, 4 & 79, 80 | 1/1              |
| <b>Grp 1</b>   | 111698045-50           | 1/1              |
| <b>Grp 2</b>   | 12039866-68, 010499021 | 1/250, 750, 1875 |
| <b>Grp 3</b>   | 112498041-46           | 1/150            |
| <b>Grp 4</b>   | 112498049-50,52        | 1/200            |
| <b>Grp 4</b>   | 12039881,83 010499020  | 1/60, 2000, 5000 |
| <b>MS, MSD</b> | 111698075-76           | 1/1              |

**Sample Data**

**Week 4 MONKEY SERA**

| Group Dose                                      | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-1         | NA                    | 0.9582                     | 1                    | <LOQ             | <LOQ                                  |                 |                          |
|                                                 | H2O Blk-2         | NA                    | 0.9582                     | 1                    | <LOQ             | <LOQ                                  | <LOQ            | NA                       |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                  | 0.9582                     | 1                    | <LOQ             | <LOQ                                  |                 |                          |
|                                                 | Rabbit Sera Blk-2 | 1.00                  | 0.9582                     | 1                    | 14.4             | 0.0138                                | <LOQ            | NA                       |
| QC - 500 ppb                                    | RBS11138-MS       | 1.00                  | NA                         | 1                    | 530              | 109% **                               |                 |                          |
|                                                 | RBS11138-MSD      | 1.00                  | NA                         | 1                    | 489              | 100% **                               | 104%            | 8%                       |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.50                  | 0.9582                     | 1                    | 156              | 0.299                                 |                 |                          |
|                                                 | I05714M           | 0.50                  | 0.9582                     | 1                    | 120              | 0.230                                 |                 |                          |
|                                                 | I05715M           | 0.60                  | 0.9582                     | 1                    | 49.1             | 0.0785                                |                 |                          |
|                                                 | I05718M           | 0.30                  | 0.9582                     | 1                    | 109              | 0.347                                 |                 |                          |
|                                                 | I05720M           | 0.30                  | 0.9582                     | 1                    | 52.2             | 0.167                                 |                 | 51.1                     |
|                                                 | I05725M           | 0.50                  | 0.9582                     | 1                    | 59.5             | 0.114                                 | 0.206           | 0.105                    |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.60                  | 0.9582                     | 750                  | 80.8             | 96.8                                  |                 |                          |
|                                                 | I05706M           | 0.40                  | 0.9582                     | 1875                 | 804              | 3611 *                                | 98.4            | 43.4*                    |
|                                                 | I05717M           | 0.50                  | 0.9582                     | 750                  | 98.7             | 142                                   | 98.4*           | 42.7*                    |
|                                                 | I05721M           | 0.40                  | 0.9582                     | 250                  | 23576            | 56.5                                  |                 | 180                      |
|                                                 | I05723M           | -                     | 0.9582                     | NR                   | NR               | NR                                    | 977             | 1757                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.50                  | 0.9582                     | 150                  | 684              | 197                                   |                 |                          |
|                                                 | I05708M           | 0.60                  | 0.9582                     | 150                  | 353              | 84.6                                  |                 |                          |
|                                                 | I05710M           | 0.30                  | 0.9582                     | 150                  | 558              | 268                                   |                 |                          |
|                                                 | I05712M           | 0.40                  | 0.9582                     | E                    | E                | E                                     |                 |                          |
|                                                 | I05716M           | 0.60                  | 0.9582                     | 150                  | 498              | 119                                   |                 | 41.7                     |
|                                                 | I05719M           | 0.50                  | 0.9582                     | 150                  | 676              | 194                                   | 172             | 71.9                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.40                  | 0.9582                     | 200                  | 598              | 286                                   |                 |                          |
|                                                 | I05704M           | 0.40                  | 0.9582                     | 200                  | 524              | 251                                   |                 |                          |
|                                                 | I05711M           | 0.30                  | 0.9582                     | 5000                 | 301              | 4802                                  |                 |                          |
|                                                 | I05713M           | 0.30                  | 0.9582                     | 60                   | 20893            | 66.7                                  |                 |                          |
|                                                 | I05722M           | 0.50                  | 0.9582                     | 200                  | 713              | 273                                   |                 | 170                      |
|                                                 | I05724M           | 0.50                  | 0.9582                     | 2000                 | 214              | 822                                   | 1084            | 1839                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\*Anomaly, was not included in these values.

\*\*Bracketed by a CCV not within method criteria (31%).

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

POAA = Perfluorooctanoate

Date Entered/By: 11/21/98, 12/07/98, 01/12/99, 10/10/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00 KJH, 11/09/00 OJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | FACT-M-3.1 & FACT-M-4.1                                        |
| Analytical Equipment System Number: | Amelia 062498                                                  |
| Instrument Software/Version:        | MassLynx 3.1                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 11/13/98 IAS                                                   |
| Date of Analysis/Analyst:           | 11/16/98, 11/24/98, 12/03/98, 01/04/99 HOJ                     |
| Date of Data Reduction/Analyst:     | 11/17/98, 11/30/98, 12/07/98, 01/07/99, 09/29/00 HOJ/KJH       |

**Sample Data**

**Week 4 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | 0.0138                                      | <LOQ                  | NA                             |
| QC - 500 ppb                             | RBS11138-MS       | 109% **                                     |                       |                                |
|                                          | RBS11138-MSD      | 100% **                                     | 104%                  | 8%                             |
| Group 1<br>Control<br>0.0 mg/kg/day      | I05709M           | 0.299                                       |                       |                                |
|                                          | I05714M           | 0.230                                       |                       |                                |
|                                          | I05715M           | 0.0785                                      |                       |                                |
|                                          | I05718M           | 0.347                                       |                       |                                |
|                                          | I05720M           | 0.167                                       |                       | 51.1                           |
|                                          | I05725M           | 0.114                                       | 0.206                 | 0.105                          |
| Group 2<br>Low Dose                      | I05702M           | 96.8                                        |                       |                                |
|                                          | I05706M           | 3611 *                                      |                       | 43.4*                          |
|                                          | I05717M           | 142                                         | 98.4*                 | 42.7*                          |
|                                          | I05721M           | 56.5                                        |                       | 180                            |
|                                          | I05723M           | NR                                          | 977                   | 1757                           |
| Group 3<br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 197                                         |                       |                                |
|                                          | I05708M           | 84.6                                        |                       |                                |
|                                          | I05710M           | 268                                         |                       |                                |
|                                          | I05712M           | E                                           |                       |                                |
|                                          | I05716M           | 119                                         |                       | 41.7                           |
| Group 4<br>High Dose<br>30 mg/kg/day     | I05719M           | 194                                         | 172                   | 71.9                           |
|                                          | I05703M           | 286                                         |                       |                                |
|                                          | I05704M           | 251                                         |                       |                                |
|                                          | I05711M           | 4802                                        |                       |                                |
|                                          | I05713M           | 66.7                                        |                       |                                |
|                                          | I05722M           | 273                                         |                       | 170                            |
|                                          | I05724M           | 822                                         | 1084                  | 1839                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\*Anomaly, was not included in these values.

\*\*Bracketed by a CCV not within method criteria (31%).

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

POAA = Perfluorooctanoate

Date Entered/By: 11/21/98, 12/07/98, 01/12/99, 10/10/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00 KJH, 11/09/00 OJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
 Product Number(Test Substance): T-6889.2 (POAA)  
 Matrix: Monkey Sera  
 Method/Revision: ETS-8-4.1 and ETS-8-5.1  
 Analytical Equipment System Number: Amelia 062498, Soup 020199  
 Instrument Software/Version: MassLynx 3.2  
 Filename: See Attachments  
 R-Squared Value: See Attachments  
 Slope: See Attachments  
 Y-Intercept: See Attachments  
 Date of Extraction/Analyst: 05/12/99 SAH  
 Date of Analysis/Analyst: 06/03/99, 06/07/99, 06/10/99, 06/16/99 HOJ/SAH/MEE/LAC  
 Date of Data Reduction/Analyst: 06/04/99, 06/08/99, 06/11/99, 06/17/99, 11/06/00 HOJ/LAC/KJH

|  |                |                                |                  |
|--|----------------|--------------------------------|------------------|
|  | <b>Blks</b>    | <b>FileNames</b>               | <b>Dilutions</b> |
|  |                | <b>POAA</b>                    |                  |
|  |                | A061099031, 32 & 99, 100       | 1/1              |
|  | <b>Grp 1</b>   | 061099043-50                   | 1/1              |
|  | <b>Grp 2</b>   | 060799015-18,64                | 1/100&1000       |
|  | <b>Grp 3</b>   | 060799022-23,27-28,67-68       | 1/100&1000       |
|  | <b>Grp 4</b>   | 060799031-32,71-73, 0616990115 | 1/100,200,&1000  |
|  | <b>MS, MSD</b> | 061099051-54                   | 1/1              |

**Sample Data**

**Week 6 MONKEY SERA**

| Group Dose                                      | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-3         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   | <LOQ            | NA                       |
|                                                 | H2O Blk-4         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
| Matrix Blk                                      | Rabbit Sera Blk-3 | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   | <LOQ            | NA                       |
|                                                 | Rabbit Sera Blk-4 | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
| QC - 250 ppb                                    | RBS05129-MS-3     | 1.00                  | NA                         | 1                    | 199              | 80%                                   | 80%             | 0%                       |
|                                                 | RBS05129-MSD-3    | 1.00                  | NA                         | 1                    | 199              | 79%                                   |                 |                          |
|                                                 | RBS05129-MS-4     | 1.00                  | NA                         | 1                    | 229              | 92%                                   | 89%             | 7%                       |
|                                                 | RBS05129-MSD-4    | 1.00                  | NA                         | 1                    | 214              | 85%                                   |                 |                          |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.54                  | 0.9582                     | 1                    | 56.3             | <LOQ (0.0983 ug/mL)                   | 0.103           | 11.0<br>0.0113           |
|                                                 | I05714M           | 0.74                  | 0.9582                     | 1                    | 100              | 0.126                                 |                 |                          |
|                                                 | I05715M           | 0.70                  | 0.9582                     | 1                    | 22.5             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05718M           | 0.55                  | 0.9582                     | 1                    | 33.0             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05720M           | 0.56                  | 0.9582                     | 1                    | 3.15             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05725M           | 0.67                  | 0.9582                     | 1                    | 21.0             | <LOQ (0.0983 ug/mL)                   |                 |                          |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.70                  | 0.9582                     | 100                  | 493              | 67.5                                  | 102             | 32.8<br>33.6             |
|                                                 | I05706M           | 0.55                  | 0.9582                     | 100                  | 662              | 115                                   |                 |                          |
|                                                 | I05717M           | 0.56                  | 0.9582                     | 100                  | 489              | 83.7                                  |                 |                          |
|                                                 | I05721M           | 0.65                  | 0.9582                     | 1000                 | 97.1             | 143                                   |                 |                          |
|                                                 | I05723M           | NR                    | 0.9582                     | 1                    | NR               | NR                                    |                 |                          |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.72                  | 0.9582                     | 1000                 | 63.4             | 84.4                                  | 95.8            | 21.5<br>20.6             |
|                                                 | I05708M           | 0.55                  | 0.9582                     | 100                  | 452              | 78.8                                  |                 |                          |
|                                                 | I05710M           | 0.53                  | 0.9582                     | 100                  | 569              | 103                                   |                 |                          |
|                                                 | I05712M           | 0.82                  | 0.9582                     | 1000                 | 75.3             | 87.9                                  |                 |                          |
|                                                 | I05716M           | 0.67                  | 0.9582                     | 100                  | 602              | 86.2                                  |                 |                          |
|                                                 | I05719M           | 0.40                  | 0.9582                     | 100                  | 562              | 135                                   |                 |                          |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.49                  | 0.9582                     | 200                  | 414              | 162                                   | 145             | 14.9<br>21.6             |
|                                                 | I05704M           | 0.39                  | 0.9582                     | 100                  | 450              | 111                                   |                 |                          |
|                                                 | I05711M           | 0.61                  | 0.9582                     | 1000                 | 102              | 161                                   |                 |                          |
|                                                 | I05713M           | 0.59                  | 0.9582                     | 1000                 | 95.1             | 154                                   |                 |                          |
|                                                 | I05722M           | 0.49                  | 0.9582                     | 1000                 | 70.0             | 137                                   |                 |                          |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

NR = Sample not received nor reported.

POAA = Perfluorooctanoate

Date Entered/By: 06/08/99, 06/14/99, 06/17/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498, Soup 020199  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/12/99 SAH  
Date of Analysis/Analyst: 06/03/99, 06/07/99, 06/10/99, 06/16/99 HOJ/SAH/MEE/LAC  
Date of Data Reduction/Analyst: 06/04/99, 06/08/99, 06/11/99, 06/17/99, 11/06/00 HOJ/LAC/KJH

**Sample Data**

**Week 6 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-3         | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | H2O Blk-4         | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-3 | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | Rabbit Sera Blk-4 | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS05129-MS-3     | 80%                                         |                       |                                |
|                                                 | RBS05129-MSD-3    | 79%                                         | 80%                   | 0%                             |
|                                                 | RBS05129-MS-4     | 92%                                         |                       |                                |
|                                                 | RBS05129-MSD-4    | 85%                                         | 89%                   | 7%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05714M           | 0.126                                       |                       |                                |
|                                                 | I05715M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05718M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05720M           | <LOQ (0.0983 ug/mL)                         |                       | 11.0                           |
|                                                 | I05725M           | <LOQ (0.0983 ug/mL)                         | 0.103                 | 0.0113                         |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 67.5                                        |                       |                                |
|                                                 | I05706M           | 115                                         |                       |                                |
|                                                 | I05717M           | 83.7                                        |                       |                                |
|                                                 | I05721M           | 143                                         |                       | 32.8                           |
|                                                 | I05723M           | NR                                          | 102                   | 33.6                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 84.4                                        |                       |                                |
|                                                 | I05708M           | 78.8                                        |                       |                                |
|                                                 | I05710M           | 103                                         |                       |                                |
|                                                 | I05712M           | 87.9                                        |                       |                                |
|                                                 | I05716M           | 86.2                                        |                       | 21.5                           |
|                                                 | I05719M           | 135                                         | 95.8                  | 20.6                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 162                                         |                       |                                |
|                                                 | I05704M           | 111                                         |                       |                                |
|                                                 | I05711M           | 161                                         |                       |                                |
|                                                 | I05713M           | 154                                         |                       | 14.9                           |
|                                                 | I05722M           | 137                                         | 145                   | 21.6                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data NR = Sample not received nor reported.

POAA = Perfluorooctanoate

Date Entered/By: 06/08/99, 06/14/99, 06/17/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
 Product Number(Test Substance): T-6889.2 (POAA)  
 Matrix: Monkey Sera  
 Method/Revision: ETS-8-4.1 and ETS-8-5.1  
 Analytical Equipment System Number: Amelia 062498  
 Instrument Software/Version: MassLynx 3.2  
 Filename: See Attachments  
 R-Squared Value: See Attachments  
 Slope: See Attachments  
 Y-Intercept: See Attachments  
 Date of Extraction/Analyst: 05/12/99 SAH  
 Date of Analysis/Analyst: 06/03/99, 06/07/99, 06/10/99 HOJ  
 Date of Data Reduction/Analyst: 06/04/99, 06/08/99, 06/11/99, 11/06/00 HOJ/KJH

|                | FileNames                | Dilutions  |
|----------------|--------------------------|------------|
| <b>Blks</b>    | <b>POAA</b>              |            |
|                | A061099031, 32 & 99, 100 | 1/1        |
| <b>Grp 1</b>   | 061099057-62             | 1/1        |
| <b>Grp 2</b>   | 060799038-41             | 1/100      |
| <b>Grp 3</b>   | 060799044-46,50-51,80    | 1/100&1000 |
| <b>Grp 4</b>   | 060799054-56,84-85       | 1/100&1000 |
| <b>MS, MSD</b> | 061099051-54             | 1/1        |

**Sample Data**

**Week 8 MONKEY SERA**

| Group Dose                                      | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-3         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | H2O Blk-4         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   | <LOQ            | NA                       |
| Matrix Blk                                      | Rabbit Sera Blk-3 | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | Rabbit Sera Blk-4 | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   | <LOQ            | NA                       |
| QC - 250 ppb                                    | RBS05129-MS-3     | 1.00                  | NA                         | 1                    | 199              | 80%                                   |                 |                          |
|                                                 | RBS05129-MSD-3    | 1.00                  | NA                         | 1                    | 199              | 79%                                   | 80%             | 0%                       |
|                                                 | RBS05129-MS-4     | 1.00                  | NA                         | 1                    | 229              | 92%                                   |                 |                          |
|                                                 | RBS05129-MSD-4    | 1.00                  | NA                         | 1                    | 214              | 85%                                   | 89%             | 7%                       |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.30                  | 0.9582                     | 1                    | 32.5             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05714M           | 0.67                  | 0.9582                     | 1                    | 86.2             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05715M           | 0.34                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05718M           | 0.26                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                                 | I05720M           | 0.60                  | 0.9582                     | 1                    | 39.3             | <LOQ (0.0983 ug/mL)                   |                 | NA                       |
|                                                 | I05725M           | 0.57                  | 0.9582                     | 1                    | 4.97             | <LOQ (0.0983 ug/mL)                   | <LOQ            | NA                       |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.54                  | 0.9582                     | 100                  | 431              | 76.4                                  |                 |                          |
|                                                 | I05706M           | 0.50                  | 0.9582                     | 100                  | 529              | 101                                   |                 |                          |
|                                                 | I05717M           | 0.57                  | 0.9582                     | 100                  | 419              | 70.5                                  |                 |                          |
|                                                 | I05721M           | 0.52                  | 0.9582                     | 100                  | 707              | 130                                   |                 | 28.8                     |
|                                                 | I05723M           | NR                    | 0.9582                     | 1                    | NR               | NR                                    | 94.7            | 27.3                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.58                  | 0.9582                     | 100                  | 588              | 97.2                                  |                 |                          |
|                                                 | I05708M           | 0.63                  | 0.9582                     | 100                  | 446              | 67.8                                  |                 |                          |
|                                                 | I05710M           | 0.41                  | 0.9582                     | 100                  | 379              | 88.5                                  |                 |                          |
|                                                 | I05712M           | 0.73                  | 0.9582                     | 1000                 | 62.7             | 82.3                                  |                 |                          |
|                                                 | I05716M           | 0.56                  | 0.9582                     | 100                  | 597              | 102                                   |                 | 24.1                     |
|                                                 | I05719M           | 0.41                  | 0.9582                     | 100                  | 567              | 133                                   | 97.6            | 23.5                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.36                  | 0.9582                     | 100                  | 659              | 175                                   |                 |                          |
|                                                 | I05704M           | 0.42                  | 0.9582                     | 100                  | 513              | 117                                   |                 |                          |
|                                                 | I05711M           | 0.64                  | 0.9582                     | 100                  | 684              | 102                                   |                 |                          |
|                                                 | I05713M           | 0.59                  | 0.9582                     | 1000                 | 61.8             | 100                                   |                 | 59.6                     |
|                                                 | I05722M           | 0.61                  | 0.9582                     | 1000                 | 213              | 334                                   | 166             | 98.9                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

NR = Sample not received nor reported.

POAA = Perfluorooctanoate

Date Entered/By: 06/08/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Amelia 062498                                                  |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/12/99 SAH                                                   |
| Date of Analysis/Analyst:           | 06/03/99, 06/07/99, 06/10/99 HOJ                               |
| Date of Data Reduction/Analyst:     | 06/04/99, 06/08/99, 06/11/99, 11/06/00 HOJ/KJH                 |

**Sample Data**

**Week 8 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-3         | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
|                                                 | H2O Blk-4         | <LOQ (0.0983 ug/mL)                         |                       |                                |
| Matrix Blk                                      | Rabbit Sera Blk-3 | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
|                                                 | Rabbit Sera Blk-4 | <LOQ (0.0983 ug/mL)                         |                       |                                |
| QC - 250 ppb                                    | RBS05129-MS-3     | 80%                                         | 80%                   | 0%                             |
|                                                 | RBS05129-MSD-3    | 79%                                         |                       |                                |
|                                                 | RBS05129-MS-4     | 92%                                         | 89%                   | 7%                             |
|                                                 | RBS05129-MSD-4    | 85%                                         |                       |                                |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
|                                                 | I05714M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05715M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05718M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05720M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05725M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 76.4                                        | 94.7                  | 28.8                           |
|                                                 | I05706M           | 101                                         |                       |                                |
|                                                 | I05717M           | 70.5                                        |                       |                                |
|                                                 | I05721M           | 130                                         |                       |                                |
|                                                 | I05723M           | NR                                          |                       |                                |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 97.2                                        | 97.6                  | 24.1                           |
|                                                 | I05708M           | 67.8                                        |                       |                                |
|                                                 | I05710M           | 88.5                                        |                       |                                |
|                                                 | I05712M           | 82.3                                        |                       |                                |
|                                                 | I05716M           | 102                                         |                       |                                |
|                                                 | I05719M           | 133                                         |                       |                                |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 175                                         | 166                   | 59.6                           |
|                                                 | I05704M           | 117                                         |                       |                                |
|                                                 | I05711M           | 102                                         |                       |                                |
|                                                 | I05713M           | 100                                         |                       |                                |
|                                                 | I05722M           | 334                                         |                       |                                |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data NR = Sample not received nor reported.

POAA = Perfluorooctanoate

Date Entered/By: 06/08/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/13/99 SRP/JCP  
Date of Analysis/Analyst: 05/17/99, 05/19/99, 06/10/99 MEE/KJH/HOJ  
Date of Data Reduction/Analyst: 05/18/99, 05/20/99, 06/11/99, 11/06/00 KJH/MEE/HOJ

|                | POAA                              | Dilutions |
|----------------|-----------------------------------|-----------|
| <b>Blks</b>    | M051799028, 29, 98-99, & 109, 110 | 1/1       |
| <b>Grp 1</b>   | 051799040-44, 47                  | 1/1       |
| <b>Grp 2</b>   | 051999016-19                      | 1/100     |
| <b>Grp 3</b>   | 051999022-27                      | 1/100     |
| <b>Grp 4</b>   | 051999030-34, 052499058           | 1/250&500 |
| <b>MS, MSD</b> | 051799102-105, 061099073          | 1/1       |

**Sample Data**

**Week 10 MONKEY SERA**

CCV outside criteria

| Group Dose                               | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                               | H2O Blk-7         | 1.00                  | 0.9582                     | 1                    | 7.56             | <LOQ                                  | <LOQ            | NA                       |
|                                          | H2O Blk-8         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
| Matrix Blk                               | Rabbit Sera Blk-7 | 1.00                  | 0.9582                     | 1                    | 30.0             | 0.0288                                | 0.0301          | 0.00192                  |
|                                          | Rabbit Sera Blk-8 | 1.00                  | 0.9582                     | 1                    | 32.8             | 0.0315                                |                 |                          |
| Matrix Blk                               | Monkey Sera Blk-7 | 1.00                  | 0.9582                     | 1                    | 85.4             | 0.0819                                | 0.0636          | 0.0258                   |
|                                          | Monkey Sera Blk-8 | 1.00                  | 0.9582                     | 1                    | 47.4             | 0.0454                                |                 |                          |
| QC - 250 ppb                             | RBS05129-MS-7     | 1.00                  | NA                         | 1                    | 257              | 103%                                  | 115%            | 20%                      |
|                                          | RBS05129-MSD-7    | 1.00                  | NA                         | 1                    | 265              | 106%                                  |                 |                          |
|                                          | RBS05129-MS-8     | 1.00                  | NA                         | 1                    | 258              | 103%                                  |                 |                          |
|                                          | RBS05129-MSD-8    | 1.00                  | NA                         | 1                    | 317              | 127%                                  |                 |                          |
| Group 1<br>Control<br>0.0 mg/kg/day      | I05709M           | 0.43                  | 0.9582                     | 1                    | 85.5             | 0.191                                 | 0.126           | 27.7<br>0.0348           |
|                                          | I05714M           | 0.83                  | 0.9582                     | 1                    | 90.7             | 0.105                                 |                 |                          |
|                                          | I05715M           | 0.79                  | 0.9582                     | 1                    | 75.7             | 0.0919                                |                 |                          |
|                                          | I05718M           | 0.57                  | 0.9582                     | 1                    | 76.6             | 0.129                                 |                 |                          |
|                                          | I05720M           | 0.53                  | 0.9582                     | 1                    | 70.9             | 0.128                                 |                 |                          |
|                                          | I05725M           | 0.45                  | 0.9582                     | 1                    | 51.5             | 0.110                                 |                 |                          |
| Group 2<br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.52                  | 0.9582                     | 100                  | 412              | 75.9                                  | 105             | 36.0<br>37.7             |
|                                          | I05706M           | 0.54                  | 0.9582                     | 100                  | 641              | 114                                   |                 |                          |
|                                          | I05717M           | 0.42                  | 0.9582                     | 100                  | 328              | 74.8                                  |                 |                          |
|                                          | I05721M           | 0.33                  | 0.9582                     | 100                  | 532              | 154                                   |                 |                          |
|                                          | I05723M           | NR                    | 0.9582                     | NR                   | NR               | NR                                    |                 |                          |
| Group 3<br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.55                  | 0.9582                     | 100                  | 559              | 97.4                                  | 93.6            | 14.6<br>13.7             |
|                                          | I05708M           | 0.77                  | 0.9582                     | 100                  | 585              | 72.8                                  |                 |                          |
|                                          | I05710M           | 0.61                  | 0.9582                     | 100                  | 560              | 87.9                                  |                 |                          |
|                                          | I05712M           | 0.69                  | 0.9582                     | 100                  | 740              | 103                                   |                 |                          |
|                                          | I05716M           | 0.66                  | 0.9582                     | 100                  | 703              | 102                                   |                 |                          |
|                                          | I05719M           | 0.63                  | 0.9582                     | 100                  | 708              | 108                                   |                 |                          |
| Group 4<br>High Dose<br>30 mg/kg/day     | I05703M           | 0.50                  | 0.9582                     | 250                  | 654              | 313                                   | 237             | 66.8<br>158              |
|                                          | I05704M           | 0.73                  | 0.9582                     | 250                  | 392              | 128                                   |                 |                          |
|                                          | I05711M           | 0.68                  | 0.9582                     | 250                  | 191              | 67.4                                  |                 |                          |
|                                          | I05713M           | 0.55                  | 0.9582                     | 250                  | 477              | 208                                   |                 |                          |
|                                          | I05722M           | 0.53                  | 0.9582                     | 500                  | 517              | 467                                   |                 |                          |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\* CCV bracketing these data was outside criteria.

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

POAA = Perfluorooctanoate

Date Entered/By: 05/20/99, 05/25/99, 06/14/99, 11/06/00, 11/09/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Madeline 041098                                                |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/13/99 SRP/JCP                                               |
| Date of Analysis/Analyst:           | 05/17/99, 05/19/99, 06/10/99 MEE/KJH/HOJ                       |
| Date of Data Reduction/Analyst:     | 05/18/99, 05/20/99, 06/11/99, 11/06/00 KJH/MEE/HOJ             |

**Sample Data**

**Week 10 MONKEY SERA**

| Group Dose                                      | Sample #          | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-7         | <LOQ                                  |                 |                          |
|                                                 | H2O Blk-8         | <LOQ *                                | <LOQ            | NA                       |
| Matrix Blk                                      | Rabbit Sera Blk-7 | 0.0288                                |                 |                          |
|                                                 | Rabbit Sera Blk-8 | 0.0315 *                              | 0.0301          | 0.00192                  |
| Matrix Blk                                      | Monkey Sera Blk-7 | 0.0819 *                              |                 |                          |
|                                                 | Monkey Sera Blk-8 | 0.0454 *                              | 0.0636          | 0.0258                   |
| QC - 250 ppb                                    | RBS05129-MS-7     | 103% *                                |                 |                          |
|                                                 | RBS05129-MSD-7    | 106% *                                | 104%            | 3%                       |
|                                                 | RBS05129-MS-8     | 103% *                                |                 |                          |
|                                                 | RBS05129-MSD-8    | 127% *                                | 115%            | 20%                      |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.191                                 |                 |                          |
|                                                 | I05714M           | 0.105                                 |                 |                          |
|                                                 | I05715M           | 0.0919                                |                 |                          |
|                                                 | I05718M           | 0.129                                 |                 |                          |
|                                                 | I05720M           | 0.128                                 |                 | 27.7                     |
|                                                 | I05725M           | 0.110                                 | 0.126           | 0.0348                   |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 75.9                                  |                 |                          |
|                                                 | I05706M           | 114                                   |                 |                          |
|                                                 | I05717M           | 74.8                                  |                 |                          |
|                                                 | I05721M           | 154                                   |                 | 36.0                     |
|                                                 | I05723M           | NR                                    | 105             | 37.7                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 97.4                                  |                 |                          |
|                                                 | I05708M           | 72.8                                  |                 |                          |
|                                                 | I05710M           | 87.9                                  |                 |                          |
|                                                 | I05712M           | 103                                   |                 |                          |
|                                                 | I05716M           | 102                                   |                 | 14.6                     |
|                                                 | I05719M           | 108                                   | 93.6            | 13.7                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 313                                   |                 |                          |
|                                                 | I05704M           | 128                                   |                 |                          |
|                                                 | I05711M           | 67.4                                  |                 |                          |
|                                                 | I05713M           | 208                                   |                 | 66.8                     |
|                                                 | I05722M           | 467                                   | 237             | 158                      |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

\* CCV bracketing these data was outside criteria.

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

POAA = Perfluorooctanoate

Date Entered/By: 05/20/99, 05/25/99, 06/14/99, 11/06/00, 11/09/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: FACT-M-3.1 & FACT-M-4.1, ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098  
Instrument Software/Version: MassLynx 3.1  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 01/08/99 SAH/JCP  
Date of Analysis/Analyst: 01/21/99, 01/27/99 HOJ/MEE  
Date of Data Reduction/Analyst: 01/25/99, 01/28/99, 10/02/00, 10/11/00 KJH/MEE

**FileNames**

|                | <b>POAA</b>                | <b>Dilutions</b>                 |
|----------------|----------------------------|----------------------------------|
| <b>Blks</b>    | M012199003, 4 & 79, 80     | 1/1                              |
| <b>Grp 1</b>   | 012199016-21               | 1/1                              |
| <b>Grp 2</b>   | 012199024-27               | 1/150                            |
| <b>Grp 3</b>   | 012199030-35, 012799026    | 1/150, 1/400                     |
| <b>Grp 4</b>   | 012199039-40, 012799027-29 | 1/50, 1/150, 1/250, 1/400, 1/500 |
| <b>MS, MSD</b> | 012199074-75               | 1/1                              |

**Sample Data**

**Week 12 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | NA                       | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | NA                       | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                     | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | 1.00                     | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS01089-MS       | 1.00                     | NA                               | 1                          | 247                    | 100%                                        |                       |                                |
|                                                 | RBS01089-MSD      | 1.00                     | NA                               | 1                          | 243                    | 98%                                         | 99%                   | 2%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.50                     | 0.9582                           | 1                          | 108                    | 0.207                                       |                       |                                |
|                                                 | I05714M           | 0.70                     | 0.9582                           | 1                          | 73.0                   | 0.0994                                      |                       |                                |
|                                                 | I05715M           | 0.60                     | 0.9582                           | 1                          | 40.2                   | 0.064                                       |                       |                                |
|                                                 | I05718M           | 0.40                     | 0.9582                           | 1                          | 58.2                   | 0.139                                       |                       |                                |
|                                                 | I05720M           | 0.60                     | 0.9582                           | 1                          | 87.9                   | 0.140                                       |                       | 41.4                           |
|                                                 | I05725M           | 0.50                     | 0.9582                           | 1                          | 45.6                   | 0.0869                                      | 0.123                 | 0.0507                         |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.60                     | 0.9582                           | 150                        | 268                    | 64.0                                        |                       |                                |
|                                                 | I05706M           | 0.60                     | 0.9582                           | 150                        | 358                    | 85.3                                        |                       |                                |
|                                                 | I05717M           | 0.70                     | 0.9582                           | 150                        | 272                    | 55.5                                        |                       |                                |
|                                                 | I05721M           | 0.50                     | 0.9582                           | 150                        | 397                    | 114                                         |                       | 32.6                           |
|                                                 | I05723M           | 1.00                     | 0.9582                           | NR                         | NR                     | NR                                          | 79.6                  | 25.9                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.50                     | 0.9582                           | 150                        | 329                    | 94.0                                        |                       |                                |
|                                                 | I05708M           | 0.60                     | 0.9582                           | 150                        | 239                    | 56.9                                        |                       |                                |
|                                                 | I05710M           | 0.50                     | 0.9582                           | 150                        | 276                    | 79.0                                        |                       |                                |
|                                                 | I05712M           | 0.70                     | 0.9582                           | 400                        | 240                    | 131                                         |                       |                                |
|                                                 | I05716M           | 0.60                     | 0.9582                           | 150                        | 354                    | 84.4                                        |                       | 27.1                           |
|                                                 | I05719M           | 0.50                     | 0.9582                           | 150                        | 344                    | 98.4                                        | 90.6                  | 24.5                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.70                     | 0.9582                           | 500                        | 345                    | 235                                         |                       |                                |
|                                                 | I05704M           | 0.60                     | 0.9582                           | 150                        | 433                    | 103                                         |                       |                                |
|                                                 | I05711M           | 0.60                     | 0.9582                           | 50                         | 405                    | 32.2                                        |                       |                                |
|                                                 | I05713M           | 0.50                     | 0.9582                           | 400                        | 243                    | 185                                         |                       |                                |
|                                                 | I05722M           | 0.70                     | 0.9582                           | 400                        | 263                    | 143                                         |                       | 55.5                           |
|                                                 | I05724M           | 1.00                     | 0.9582                           | NR                         | NR                     | NR                                          | 140                   | 77.5                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 01/27/99, 01/28/99, 10/10/00, 10/13/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00, 10/19/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | FACT-M-3.1 & FACT-M-4.1, ETS-8-5.1                             |
| Analytical Equipment System Number: | Madeline 041098                                                |
| Instrument Software/Version:        | MassLynx 3.1                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 01/08/99 SAH/JCP                                               |
| Date of Analysis/Analyst:           | 01/21/99, 01/27/99 HOJ/MEE                                     |
| Date of Data Reduction/Analyst:     | 01/25/99, 01/28/99, 10/02/00, 10/11/00 KJH/MEE                 |

**Sample Data**

**Week 12 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS01089-MS       | 100%                                        |                       |                                |
|                                                 | RBS01089-MSD      | 98%                                         | 99%                   | 2%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.207                                       |                       |                                |
|                                                 | I05714M           | 0.0994                                      |                       |                                |
|                                                 | I05715M           | 0.0639                                      |                       |                                |
|                                                 | I05718M           | 0.139                                       |                       |                                |
|                                                 | I05720M           | 0.140                                       |                       | 41.4                           |
|                                                 | I05725M           | 0.0869                                      | 0.123                 | 0.0507                         |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 64.0                                        |                       |                                |
|                                                 | I05706M           | 85.3                                        |                       |                                |
|                                                 | I05717M           | 55.5                                        |                       |                                |
|                                                 | I05721M           | 114                                         |                       | 32.6                           |
|                                                 | I05723M           | NR                                          | 79.6                  | 25.9                           |
|                                                 |                   |                                             |                       |                                |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 94.0                                        |                       |                                |
|                                                 | I05708M           | 56.9                                        |                       |                                |
|                                                 | I05710M           | 79.0                                        |                       |                                |
|                                                 | I05712M           | 131                                         |                       |                                |
|                                                 | I05716M           | 84.4                                        |                       | 27.1                           |
|                                                 | I05719M           | 98                                          | 90.6                  | 24.5                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 235                                         |                       |                                |
|                                                 | I05704M           | 103                                         |                       |                                |
|                                                 | I05711M           | 32.2                                        |                       |                                |
|                                                 | I05713M           | 185                                         |                       |                                |
|                                                 | I05722M           | 143                                         |                       | 55.5                           |
|                                                 | I05724M           | NR                                          | 140                   | 77.5                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 01/27/99, 01/28/99, 10/10/00, 10/13/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00, 10/19/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: FACT-M-3.1 & FACT-M-4.1, ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098  
Instrument Software/Version: MassLynx 3.1  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 01/08/99 SAH/JCP  
Date of Analysis/Analyst: 01/21/99, 01/27/99 HOJ/MEE  
Date of Data Reduction/Analyst: 01/25/99, 01/28/99, 10/02/00, 10/11/00 KJH/MEE

| FileNames      | POAA                          | Dilutions    |
|----------------|-------------------------------|--------------|
| <b>Blks</b>    | M012199003, 4 & 79, 80        | 1/1          |
| <b>Grp 1</b>   | 012199045-50                  | 1/1          |
| <b>Grp 2</b>   | 012199053-56, 012799033       | 1/100, 1/200 |
| <b>Grp 3</b>   | 012199061-62, 64 012799034-36 | 1/100, 1/200 |
| <b>Grp 4</b>   | 012199067-71                  | 1/150        |
| <b>MS, MSD</b> | 012199074-75                  | 1/1          |

**Sample Data**

**Week 14 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | NA                       | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | NA                       | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                     | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | 1.00                     | 0.9582                           | 1                          | <LOQ                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS01089-MS       | 1.00                     | NA                               | 1                          | 247                    | 100%                                        |                       |                                |
|                                                 | RBS01089-MSD      | 1.00                     | NA                               | 1                          | 243                    | 98%                                         | 99%                   | 2%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.50                     | 0.9582                           | 1                          | 144                    | 0.275                                       |                       |                                |
|                                                 | I05714M           | 0.50                     | 0.9582                           | 1                          | 81.6                   | 0.156                                       |                       |                                |
|                                                 | I05715M           | 0.50                     | 0.9582                           | 1                          | 51.4                   | 0.0981                                      |                       |                                |
|                                                 | I05718M           | 0.50                     | 0.9582                           | 1                          | 80.3                   | 0.153                                       |                       |                                |
|                                                 | I05720M           | 0.30                     | 0.9582                           | 1                          | 57.3                   | 0.182                                       |                       | 39.8                           |
|                                                 | I05725M           | 0.50                     | 0.9582                           | 1                          | 55.0                   | 0.105                                       | 0.162                 | 0.0643                         |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.50                     | 0.9582                           | 100                        | 381                    | 72.7                                        |                       |                                |
|                                                 | I05706M           | 0.50                     | 0.9582                           | 200                        | 294                    | 112                                         |                       |                                |
|                                                 | I05717M           | 0.50                     | 0.9582                           | 100                        | 306                    | 58.4                                        |                       |                                |
|                                                 | I05721M           | 0.35                     | 0.9582                           | 100                        | 428                    | 117                                         |                       | 32.1                           |
|                                                 | I05723M           | 1.00                     | 0.9582                           | NR                         | NR                     | NR                                          | 90.0                  | 28.9                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.50                     | 0.9582                           | 200                        | 178                    | 68.0                                        |                       |                                |
|                                                 | I05708M           | 0.50                     | 0.9582                           | 200                        | 270                    | 103                                         |                       |                                |
|                                                 | I05710M           | 0.50                     | 0.9582                           | 100                        | 322                    | 61.3                                        |                       |                                |
|                                                 | I05712M           | 0.50                     | 0.9582                           | 100                        | 415                    | 79.2                                        |                       |                                |
|                                                 | I05716M           | 0.45                     | 0.9582                           | 200                        | 316                    | 134                                         |                       | 30.0                           |
|                                                 | I05719M           | 0.40                     | 0.9582                           | 100                        | 451                    | 108                                         | 92.2                  | 27.6                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.50                     | 0.9582                           | 150                        | 295                    | 84.4                                        |                       |                                |
|                                                 | I05704M           | 0.50                     | 0.9582                           | 150                        | 380                    | 109                                         |                       |                                |
|                                                 | I05711M           | 0.45                     | 0.9582                           | 150                        | 215                    | 68.2                                        |                       |                                |
|                                                 | I05713M           | 0.50                     | 0.9582                           | 150                        | 129                    | 36.9                                        |                       |                                |
|                                                 | I05722M           | 0.50                     | 0.9582                           | 150                        | 346                    | 98.8                                        |                       | 35.6                           |
|                                                 | I05724M           | 1.00                     | 0.9582                           | NR                         | NR                     | NR                                          | 79.4                  | 28.3                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 01/27/99, 01/28/99, 10/10/00, 10/13/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00, 10/19/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: FACT-M-3.1 & FACT-M-4.1, ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098  
Instrument Software/Version: MassLynx 3.1  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 01/08/99 SAH/JCP  
Date of Analysis/Analyst: 01/21/99, 01/27/99 HOJ/MEE  
Date of Data Reduction/Analyst: 01/25/99, 01/28/99, 10/02/00, 10/11/00 KJH/MEE

**Sample Data**

**Week 14 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS01089-MS       | 100%                                        |                       |                                |
|                                                 | RBS01089-MSD      | 98%                                         | 99%                   | 2%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.275                                       |                       |                                |
|                                                 | I05714M           | 0.156                                       |                       |                                |
|                                                 | I05715M           | 0.0981                                      |                       |                                |
|                                                 | I05718M           | 0.153                                       |                       |                                |
|                                                 | I05720M           | 0.182                                       |                       | 39.8                           |
|                                                 | I05725M           | 0.105                                       | 0.162                 | 0.0643                         |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 72.7                                        |                       |                                |
|                                                 | I05706M           | 112                                         |                       |                                |
|                                                 | I05717M           | 58.4                                        |                       |                                |
|                                                 | I05721M           | 117                                         |                       | 32.1                           |
|                                                 | I05723M           | NR                                          | 90.0                  | 28.9                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 68.0                                        |                       |                                |
|                                                 | I05708M           | 103                                         |                       |                                |
|                                                 | I05710M           | 61.3                                        |                       |                                |
|                                                 | I05712M           | 79.2                                        |                       |                                |
|                                                 | I05716M           | 134                                         |                       | 30.0                           |
|                                                 | I05719M           | 108                                         | 92.2                  | 27.6                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 84.4                                        |                       |                                |
|                                                 | I05704M           | 109                                         |                       |                                |
|                                                 | I05711M           | 68.2                                        |                       |                                |
|                                                 | I05713M           | 36.9                                        |                       |                                |
|                                                 | I05722M           | 98.8                                        |                       | 35.6                           |
|                                                 | I05724M           | NR                                          | 79.4                  | 28.3                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 01/27/99, 01/28/99, 10/10/00, 10/13/00 LAC

Date Verified/ By: 06/21/99 EAD, 10/11/00, 10/19/00 KJH, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
 Product Number(Test Substance): T-6889.2 (POAA)  
 Matrix: Monkey Sera  
 Method/Revision: ETS-8-4.1 and ETS-8-5.1  
 Analytical Equipment System Number: Madeline 041098  
 Instrument Software/Version: MassLynx 3.1  
 Filename: See Attachments  
 R-Squared Value: See Attachments  
 Slope: See Attachments  
 Y-Intercept: See Attachments  
 Date of Extraction/Analyst: 05/13/99 SRP/JCP  
 Date of Analysis/Analyst: 05/17/99, 05/19/99, 06/10/99 MEE  
 Date of Data Reduction/Analyst: 05/18/99, 05/20/99, 06/11/99, 11/06/00 KJH/MEE/HOJ

|         | Blks | POAA                              | Dilutions |
|---------|------|-----------------------------------|-----------|
|         |      | M051799028, 29, 98-99, & 109, 110 | 1/1       |
| Grp 1   |      | 061099065-69, 72                  | 1/1       |
| Grp 2   |      | 051999037-40                      | 1/100     |
| Grp 3   |      | 051999043-45, 47-48, 052499059    | 1/100     |
| Grp 4   |      | 051999051-52, 54-55, 061099074    | 1/10&250  |
| MS, MSD |      | 051799102-105, 061099073          | 1/1       |

**Sample Data**

**Week 16 MONKEY SERA**

| Group Dose                               | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                               | H2O Blk-7         | 1.00                  | 0.9582                     | 1                    | 7.56             | <LOQ                                  |                 |                          |
|                                          | H2O Blk-8         | 1.00                  | 0.9582                     | 1                    | 0.00             | <LOQ *                                | <LOQ            | NA                       |
| Matrix Blk                               | Rabbit Sera Blk-7 | 1.00                  | 0.9582                     | 1                    | 30.0             | 0.0288                                |                 |                          |
|                                          | Rabbit Sera Blk-8 | 1.00                  | 0.9582                     | 1                    | 32.8             | 0.0315 *                              | 0.0301          | 0.00192                  |
| Matrix Blk                               | Monkey Sera Blk-7 | 1.00                  | 0.9582                     | 1                    | 85.4             | 0.0819 *                              |                 |                          |
|                                          | Monkey Sera Blk-8 | 1.00                  | 0.9582                     | 1                    | 47.4             | 0.0454 *                              | 0.0636          | 0.0258                   |
| QC - 250 ppb                             | RBS05129-MS-7     | 1.00                  | NA                         | 1                    | 257              | 103% *                                |                 |                          |
|                                          | RBS05129-MSD-7    | 1.00                  | NA                         | 1                    | 265              | 106% *                                | 104%            | 3%                       |
|                                          | RBS05129-MS-8     | 1.00                  | NA                         | 1                    | 258              | 103% *                                |                 |                          |
|                                          | RBS05129-MSD-8    | 1.00                  | NA                         | 1                    | 317              | 127% *                                | 115%            | 20%                      |
| Group 1<br>Control<br>0.0 mg/kg/day      | I05709M           | 0.54                  | 0.9582                     | 1                    | 155              | 0.275                                 |                 |                          |
|                                          | I05714M           | 0.66                  | 0.9582                     | 1                    | 70.7             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                          | I05715M           | 0.64                  | 0.9582                     | 1                    | 6.40             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                          | I05718M           | 0.51                  | 0.9582                     | 1                    | 50.6             | <LOQ (0.0983 ug/mL)                   |                 |                          |
|                                          | I05720M           | 0.50                  | 0.9582                     | 1                    | 48.3             | <LOQ (0.0983 ug/mL)                   |                 | 56.4                     |
|                                          | I05725M           | 0.59                  | 0.9582                     | 1                    | 3.07             | <LOQ (0.0983 ug/mL)                   | 0.128           | 0.0721                   |
| Group 2<br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.60                  | 0.9582                     | 100                  | 346              | 55.3                                  |                 |                          |
|                                          | I05706M           | 0.55                  | 0.9582                     | 100                  | 417              | 72.6                                  |                 |                          |
|                                          | I05717M           | 0.59                  | 0.9582                     | 100                  | 267              | 43.4                                  |                 |                          |
|                                          | I05721M           | 0.52                  | 0.9582                     | 100                  | 560              | 103                                   |                 | 37.9                     |
|                                          | I05723M           | NR                    | 0.9582                     | NR                   | NR               | NR                                    | 68.6            | 26.0                     |
| Group 3<br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.57                  | 0.9582                     | 100                  | 592              | 99.5                                  |                 |                          |
|                                          | I05708M           | 0.68                  | 0.9582                     | 100                  | 427              | 60.2                                  |                 |                          |
|                                          | I05710M           | 0.56                  | 0.9582                     | 100                  | 428              | 73.2                                  |                 |                          |
|                                          | I05712M           | 0.59                  | 0.9582                     | 500                  | 222              | 180                                   |                 |                          |
|                                          | I05716M           | 0.57                  | 0.9582                     | 100                  | 543              | 91.3                                  |                 | 43.0                     |
|                                          | I05719M           | 0.64                  | 0.9582                     | 100                  | 578              | 86.5                                  | 98.5            | 42.3                     |
| Group 4<br>High Dose<br>30 mg/kg/day     | I05703M           | 0.57                  | 0.9582                     | 250                  | 162              | 68.2                                  |                 |                          |
|                                          | I05704M           | 0.75                  | 0.9582                     | 250                  | 355              | 113                                   |                 |                          |
|                                          | I05711M           | 0.60                  | 0.9582                     | 10                   | 835              | 13.3                                  |                 |                          |
|                                          | I05713M           | 0.74                  | 0.9582                     | 250                  | 516              | 167                                   |                 |                          |
|                                          | I05722M           | 0.54                  | 0.9582                     | 250                  | 130              | 57.7                                  |                 | 69.7                     |
|                                          | I05724M           | NR                    | 0.9582                     | NR                   | NR               | NR                                    | 83.9            | 58.5                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

\* CCV bracketing these data was not within criteria.

POAA = Perfluorooctanoate

Date Entered/By: 05/20/99, 06/14/99, 11/06/00 LAC

ETS-8-5.1 Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

Sera Week16

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Madeline 041098                                                |
| Instrument Software/Version:        | MassLynx 3.1                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/13/99 SRP/JCP                                               |
| Date of Analysis/Analyst:           | 05/17/99, 05/19/99, 06/10/99 MEE                               |
| Date of Data Reduction/Analyst:     | 05/18/99, 05/20/99, 06/11/99, 11/06/00 KJH/MEE/HOJ             |

**Sample Data**

**Week 16 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-7         | <LOQ                                        | <LOQ                  | NA                             |
|                                          | H2O Blk-8         | <LOQ *                                      |                       |                                |
| Matrix Blk                               | Rabbit Sera Blk-7 | 0.0288                                      | 0.0301                | 0.00192                        |
|                                          | Rabbit Sera Blk-8 | 0.0315 *                                    |                       |                                |
| Matrix Blk                               | Monkey Sera Blk-7 | 0.0819 *                                    | 0.0636                | 0.02578                        |
|                                          | Monkey Sera Blk-8 | 0.0454 *                                    |                       |                                |
| QC - 250 ppb                             | RBS05129-MS-7     | 103% *                                      | 104%                  | 3%                             |
|                                          | RBS05129-MSD-7    | 106% *                                      |                       |                                |
|                                          | RBS05129-MS-8     | 103% *                                      |                       |                                |
| Group 1<br>Control<br>0.0 mg/kg/day      | RBS05129-MSD-8    | 127% *                                      | 115%                  | 20%                            |
|                                          | I05709M           | 0.275                                       |                       |                                |
|                                          | I05714M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                          | I05715M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                          | I05718M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                          | I05720M           | <LOQ (0.0983 ug/mL)                         |                       |                                |
| Group 2<br>Low Dose                      | I05725M           | <LOQ (0.0983 ug/mL)                         | 0.128                 | 56.4<br>0.0721                 |
|                                          | I05702M           | 55.3                                        |                       |                                |
|                                          | I05706M           | 72.6                                        |                       |                                |
|                                          | I05717M           | 43.4                                        |                       |                                |
|                                          | I05721M           | 103                                         |                       |                                |
|                                          | I05723M           | NR                                          |                       |                                |
| Group 3<br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 100                                         | 68.6                  | 37.9<br>26.0                   |
|                                          | I05708M           | 60.2                                        |                       |                                |
|                                          | I05710M           | 73.2                                        |                       |                                |
|                                          | I05712M           | 180                                         |                       |                                |
|                                          | I05716M           | 91.3                                        |                       |                                |
|                                          | I05719M           | 86.5                                        |                       |                                |
| Group 4<br>High Dose<br>30 mg/kg/day     | I05703M           | 68.2                                        | 98.5                  | 43.0<br>42.3                   |
|                                          | I05704M           | 113                                         |                       |                                |
|                                          | I05711M           | 13.3                                        |                       |                                |
|                                          | I05713M           | 167                                         |                       |                                |
|                                          | I05722M           | 57.7                                        |                       |                                |
|                                          | I05724M           | NR                                          |                       |                                |
|                                          |                   |                                             | 83.9                  | 69.7<br>58.5                   |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

NR = Sample not received nor reported.

E = Sample evaporated, not analyzed.

\* CCV bracketing these data was not within criteria.

POAA = Perfluorooctanoate

Date Entered/By: 05/20/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ Sera Week16

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/14/99 SEE  
Date of Analysis/Analyst: 05/18/99, 05/24/99, 05/25/99, 06/07/99 MEE/HOJ/SAH  
Date of Data Reduction/Analyst: 05/19/99, 05/25/99, 05/27/99, 06/08/99 MEE/HOJ

**FileNames**

|                | <b>POAA</b>                       | <b>POAA</b> | <b>Dilutions</b> |
|----------------|-----------------------------------|-------------|------------------|
| <b>Blks</b>    | M051899013, 14 & 89, 90           |             | 1/1              |
| <b>Grp 1</b>   | 051899026-33                      |             | 1/1              |
| <b>Grp 2</b>   | 052599086-87, 89                  | 60799061    | 1/50&250         |
| <b>Grp 3</b>   | 052599092-97                      |             | 1/50             |
| <b>Grp 4</b>   | 052499030, 32, 34, 052599101, 103 |             | 1/50             |
| <b>MS, MSD</b> | 051899084-87                      |             | 1/1              |

**Sample Data**

**Week 18 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | NA                       | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | NA                       | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                     | 0.9582                           | 1                          | 8.69                   | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | 1.00                     | 0.9582                           | 1                          | 1.76                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-11    | 1.00                     | NA                               | 1                          | 290                    | 116%                                        |                       |                                |
|                                                 | RBS05149-MSD-11   | 1.00                     | NA                               | 1                          | 236                    | 95%                                         | 105%                  | 20%                            |
|                                                 | RBS05149-MS-12    | 1.00                     | NA                               | 1                          | 247                    | 99%                                         |                       |                                |
|                                                 | RBS05149-MSD-12   | 1.00                     | NA                               | 1                          | 201                    | 80%                                         | 90%                   | 21%                            |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.63                     | 0.9582                           | 1                          | 173                    | 0.263                                       |                       |                                |
|                                                 | I05714M           | 0.57                     | 0.9582                           | 1                          | 145                    | 0.244                                       |                       |                                |
|                                                 | I05715M           | 0.57                     | 0.9582                           | 1                          | 56.5                   | 0.0950                                      |                       |                                |
|                                                 | I05718M           | 0.48                     | 0.9582                           | 1                          | 82.7                   | 0.165                                       |                       |                                |
|                                                 | I05720M           | 0.43                     | 0.9582                           | 1                          | 86.7                   | 0.193                                       |                       | 34.8                           |
|                                                 | I05725M           | 0.52                     | 0.9582                           | 1                          | 75.1                   | 0.138                                       | 0.183                 | 0.0637                         |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.69                     | 0.9582                           | 50                         | 160                    | 11.1                                        |                       |                                |
|                                                 | I05706M           | 0.63                     | 0.9582                           | 50                         | 210                    | 16.0                                        |                       |                                |
|                                                 | I05717M           | 0.45                     | 0.9582                           | 250                        | 125                    | 66.8                                        |                       | 86.5                           |
|                                                 | I05721M           | 0.47                     | 0.9582                           | 50                         | 234                    | 23.8                                        | 29.4                  | 25.4                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.52                     | 0.9582                           | 50                         | 145                    | 13.4                                        |                       |                                |
|                                                 | I05708M           | 0.68                     | 0.9582                           | 50                         | 137                    | 9.67                                        |                       |                                |
|                                                 | I05710M           | 0.48                     | 0.9582                           | 50                         | 465                    | 46.4                                        |                       |                                |
|                                                 | I05712M           | 0.60                     | 0.9582                           | 50                         | 171                    | 13.7                                        |                       |                                |
|                                                 | I05716M           | 0.70                     | 0.9582                           | 50                         | 147                    | 10.1                                        |                       | 82.1                           |
|                                                 | I05719M           | 0.75                     | 0.9582                           | 50                         | 178                    | 11.3                                        | 17.4                  | 14.3                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.46                     | 0.9582                           | 50                         | 660                    | 68.8                                        |                       |                                |
|                                                 | I05704M           | 0.66                     | 0.9582                           | 50                         | 276                    | 20.0                                        |                       |                                |
|                                                 | I05711M           | 0.32                     | 0.9582                           | 50                         | 127                    | 19.0                                        |                       |                                |
|                                                 | I05713M           | 0.70                     | 0.9582                           | 50                         | 399                    | 27.3                                        |                       | 58.5                           |
|                                                 | I05722M           | 0.60                     | 0.9582                           | 50                         | 575                    | 45.9                                        | 36.2                  | 21.2                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 05/19/99, 05/25/99, 05/27/99, 06/08/99 LAC

Date Verified/ By: 06/21/99 EAD

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Madeline 041098, Amelia 062498                                 |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/14/99 SEE                                                   |
| Date of Analysis/Analyst:           | 05/18/99, 05/24/99, 05/25/99, 06/07/99 MEE/HOJ/SAH             |
| Date of Data Reduction/Analyst:     | 05/19/99, 05/25/99, 05/27/99, 06/08/99 MEE/HOJ                 |

**Sample Data**

**Week 18 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | RBS05149-MS-11    | 116%                                        |                       |                                |
|                                          | RBS05149-MSD-11   | 95%                                         | 105%                  | 20%                            |
|                                          | RBS05149-MS-12    | 99%                                         |                       |                                |
|                                          | RBS05149-MSD-12   | 80%                                         | 90%                   | 21%                            |
| Group 1<br>Control<br>0.0 mg/kg/day      | I05709M           | 0.263                                       |                       |                                |
|                                          | I05714M           | 0.244                                       |                       |                                |
|                                          | I05715M           | 0.0950                                      |                       |                                |
|                                          | I05718M           | 0.165                                       |                       |                                |
|                                          | I05720M           | 0.193                                       |                       | 34.8                           |
|                                          | I05725M           | 0.138                                       | 0.183                 | 0.0637                         |
| Group 2<br>Low Dose                      | I05702M           | 11.1                                        |                       |                                |
|                                          | I05706M           | 16.0                                        |                       |                                |
|                                          | I05717M           | 66.8                                        |                       | 86.5                           |
|                                          | I05721M           | 23.8                                        | 29.4                  | 25.4                           |
| Group 3<br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 13.4                                        |                       |                                |
|                                          | I05708M           | 9.67                                        |                       |                                |
|                                          | I05710M           | 46.4                                        |                       |                                |
|                                          | I05712M           | 13.7                                        |                       |                                |
|                                          | I05716M           | 10.1                                        |                       | 82.1                           |
|                                          | I05719M           | 11.3                                        | 17.4                  | 14.3                           |
| Group 4<br>High Dose<br>30 mg/kg/day     | I05703M           | 68.8                                        |                       |                                |
|                                          | I05704M           | 20.0                                        |                       |                                |
|                                          | I05711M           | 19.0                                        |                       |                                |
|                                          | I05713M           | 27.3                                        |                       | 58.5                           |
|                                          | I05722M           | 45.9                                        | 36.2                  | 21.2                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/19/99, 05/25/99, 05/27/99, 06/08/99 LAC

Date Verified/ By: 06/21/99 EAD

**FACT-TOX-026**  
**Covance# 6329-231**

0.250746269

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Madeline 041098, Amelia 062498, Soup 020199  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/14/99 SEE  
Date of Analysis/Analyst: 05/18/99, 05/24/99, 05/25/99, 06/10/99, 06/16/99 MEE/HOJ/SAH/LAC  
Date of Data Reduction/Analyst: 05/19/99, 05/25/99, 05/27/99, 06/11/99, 06/17/99, 11/06/00 MEE/HOJ/LAC/KJH

**FileNames**

|                | <b>POAA</b>                           | <b>Dilutions</b> |
|----------------|---------------------------------------|------------------|
| <b>Blks</b>    | M051899013, 14 & 89, 90               | 1/1              |
| <b>Grp 1</b>   | 051899055-62                          | 1/1              |
| <b>Grp 2</b>   | 052599107-110                         | 1/50             |
| <b>Grp 3</b>   | 052499045, 061099075-76, 061699018-20 | 1/50&500         |
| <b>Grp 4</b>   | 052499051, 53, 55, 061699021-22       | 1/50&500         |
| <b>MS, MSD</b> | 051899084-87                          | 1/1              |

**Sample Data**

**Week 20 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | NA                       | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | NA                       | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | 1.00                     | 0.9582                           | 1                          | 8.69                   | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | 1.00                     | 0.9582                           | 1                          | 1.76                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-11    | 1.00                     | NA                               | 1                          | 290                    | 116%                                        |                       |                                |
|                                                 | RBS05149-MSD-11   | 1.00                     | NA                               | 1                          | 236                    | 95%                                         | 105%                  | 20%                            |
|                                                 | RBS05149-MS-12    | 1.00                     | NA                               | 1                          | 247                    | 99%                                         |                       |                                |
|                                                 | RBS05149-MSD-12   | 1.00                     | NA                               | 1                          | 201                    | 80%                                         | 90%                   | 21%                            |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.59                     | 0.9582                           | 1                          | 211                    | 0.342                                       |                       |                                |
|                                                 | I05714M           | 0.66                     | 0.9582                           | 1                          | 146                    | 0.212                                       |                       |                                |
|                                                 | I05715M           | 0.62                     | 0.9582                           | 1                          | 74.3                   | 0.115                                       |                       |                                |
|                                                 | I05718M           | 0.33                     | 0.9582                           | 1                          | 77.2                   | 0.224                                       |                       |                                |
|                                                 | I05720M           | 0.53                     | 0.9582                           | 1                          | 134                    | 0.242                                       |                       | 32.6                           |
|                                                 | I05725M           | 0.58                     | 0.9582                           | 1                          | 126                    | 0.207                                       | 0.224                 | 0.0730                         |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M           | 0.73                     | 0.9582                           | 50                         | 271                    | 17.8                                        |                       |                                |
|                                                 | I05706M           | 0.65                     | 0.9582                           | 50                         | 155                    | 11.4                                        |                       |                                |
|                                                 | I05717M           | 0.63                     | 0.9582                           | 50                         | 438                    | 33.3                                        |                       | 78.8                           |
|                                                 | I05721M           | 0.24                     | 0.9582                           | 50                         | 348                    | 69.6                                        | 33.0                  | 26.0                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 0.43                     | 0.9582                           | 50                         | 964                    | 107                                         |                       |                                |
|                                                 | I05708M           | 0.70                     | 0.9582                           | 50                         | 905                    | 61.9                                        |                       |                                |
|                                                 | I05710M           | 0.42                     | 0.9582                           | 50                         | 660                    | 75.2                                        |                       |                                |
|                                                 | I05712M           | 0.65                     | 0.9582                           | 500                        | 186                    | 137                                         |                       |                                |
|                                                 | I05716M           | 0.55                     | 0.9582                           | 500                        | 105                    | 91.4                                        |                       | 27.5                           |
|                                                 | I05719M           | 0.62                     | 0.9582                           | 500                        | 138                    | 107                                         | 96.6                  | 26.6                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 0.49                     | 0.9582                           | 50                         | 398                    | 38.9                                        |                       |                                |
|                                                 | I05704M           | 0.53                     | 0.9582                           | 500                        | 102                    | 92.5                                        |                       |                                |
|                                                 | I05711M           | 0.38                     | 0.9582                           | 50                         | 18.9                   | 2.39                                        |                       |                                |
|                                                 | I05713M           | 0.68                     | 0.9582                           | 500                        | 456                    | 322                                         |                       | 132                            |
|                                                 | I05722M           | 0.36                     | 0.9582                           | 50                         | 248                    | 32.9                                        | 97.7                  | 129                            |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 05/20/99, 05/25/99, 05/27/99, 06/14/99, 06/17/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                            |
|-------------------------------------|----------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys             |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                            |
| Matrix:                             | Monkey Sera                                                                |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                                    |
| Analytical Equipment System Number: | Madeline 041098, Amelia 062498, Soup 020199                                |
| Instrument Software/Version:        | MassLynx 3.2                                                               |
| Filename:                           | See Attachments                                                            |
| R-Squared Value:                    | See Attachments                                                            |
| Slope:                              | See Attachments                                                            |
| Y-Intercept:                        | See Attachments                                                            |
| Date of Extraction/Analyst:         | 05/14/99 SEE                                                               |
| Date of Analysis/Analyst:           | 05/18/99, 05/24/99, 05/25/99, 06/10/99, 06/16/99 MEE/HOJ/SAH/LAC           |
| Date of Data Reduction/Analyst:     | 05/19/99, 05/25/99, 05/27/99, 06/11/99, 06/17/99, 11/06/00 MEE/HOJ/LAC/KJH |

**Sample Data**

**Week 20 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-11    | 116%                                        |                       |                                |
|                                                 | RBS05149-MSD-11   | 95%                                         | 105%                  | 20%                            |
|                                                 | RBS05149-MS-12    | 99%                                         |                       |                                |
|                                                 | RBS05149-MSD-12   | 80%                                         | 90%                   | 21%                            |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.342                                       |                       |                                |
|                                                 | I05714M           | 0.212                                       |                       |                                |
|                                                 | I05715M           | 0.115                                       |                       |                                |
|                                                 | I05718M           | 0.224                                       |                       |                                |
|                                                 | I05720M           | 0.242                                       |                       | 32.6                           |
|                                                 | I05725M           | 0.207                                       | 0.224                 | 0.0730                         |
| <b>Group 2</b><br>Low Dose                      | I05702M           | 17.8                                        |                       |                                |
|                                                 | I05706M           | 11.4                                        |                       |                                |
|                                                 | I05717M           | 33.3                                        |                       | 78.8                           |
|                                                 | I05721M           | 69.6                                        | 33.0                  | 26.0                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 107                                         |                       |                                |
|                                                 | I05708M           | 61.9                                        |                       |                                |
|                                                 | I05710M           | 75.2                                        |                       |                                |
|                                                 | I05712M           | 137                                         |                       |                                |
|                                                 | I05716M           | 91.4                                        |                       | 27.5                           |
|                                                 | I05719M           | 107                                         | 96.6                  | 26.6                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 38.9                                        |                       |                                |
|                                                 | I05704M           | 92.5                                        |                       |                                |
|                                                 | I05711M           | 2.39                                        |                       |                                |
|                                                 | I05713M           | 322                                         |                       | 132                            |
|                                                 | I05722M           | 32.9                                        | 97.7                  | 129                            |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/20/99, 05/25/99, 05/27/99, 06/14/99, 06/17/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498, Madeline 041098  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/14/99 MCH  
Date of Analysis/Analyst: 05/20/99, 05/26/99, 05/28/99, 06/10/99 HOJ/MEE  
Date of Data Reduction/Analyst: 05/24/99, 05/27/99, 06/01/99, 06/11/99, 11/06/00 HOJ/MEE/KJH

**Filenames**

|                | <b>POAA</b>                    | <b>Dilutions</b> |
|----------------|--------------------------------|------------------|
| <b>Blks</b>    | A052099035, 36 & 98, 99        | 1/1              |
| <b>Grp 1</b>   | 052099047-51, 061099086        | 1/1              |
| <b>Grp 2</b>   | 052699025-27                   | 1/100            |
| <b>Grp 3</b>   | 052699028-32, 34-35, 052899104 | 1/100&200        |
| <b>Grp 4</b>   | 052699036-42                   | 1/10&100         |
| <b>MS, MSD</b> | 061099095-96                   | 1/1              |

**Sample Data**

**Week 22 MONKEY SERA**

| Group Dose                                      | Sample #           | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------------------------------------|--------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                      | H2O Blk-9          | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                                 | H2O Blk-10         | 1.0                   | 0.9582                     | 1                    | 42.5             | 0.0407                                | NA              | NA                       |
| Matrix Blk                                      | Rabbit Sera Blk-9  | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                                 | Rabbit Sera Blk-10 | 1.0                   | 0.9582                     | 1                    | 32.9             | 0.0315                                | NA              | NA                       |
| QC - 250 ppb                                    | RBS05149-MS-9      | 1.0                   | NA                         | 1                    | 261              | 104%                                  |                 |                          |
|                                                 | RBS05149-MSD-9     | 1.0                   | NA                         | 1                    | 273              | 109%                                  | 107%            | 4%                       |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M            | 0.62                  | 0.9582                     | 1                    | 254              | 0.393                                 |                 |                          |
|                                                 | I05714M            | 0.68                  | 0.9582                     | 1                    | 111              | 0.157                                 |                 |                          |
|                                                 | I05715M            | 0.53                  | 0.9582                     | 1                    | 58.8             | 0.106                                 |                 |                          |
|                                                 | I05718M            | 0.49                  | 0.9582                     | 1                    | 137              | 0.269                                 |                 |                          |
|                                                 | I05720M            | 0.31                  | 0.9582                     | 1                    | 120              | 0.372                                 |                 | 56.5                     |
|                                                 | I05725M            | 0.28                  | 0.9582                     | 1                    | 0.00             | <LOQ (0.0983 ug/mL)                   | 0.232           | 0.131                    |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M            | 0.75                  | 0.9582                     | 100                  | 547              | 69.8                                  |                 |                          |
|                                                 | I05706M            | 0.32                  | 0.9582                     | 100                  | 352              | 105                                   |                 | 32.1                     |
|                                                 | I05717M            | 0.68                  | 0.9582                     | 100                  | 408              | 57.5                                  | 77.6            | 24.9                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M            | 0.42                  | 0.9582                     | 100                  | 533              | 122                                   |                 |                          |
|                                                 | I05708M            | 0.55                  | 0.9582                     | 100                  | 366              | 63.7                                  |                 |                          |
|                                                 | I05710M            | 0.65                  | 0.9582                     | 100                  | 595              | 87.7                                  |                 |                          |
|                                                 | I05712M            | 0.54                  | 0.9582                     | 200                  | 472              | 168                                   |                 |                          |
|                                                 | I05716M            | 0.46                  | 0.9582                     | 100                  | 510              | 106                                   |                 | 34.5                     |
|                                                 | I05719M            | 0.60                  | 0.9582                     | 100                  | 533              | 85.2                                  | 105             | 36.3                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M            | 0.35                  | 0.9582                     | 10                   | 601              | 16.4                                  |                 |                          |
|                                                 | I05704M            | 0.28                  | 0.9582                     | 100                  | 271              | 92.7                                  |                 |                          |
|                                                 | I05711M            | 0.33                  | 0.9582                     | 10                   | 63.2             | 1.83                                  |                 |                          |
|                                                 | I05713M            | 0.56                  | 0.9582                     | 100                  | 563              | 96.3                                  |                 | 77.8                     |
|                                                 | I05722M            | 0.50                  | 0.9582                     | 100                  | 449              | 86.0                                  | 58.6            | 45.6                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 05/25/99, 05/27/99, 06/02/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Amelia 062498, Madeline 041098                                 |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/14/99 MCH                                                   |
| Date of Analysis/Analyst:           | 05/20/99, 05/26/99, 05/28/99, 06/10/99 HOJ/MEE                 |
| Date of Data Reduction/Analyst:     | 05/24/99, 05/27/99, 06/01/99, 06/11/99, 11/06/00 HOJ/MEE/KJH   |

**Sample Data**

**Week 22 MONKEY SERA**

| Group<br>Dose                                   | Sample #           | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|--------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-9          | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-10         | 0.0407                                      | NA                    | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-9  | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-10 | 0.0315                                      | NA                    | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-9      | 104%                                        |                       |                                |
|                                                 | RBS05149-MSD-9     | 109%                                        | 107%                  | 4%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M            | 0.393                                       |                       |                                |
|                                                 | I05714M            | 0.157                                       |                       |                                |
|                                                 | I05715M            | 0.106                                       |                       |                                |
|                                                 | I05718M            | 0.269                                       |                       |                                |
|                                                 | I05720M            | 0.372                                       |                       | 56.5                           |
|                                                 | I05725M            | <LOQ (0.0983 ug/mL)                         | 0.232                 | 0.131                          |
| <b>Group 2</b><br>Low Dose                      | I05702M            | 69.8                                        |                       |                                |
|                                                 | I05706M            | 105                                         |                       | 32.1                           |
|                                                 | I05717M            | 57.5                                        | 77.6                  | 24.9                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M            | 122                                         |                       |                                |
|                                                 | I05708M            | 63.7                                        |                       |                                |
|                                                 | I05710M            | 87.7                                        |                       |                                |
|                                                 | I05712M            | 168                                         |                       |                                |
|                                                 | I05716M            | 106                                         |                       | 34.5                           |
|                                                 | I05719M            | 85.2                                        | 105                   | 36.3                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M            | 16.4                                        |                       |                                |
|                                                 | I05704M            | 92.7                                        |                       |                                |
|                                                 | I05711M            | 1.83                                        |                       |                                |
|                                                 | I05713M            | 96.3                                        |                       | 77.8                           |
|                                                 | I05722M            | 86.0                                        | 58.6                  | 45.6                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/25/99, 05/27/99, 06/02/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 and ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 05/14/99 MCH  
Date of Analysis/Analyst: 05/20/99, 05/26/99, 05/28/99 HOJ/SAH/MEE  
Date of Data Reduction/Analyst: 05/24/99, 05/27/99, 06/01/99, 11/06/00 HOJ/MEE/KJH

**FileNames**

|                | <b>POAA</b>                     | <b>Dilutions</b> |
|----------------|---------------------------------|------------------|
| <b>Blks</b>    | A052099035, 36 & 98, 99         | 1/1              |
| <b>Grp 1</b>   | 061099087-90                    | 1/1              |
| <b>Grp 2</b>   | 052699045, 47, 052899105        | 1/100&200        |
| <b>Grp 3</b>   | 052699052-59                    | 1/100            |
| <b>Grp 4</b>   | 052699051, 63-64, 052899106-107 | 1/1,10,20,&200   |
| <b>MS, MSD</b> | 061099095-96                    | 1/1              |

**Sample Data**

**Week 24 MONKEY SERA**

| Group<br>Dose                                   | Sample #           | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|--------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-9          | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                                 | H2O Blk-10         | 1.0                      | 0.9582                           | 1                          | 42.5                   | 0.0407                                      | NA                    | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-9  | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                                 | Rabbit Sera Blk-10 | 1.0                      | 0.9582                           | 1                          | 32.9                   | 0.0315                                      | NA                    | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-9      | 1.0                      | NA                               | 1                          | 261                    | 104%                                        |                       |                                |
|                                                 | RBS05149-MSD-9     | 1.0                      | NA                               | 1                          | 273                    | 109%                                        | 107%                  | 4%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M            | 0.40                     | 0.9582                           | 1                          | Ext.                   | Ext                                         |                       |                                |
|                                                 | I05714M            | 0.58                     | 0.9582                           | 1                          | Ext                    | Ext                                         |                       |                                |
|                                                 | I05715M            | 0.31                     | 0.9582                           | 1                          | 5.55                   | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05718M            | 0.47                     | 0.9582                           | 1                          | 55.5                   | <LOQ (0.0983 ug/mL)                         |                       |                                |
|                                                 | I05720M            | 0.29                     | 0.9582                           | 1                          | 77.5                   | <LOQ (0.0983 ug/mL)                         |                       | NA                             |
|                                                 | I05725M            | 0.38                     | 0.9582                           | 1                          | 5.08                   | <LOQ (0.0983 ug/mL)                         | <LOQ                  | NA                             |
| <b>Group 2</b><br>Low Dose<br>3.0 mg/kg/day     | I05702M            | 0.61                     | 0.9582                           | 100                        | 64.9                   | 10.2                                        |                       |                                |
|                                                 | I05706M            | 0.69                     | 0.9582                           | 200                        | 527                    | 146                                         |                       | 95.3                           |
|                                                 | I05717M            | 0.43                     | 0.9582                           | 100                        | 270                    | 60.2                                        | 72.2                  | 68.8                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M            | 0.71                     | 0.9582                           | 100                        | 660                    | 89.1                                        |                       |                                |
|                                                 | I05708M            | 0.64                     | 0.9582                           | 100                        | 410                    | 61.4                                        |                       |                                |
|                                                 | I05710M            | 0.61                     | 0.9582                           | 100                        | 521                    | 81.8                                        |                       |                                |
|                                                 | I05712M            | 0.54                     | 0.9582                           | 100                        | 656                    | 116                                         |                       |                                |
|                                                 | I05716M            | 0.59                     | 0.9582                           | 100                        | 507                    | 82.3                                        |                       | 23.3                           |
|                                                 | I05719M            | 0.54                     | 0.9582                           | 100                        | 646                    | 115                                         | 90.9                  | 21.2                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M            | 0.57                     | 0.9582                           | 20                         | 606                    | 20.4                                        |                       |                                |
|                                                 | I05704M            | 0.72                     | 0.9582                           | 100                        | 679                    | 90.4                                        |                       |                                |
|                                                 | I05711M            | 0.52                     | 0.9582                           | 10                         | 233                    | 4.29                                        |                       |                                |
|                                                 | I05713M            | 0.63                     | 0.9582                           | 200                        | 596                    | 181                                         |                       | 119                            |
|                                                 | I05722M            | 0.43                     | 0.9582                           | 10                         | 765                    | 17.0                                        | 62.7                  | 74.3                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Ext: Samples lost during sample extraction.

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/25/99, 05/27/99, 06/02/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 and ETS-8-5.1                                        |
| Analytical Equipment System Number: | Amelia 062498                                                  |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 05/14/99 MCH                                                   |
| Date of Analysis/Analyst:           | 05/20/99, 05/26/99, 05/28/99 HOJ/SAH/MEE                       |
| Date of Data Reduction/Analyst:     | 05/24/99, 05/27/99, 06/01/99, 11/06/00 HOJ/MEE/KJH             |

**Sample Data**

**Week 24 MONKEY SERA**

| Group<br>Dose                                   | Sample #                                                       | Concentration<br>of POAA<br>ug/mL or % Rec.                                                            | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-9<br>H2O Blk-10                                        | <LOQ<br>0.0407                                                                                         | NA                    | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-9<br>Rabbit Sera Blk-10                        | <LOQ<br>0.0315                                                                                         | NA                    | NA                             |
| QC - 250 ppb                                    | RBS05149-MS-9<br>RBS05149-MSD-9                                | 104%<br>109%                                                                                           | 107%                  | 4%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M<br>I05714M<br>I05715M<br>I05718M<br>I05720M<br>I05725M | Ext<br>Ext<br><LOQ (0.0983 ug/mL)<br><LOQ (0.0983 ug/mL)<br><LOQ (0.0983 ug/mL)<br><LOQ (0.0983 ug/mL) |                       | NA<br>NA                       |
| <b>Group 2</b><br>Low Dose                      | I05702M<br>I05706M<br>I05717M                                  | 10.2<br>146<br>60.2                                                                                    | 72.2                  | 95.3<br>68.8                   |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M<br>I05708M<br>I05710M<br>I05712M<br>I05716M<br>I05719M | 89.1<br>61.4<br>81.8<br>116<br>82.3<br>115                                                             | 90.9                  | 23.3<br>21.2                   |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M<br>I05704M<br>I05711M<br>I05713M<br>I05722M            | 20.4<br>90.4<br>4.29<br>181<br>17.0                                                                    | 62.7                  | 119<br>74.3                    |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Ext: Samples lost during sample extraction.

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/25/99, 05/27/99, 06/02/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 04/29/99, 05/14/99 SAH  
Date of Analysis/Analyst: 05/17/99, 05/20/99, 05/26/99, 06/10/99 HOJ/SAH/MEE  
Date of Data Reduction/Analyst: 05/19/99, 05/24/99, 05/27/99, 06/11/99, 11/06/00 HOJ/MEE/KJH

**FileNames**

|                | <b>POAA</b>                              | <b>Dilutions</b>   |
|----------------|------------------------------------------|--------------------|
| <b>Blks</b>    | A051799055, 56 & 95, 96                  | 1/1                |
| <b>Grp 1</b>   | 051799067-70                             | 1/1                |
| <b>Grp 2</b>   | 052699015-17                             | 1/200              |
| <b>Grp 3</b>   | 052099120-121, 123 & 052699020           | 1/100&200          |
| <b>Grp 4</b>   | 051799086, 052099111, 126 & 052699021-22 | 1/1,10,20,100,&500 |
| <b>MS, MSD</b> | 061099093-94                             | 1/1                |

**Sample Data**

**Week 26 MONKEY SERA**

| Group Dose     | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|----------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk     | H2O Blk-3         | NA                    | 0.9582                     | 1                    | 0.00             | <LOQ (0.0250 ug/mL)                   |                 |                          |
| Method Blk     | H2O Blk-4         | NA                    | 0.9582                     | 1                    | 0.00             | <LOQ (0.0250 ug/mL)                   | <LOQ            | NA                       |
| Matrix Blk     | Rabbit Sera Blk-3 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0250 ug/mL)                   |                 |                          |
| Matrix Blk     | Rabbit Sera Blk-4 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0250 ug/mL)                   | <LOQ            | NA                       |
| QC - 250 ppb   | MKS05149-MS-3     | 1.0                   | NA                         | 1                    | 208              | 83%                                   |                 |                          |
|                | MKS05149-MSD-3    | 1.0                   | NA                         | 1                    | 226              | 91%                                   | 87%             | 9%                       |
| <b>Group 1</b> | I05709M           | 0.58                  | 0.9582                     | 1                    | 285              | 0.471                                 |                 |                          |
| Control        | I05714M           | 0.66                  | 0.9582                     | 1                    | 74.1             | 0.108                                 |                 |                          |
| 0.0 mg/kg/day  | I05715M           | 0.57                  | 0.9582                     | 1                    | 60.3             | 0.101                                 |                 |                          |
|                | I05718M           | 0.50                  | 0.9582                     | 1                    | 108              | 0.206                                 |                 |                          |
|                | I05720M           | 0.50                  | 0.9582                     | 1                    | 161              | 0.308                                 |                 | 74.5                     |
|                | I05725M           | 0.52                  | 0.9582                     | 1                    | 33.8             | 0.0623                                | 0.209           | 0.156                    |
| <b>Group 2</b> | I05702M           | 0.62                  | 0.9582                     | 200                  | 403              | 125                                   |                 |                          |
| Mid Dose       | I05706M           | 0.61                  | 0.9582                     | 200                  | 450              | 141                                   |                 | 23.3                     |
| 3.0 mg/kg/day  | I05717M           | 0.61                  | 0.9582                     | 200                  | 279              | 87.6                                  | 118             | 27.5                     |
| <b>Group 3</b> | I05707M           | 0.49                  | 0.9582                     | 100                  | 399              | 77.9                                  |                 |                          |
| Mid-High Dose  | I05708M           | 0.58                  | 0.9582                     | 100                  | 335              | 55.4                                  |                 |                          |
| 10 mg/kg/day   | I05710M           | 0.65                  | 0.9582                     | 200                  | 327              | 96.5                                  |                 | 21.8                     |
|                | I05719M           | 0.38                  | 0.9582                     | 100                  | 316              | 79.6                                  | 77.4            | 16.9                     |
| <b>Group 4</b> | I05703M           | 0.37                  | 0.9582                     | 10                   | 429              | 11.1                                  |                 |                          |
| High Dose      | I05704M           | 0.70                  | 0.9582                     | 100                  | 451              | 61.7                                  |                 |                          |
| 30 mg/kg/day   | I05711M           | 0.36                  | 0.9582                     | 1                    | 565              | 1.50                                  |                 |                          |
|                | I05713M           | 0.63                  | 0.9582                     | 500                  | 393              | 299                                   |                 | 162                      |
|                | I05722M           | 0.51                  | 0.9582                     | 20                   | 422              | 15.8                                  | 77.8            | 126                      |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 05/19/98, 05/27/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 04/29/99, 05/14/99 SAH  
Date of Analysis/Analyst: 05/17/99, 05/20/99, 05/26/99, 06/10/99 HOJ/SAH/MEE  
Date of Data Reduction/Analyst: 05/19/99, 05/24/99, 05/27/99, 06/11/99, 11/06/00 HOJ/MEE/KJH

**Sample Data**

**Week 26 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-3         | <LOQ (0.0250 ug/mL)                         |                       |                                |
| Method Blk                                      | H2O Blk-4         | <LOQ (0.0250 ug/mL)                         | <LOQ                  | NA                             |
| Matrix Blk                                      | Rabbit Sera Blk-3 | <LOQ (0.0250 ug/mL)                         |                       |                                |
| Matrix Blk                                      | Rabbit Sera Blk-4 | <LOQ (0.0250 ug/mL)                         | <LOQ                  | NA                             |
| QC - 250 ppb                                    | MKS05149-MS-3     | 83%                                         |                       |                                |
|                                                 | MKS05149-MSD-3    | 91%                                         | 87%                   | 9%                             |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M           | 0.471                                       |                       |                                |
|                                                 | I05714M           | 0.108                                       |                       |                                |
|                                                 | I05715M           | 0.101                                       |                       |                                |
|                                                 | I05718M           | 0.206                                       |                       |                                |
|                                                 | I05720M           | 0.308                                       |                       | 74.5                           |
|                                                 | I05725M           | 0.0623                                      | 0.209                 | 0.156                          |
| <b>Group 2</b><br>Mid Dose                      | I05702M           | 125                                         |                       |                                |
|                                                 | I05706M           | 141                                         |                       | 23.3                           |
|                                                 | I05717M           | 87.6                                        | 118                   | 27.5                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M           | 77.9                                        |                       |                                |
|                                                 | I05708M           | 55.4                                        |                       |                                |
|                                                 | I05710M           | 96.5                                        |                       | 21.8                           |
|                                                 | I05719M           | 79.6                                        | 77.4                  | 16.9                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M           | 11.1                                        |                       |                                |
|                                                 | I05704M           | 61.7                                        |                       |                                |
|                                                 | I05711M           | 1.50                                        |                       |                                |
|                                                 | I05713M           | 299                                         |                       | 162                            |
|                                                 | I05722M           | 15.8                                        | 77.8                  | 126                            |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/19/98, 05/27/99, 06/14/99, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 06/22/99 LAC

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 04/29/99 SAH  
Date of Analysis/Analyst: 04/30/99, 05/03/99, 05/04/99, 05/05/99 KJH/MEE/HOJ  
Date of Data Reduction/Analyst: 05/03/99, 05/04/99, 05/05/99, 05/25/99, 11/02/00, 11/06/00 HOJ/KJH

**FileNames**

**Blks**  
**Grp 1**  
**Grp 2**  
**Grp 3**  
**Grp 4**  
**MS, MSD**

**POAA**

A050399003, 4 & 42, 43  
043099065-70  
050399016-18,36-37  
050399021-28, 050599116  
050399029-32, 050499016, 050599117  
043099073-74

**Dilutions**

1/1  
1/1  
1/100  
1/100&1000  
1/100&1000  
1/1

**Sample Data**

**Week 26, 27 MONKEY SERA**

| Group Dose                                                           | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|----------------------------------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                                                           | H2O Blk-1         | NA                    | 0.9582                     | 1                    | 0.00             | <LOQ (0.00983 ug/mL)                  |                 |                          |
|                                                                      | H2O Blk-2         | NA                    | 0.9582                     | 1                    | 0.00             | <LOQ (0.00983 ug/mL)                  | <LOQ            | NA                       |
| Matrix Blk                                                           | Rabbit Sera Blk-1 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.00983 ug/mL)                  |                 |                          |
|                                                                      | Rabbit Sera Blk-2 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.00983 ug/mL)                  | <LOQ            | NA                       |
| QC - 250 ppb                                                         | I05709M-MS        | 1.0                   | NA                         | 1                    | 592              | 79%                                   |                 |                          |
|                                                                      | I05714M-MS        | 1.0                   | NA                         | 1                    | 500              | 108%                                  | 94%             | 17%                      |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day                           | I05709M Wk27      | 1.0                   | 0.9582                     | 1                    | 393              | 0.377                                 |                 |                          |
|                                                                      | I05714M Wk27      | 1.0                   | 0.9582                     | 1                    | 229              | 0.219                                 |                 |                          |
|                                                                      | I05715M Wk27      | 1.0                   | 0.9582                     | 1                    | 98.7             | 0.0945                                |                 |                          |
|                                                                      | I05718M Wk26      | 0.50                  | 0.9582                     | 1                    | 108              | 0.206                                 |                 |                          |
|                                                                      | I05720M Wk26      | 0.50                  | 0.9582                     | 1                    | 161              | 0.308                                 |                 | 47.1                     |
|                                                                      | I05725M Wk27      | 1.0                   | 0.9582                     | 1                    | 142              | 0.136                                 | 0.223           | 0.105                    |
| <b>Group 2</b><br>Mid Dose<br>3.0 mg/kg/day<br><br>5721M replacement | I05702M Wk27      | 1.0                   | 0.9582                     | 100                  | 500              | 47.9                                  |                 |                          |
|                                                                      | I05706M Wk27      | 1.0                   | 0.9582                     | 100                  | 673              | 64.5                                  |                 |                          |
|                                                                      | I05717M Wk27      | 1.0                   | 0.9582                     | 100                  | 460              | 44.1                                  |                 |                          |
|                                                                      | I05721M Moribund  | 1.0                   | 0.9582                     | 100                  | 480              | 46.0                                  |                 | 17.4                     |
|                                                                      | I05723M           | 1.0                   | 0.9582                     | 100                  | 626              | 60.0                                  | 52.5            | 9.14                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day                      | I05707M Wk27      | 1.0                   | 0.9582                     | 1000                 | 126              | 121                                   |                 |                          |
|                                                                      | I05708M Wk27      | 1.0                   | 0.9582                     | 100                  | 335              | 32.1                                  |                 |                          |
|                                                                      | I05710M Wk27      | 1.0                   | 0.9582                     | 100                  | 497              | 47.6                                  |                 |                          |
|                                                                      | I05712M Wk26      | 0.50                  | 0.9582                     | 100                  | 525              | 101                                   |                 |                          |
|                                                                      | I05716M Wk26      | 0.60                  | 0.9582                     | 100                  | 489              | 78.1                                  |                 | 44.7                     |
|                                                                      | I05719M Wk27      | 1.0                   | 0.9582                     | 100                  | 680              | 65.2                                  | 74.1            | 33.1                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day                          | I05703M Wk27      | 1.0                   | 0.9582                     | 100                  | 78.1             | 7.48                                  |                 |                          |
|                                                                      | I05704M Wk27      | 1.0                   | 0.9582                     | 100                  | 612              | 58.6                                  |                 |                          |
|                                                                      | I05711M Wk27      | 1.0                   | 0.9582                     | 100                  | 9.15             | 0.877                                 |                 |                          |
|                                                                      | I05713M Wk27      | 1.0                   | 0.9582                     | 1000                 | 192              | 184                                   |                 |                          |
|                                                                      | I05722M Wk27      | 1.0                   | 0.9582                     | 100                  | 69.4             | 6.65                                  |                 | 151                      |
|                                                                      | I05724M Moribund  | 1.0                   | 0.9582                     | 1000                 | 511              | 489                                   | 51.5            | 77.6                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA = Perfluorooctanoate**

Date Entered/By: 05/04/99, 05/05/99, 05/25/99, 11/03/00, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                    |
|-------------------------------------|--------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys     |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                    |
| Matrix:                             | Monkey Sera                                                        |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                              |
| Analytical Equipment System Number: | Amelia 062498                                                      |
| Instrument Software/Version:        | MassLynx 3.2                                                       |
| Filename:                           | See Attachments                                                    |
| R-Squared Value:                    | See Attachments                                                    |
| Slope:                              | See Attachments                                                    |
| Y-Intercept:                        | See Attachments                                                    |
| Date of Extraction/Analyst:         | 04/29/99 SAH                                                       |
| Date of Analysis/Analyst:           | 04/30/99, 05/03/99, 05/04/99, 05/05/99 KJH/MEE/HOJ                 |
| Date of Data Reduction/Analyst:     | 05/03/99, 05/04/99, 05/05/99, 05/25/99, 11/02/00, 11/06/00 HOJ/KJH |

**Sample Data**

**Week 26, 27 MONKEY SERA**

| Group<br>Dose                                   | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|-------------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                                      | H2O Blk-1         | <LOQ (0.00983 ug/mL)                        | <LOQ                  | NA                             |
|                                                 | H2O Blk-2         | <LOQ (0.00983 ug/mL)                        |                       |                                |
| Matrix Blk                                      | Rabbit Sera Blk-1 | <LOQ (0.00983 ug/mL)                        | <LOQ                  | NA                             |
|                                                 | Rabbit Sera Blk-2 | <LOQ (0.00983 ug/mL)                        |                       |                                |
| QC - 250 ppb                                    | I05709M-MS        | 79%                                         | 94%                   | 17%                            |
|                                                 | I05714M-MS        | 108%                                        |                       |                                |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day      | I05709M Wk27      | 0.377                                       | 0.223                 | 47.1<br>0.105                  |
|                                                 | I05714M Wk27      | 0.219                                       |                       |                                |
|                                                 | I05715M Wk27      | 0.0945                                      |                       |                                |
|                                                 | I05718M Wk26      | 0.206                                       |                       |                                |
|                                                 | I05720M Wk26      | 0.308                                       |                       |                                |
|                                                 | I05725M Wk27      | 0.136                                       |                       |                                |
| <b>Group 2</b><br>Mid Dose                      | I05702M Wk27      | 47.9                                        | 52.5                  | 17.4<br>9.14                   |
|                                                 | I05706M Wk27      | 64.5                                        |                       |                                |
|                                                 | I05717M Wk27      | 44.1                                        |                       |                                |
|                                                 | I05721M Moribund  | 46.0                                        |                       |                                |
|                                                 | I05723M           | 60.0                                        |                       |                                |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day | I05707M Wk27      | 121                                         | 74.1                  | 44.7<br>33.1                   |
|                                                 | I05708M Wk27      | 32.1                                        |                       |                                |
|                                                 | I05710M Wk27      | 47.6                                        |                       |                                |
|                                                 | I05712M Wk26      | 101                                         |                       |                                |
|                                                 | I05716M Wk26      | 78.1                                        |                       |                                |
|                                                 | I05719M Wk27      | 65.2                                        |                       |                                |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day     | I05703M Wk27      | 7.48                                        | 51.5                  | 151<br>77.6                    |
|                                                 | I05704M Wk27      | 58.6                                        |                       |                                |
|                                                 | I05711M Wk27      | 0.877                                       |                       |                                |
|                                                 | I05713M Wk27      | 184                                         |                       |                                |
|                                                 | I05722M Wk27      | 6.65                                        |                       |                                |
|                                                 | I05724M Moribund  | 489                                         |                       |                                |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 05/04/99, 05/05/99, 05/25/99, 11/03/00, 11/06/00 LAC

Date Verified/ By: 06/21/99 EAD, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 06/11/99 RWW  
Date of Analysis/Analyst: 06/22/99, 06/23/99 DRB/MEE  
Date of Data Reduction/Analyst: 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH

**FileNames**

|                | <b>POAA</b>          | <b>Dilutions</b> |
|----------------|----------------------|------------------|
| <b>Blks</b>    | S062299003-5 & 44-46 | 1/1              |
| <b>Grp 1</b>   | 062299016-17         | 1/1              |
| <b>Grp 3</b>   | 062399016-17         | 1/50             |
| <b>MS, MSD</b> | 062299040-41         | 1/1              |

**Sample Data**

**Week 28 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.150                  | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 1.0                      | NA                               | 1                          | 264                    | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 1.0                      | NA                               | 1                          | 261                    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.53                     | 0.9582                           | 1                          | 84.8                   | 0.153                                       |                       | 21.6                           |
|                                          | I05720M           | 0.56                     | 0.9582                           | 1                          | 122                    | 0.209                                       | 0.181                 | 0.0391                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 0.61                     | 0.9582                           | 50                         | 394                    | 30.9                                        |                       | 27.2                           |
|                                          | I05716M           | 0.60                     | 0.9582                           | 50                         | 262                    | 21.0                                        | 25.9                  | 7.07                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA = Perfluorooctanoate**

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 06/11/99 RWW                                                   |
| Date of Analysis/Analyst:           | 06/22/99, 06/23/99 DRB/MEE                                     |
| Date of Data Reduction/Analyst:     | 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH                       |

**Sample Data**

**Week 28 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.153                                       |                       | 21.6                           |
|                                          | I05720M           | 0.209                                       | 0.181                 | 0.0391                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 30.9                                        |                       | 27.2                           |
|                                          | I05716M           | 21.0                                        | 25.9                  | 7.07                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 06/11/99 RWW  
Date of Analysis/Analyst: 06/22/99, 06/23/99 DRB/MEE  
Date of Data Reduction/Analyst: 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH

| FileNames | POAA                 | Dilutions |
|-----------|----------------------|-----------|
| Blks      | S062299003-5 & 44-46 | 1/1       |
| Grp 1     | 062299018-19         | 1/1       |
| Grp 3     | 062399020-21         | 1/50      |
| MS, MSD   | 062299040-41         | 1/1       |

**Sample Data**

**Week 30 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
|                                          | H2O Blk-2         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
| Matrix Blk                               | Rabbit Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
|                                          | Rabbit Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
| Matrix Blk                               | Monkey Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.150                  | <LOQ                                        | <LOQ                  | NA                             |
|                                          | Monkey Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
| QC - 250 ppb                             | MKS06119-MS-1     | 1.0                      | NA                               | 1                          | 264                    | 106%                                        | 105%                  | 1%                             |
|                                          | MKS06119-MSD-1    | 1.0                      | NA                               | 1                          | 261                    | 104%                                        |                       |                                |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.65                     | 0.9582                           | 1                          | 86.1                   | 0.127                                       | 0.144                 | 16.6                           |
|                                          | I05720M           | 0.62                     | 0.9582                           | 1                          | 104                    | 0.161                                       |                       |                                |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 0.75                     | 0.9582                           | 50                         | 234                    | 14.9                                        | 11.3                  | 45.2                           |
|                                          | I05716M           | 0.55                     | 0.9582                           | 50                         | 88.4                   | 7.70                                        |                       |                                |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 06/11/99 RWW                                                   |
| Date of Analysis/Analyst:           | 06/22/99, 06/23/99 DRB/MEE                                     |
| Date of Data Reduction/Analyst:     | 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH                       |

**Sample Data**

**Week 30 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.127                                       |                       | 16.6                           |
|                                          | I05720M           | 0.161                                       | 0.144                 | 0.0238                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 14.9                                        |                       | 45.2                           |
|                                          | I05716M           | 7.70                                        | 11.3                  | 5.11                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 06/11/99 RWW  
Date of Analysis/Analyst: 06/22/99, 06/23/99 DRB/MEE  
Date of Data Reduction/Analyst: 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH

| FileNames      | POAA                 | Dilutions |
|----------------|----------------------|-----------|
| <b>Blks</b>    | S062299003-5 & 44-46 | 1/1       |
| <b>Grp 1</b>   | 062299022-23         | 1/1       |
| <b>Grp 3</b>   | 062399024-25         | 1/50      |
| <b>MS, MSD</b> | 062299040-41         | 1/1       |

**Sample Data**

**Week 32 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.150                  | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 1.0                      | NA                               | 1                          | 264                    | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 1.0                      | NA                               | 1                          | 261                    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.38                     | 0.9582                           | 1                          | 37.4                   | 0.0943                                      |                       | 19.8                           |
|                                          | I05720M           | 0.48                     | 0.9582                           | 1                          | 62.6                   | 0.125                                       | 0.110                 | 0.0216                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 0.72                     | 0.9582                           | 50                         | 156                    | 10.4                                        |                       | 51.2                           |
|                                          | I05716M           | 0.80                     | 0.9582                           | 50                         | 80.9                   | 4.85                                        | 7.60                  | 3.90                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 06/11/99 RWW                                                   |
| Date of Analysis/Analyst:           | 06/22/99, 06/23/99 DRB/MEE                                     |
| Date of Data Reduction/Analyst:     | 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH                       |

**Sample Data**

**Week 32 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.0943                                      |                       | 19.8                           |
|                                          | I05720M           | 0.125                                       | 0.110                 | 0.0216                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 10.4                                        |                       | 51.2                           |
|                                          | I05716M           | 4.85                                        | 7.60                  | 3.90                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 06/11/99 RWW  
Date of Analysis/Analyst: 06/22/99, 06/23/99 DRB/MEE  
Date of Data Reduction/Analyst: 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH

**FileNames**

|                | <b>POAA</b>          | <b>Dilutions</b> |
|----------------|----------------------|------------------|
| <b>Blks</b>    | S062299003-5 & 44-46 | 1/1              |
| <b>Grp 1</b>   | 062299024-25         | 1/1              |
| <b>Grp 3</b>   | 062399028-29         | 1/50             |
| <b>MS, MSD</b> | 062299040-41         | 1/1              |

**Sample Data**

**Week 34 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.150                  | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 1.0                      | NA                               | 1                          | 264                    | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 1.0                      | NA                               | 1                          | 261                    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.43                     | 0.9582                           | 1                          | 30.5                   | 0.0680                                      |                       | 29.7                           |
|                                          | I05720M           | 0.45                     | 0.9582                           | 1                          | 49.0                   | 0.104                                       | 0.0861                | 0.0256                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 0.76                     | 0.9582                           | 50                         | 86.4                   | 5.45                                        |                       | 52.7                           |
|                                          | I05716M           | 0.64                     | 0.9582                           | 50                         | 33.3                   | 2.49                                        | 3.97                  | 2.09                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 06/11/99 RWW                                                   |
| Date of Analysis/Analyst:           | 06/22/99, 06/23/99 DRB/MEE                                     |
| Date of Data Reduction/Analyst:     | 06/23/99, 06/24/99, 11/06/00 DRB/MEE/KJH                       |

**Sample Data**

**Week 34 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ                                        |                       |                                |
|                                          | H2O Blk-2         | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Rabbit Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ                                        |                       |                                |
|                                          | Monkey Sera Blk-2 | <LOQ                                        | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS06119-MS-1     | 106%                                        |                       |                                |
|                                          | MKS06119-MSD-1    | 104%                                        | 105%                  | 1%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.0680                                      |                       | 29.7                           |
|                                          | I05720M           | 0.104                                       | 0.0861                | 0.0256                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 5.45                                        |                       | 52.7                           |
|                                          | I05716M           | 2.49                                        | 3.97                  | 2.09                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 06/23/99, 06/25/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 07/13/99 MCH  
Date of Analysis/Analyst: 07/14/99, 07/21/99 GML/DRB  
Date of Data Reduction/Analyst: 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH

|                |                                |                  |
|----------------|--------------------------------|------------------|
| FileNames      | <b>POAA</b>                    | <b>Dilutions</b> |
| <b>Blks</b>    | S071499003, 4, 16-17, & 39, 40 | 1/1              |
| <b>Grp 1</b>   | 071499021-22                   | 1/1              |
| <b>Grp 3</b>   | 072199096-97                   | 1/10             |
| <b>MS, MSD</b> | 071499018-19                   | 1/1              |
|                | <b>Box 99-130</b>              |                  |

**Sample Data**

**Week 36 MONKEY SERA**

| Group<br>Dose          | Sample #          | Extraction<br>Vol. Ratio | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------|-------------------|--------------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk             | H2O Blk-1         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                        | H2O Blk-2         | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk             | Rabbit Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                        | Rabbit Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk             | Monkey Sera Blk-1 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                        | Monkey Sera Blk-2 | 1.0                      | 0.9582                           | 1                          | 0.00                   | <LOQ (0.0248 ug/mL)                         |                       |                                |
| QC - 250 ppb           | MKS07139-MS-1     | 1.0                      | NA                               | 1                          | 228                    | 92%                                         | 88%                   | 9%                             |
|                        | MKS07139-MSD-1    | 1.0                      | NA                               | 1                          | 208                    | 84%                                         |                       |                                |
| <b>Group 1</b>         | I05718M           | 0.45                     | 0.9582                           | 1                          | 37.3                   | 0.0795                                      | 0.111                 | 40.1                           |
| Control, 0.0 mg/kg/day | I05720M           | 0.48                     | 0.9582                           | 1                          | 71.4                   | 0.142                                       |                       | 0.0445                         |
| <b>Group 3</b>         | I05712M           | 0.64                     | 0.9582                           | 10                         | 273                    | 4.08                                        | 2.75                  | 68.4                           |
|                        | I05716M           | 0.65                     | 0.9582                           | 10                         | 96.3                   | 1.42                                        |                       | 1.88                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA = Perfluorooctanoate**

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 07/13/99 MCH                                                   |
| Date of Analysis/Analyst:           | 07/14/99, 07/21/99 GML/DRB                                     |
| Date of Data Reduction/Analyst:     | 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH                       |

**Sample Data**

**Week 36 MONKEY SERA**

| Group<br>Dose                            | Sample #                               | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|----------------------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1<br>H2O Blk-2                 | <LOQ (0.0248 ug/mL)<br><LOQ (0.0248 ug/mL)  | <LOQ                  | NA                             |
| Matrix Blk                               | Rabbit Sera Blk-1<br>Rabbit Sera Blk-2 | <LOQ (0.0248 ug/mL)<br><LOQ (0.0248 ug/mL)  | <LOQ                  | NA                             |
| Matrix Blk                               | Monkey Sera Blk-1<br>Monkey Sera Blk-2 | <LOQ (0.0248 ug/mL)<br><LOQ (0.0248 ug/mL)  | <LOQ                  | NA                             |
| QC - 250 ppb                             | MKS07139-MS-1<br>MKS07139-MSD-1        | 92%<br>84%                                  | 88%                   | 9%                             |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M<br>I05720M                     | 0.0795<br>0.142                             | 0.111                 | 40.1<br>0.0445                 |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M<br>I05716M                     | 4.08<br>1.42                                | 2.75                  | 68.4<br>1.88                   |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 07/13/99 MCH  
Date of Analysis/Analyst: 07/14/99, 07/21/99 GML/DRB  
Date of Data Reduction/Analyst: 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH

FileNames  
**POAA**  
**Blks** S071499003, 4, 16-17, & 39, 40  
**Grp 1** 071499027-28  
**Grp 3** 071499030, 072199098  
**MS, MSD** 071499018-19  
**Box** 99-130  
**Dilutions**  
1/1  
1/1  
1/1&10  
1/1

**Sample Data**

**Week 38 MONKEY SERA**

| Group Dose             | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk             | H2O Blk-1         | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                        | H2O Blk-2         | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| Matrix Blk             | Rabbit Sera Blk-1 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                        | Rabbit Sera Blk-2 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| Matrix Blk             | Monkey Sera Blk-1 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                        | Monkey Sera Blk-2 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| QC - 250 ppb           | MKS07139-MS-1     | 1.0                   | NA                         | 1                    | 228              | 92%                                   | 88%             | 9%                       |
|                        | MKS07139-MSD-1    | 1.0                   | NA                         | 1                    | 208              | 84%                                   |                 |                          |
| <b>Group 1</b>         | I05718M           | 0.63                  | 0.9582                     | 1                    | 46.8             | 0.0711                                | 0.0941          | 34.5                     |
| Control, 0.0 mg/kg/day | I05720M           | 0.49                  | 0.9582                     | 1                    | 59.8             | 0.117                                 |                 | 0.0324                   |
| <b>Group 3</b>         | I05712M           | 0.75                  | 0.9582                     | 10                   | 221              | 2.83                                  | 1.84            | 76.5                     |
| 10 mg/kg/day           | I05716M           | 0.75                  | 0.9582                     | 1                    | 659              | 0.842                                 |                 | 1.40                     |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 07/13/99 MCH                                                   |
| Date of Analysis/Analyst:           | 07/14/99, 07/21/99 GML/DRB                                     |
| Date of Data Reduction/Analyst:     | 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH                       |

**Sample Data**

**Week 38 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | H2O Blk-2         | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | Rabbit Sera Blk-2 | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | Monkey Sera Blk-2 | <LOQ (0.0248 ug/mL)                         |                       |                                |
| QC - 250 ppb                             | MKS07139-MS-1     | 92%                                         | 88%                   | 9%                             |
|                                          | MKS07139-MSD-1    | 84%                                         |                       |                                |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.0711                                      | 0.0941                | 34.5                           |
|                                          | I05720M           | 0.117                                       |                       | 0.0324                         |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 2.83                                        | 1.84                  | 76.5                           |
|                                          | I05716M           | 0.842                                       |                       | 1.40                           |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Sera  
Method/Revision: ETS-8-4.1 & ETS-8-5.1  
Analytical Equipment System Number: Soup 020199, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Date of Extraction/Analyst: 07/13/99 MCH  
Date of Analysis/Analyst: 07/14/99, 07/21/99 GML/DRB  
Date of Data Reduction/Analyst: 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH

|                |                                |                  |
|----------------|--------------------------------|------------------|
| FileNames      | <b>POAA</b>                    | <b>Dilutions</b> |
| <b>Blks</b>    | S071499003, 4, 16-17, & 39, 40 | 1/1              |
| <b>Grp 1</b>   | 071499033-34                   | 1/1              |
| <b>Grp 3</b>   | 071499036, 072199099           | 1/1&2            |
| <b>MS, MSD</b> | 071499018-19                   | 1/1              |
|                | <b>Box 99-130</b>              |                  |

**Sample Data**

**Week 40 MONKEY SERA**

| Group Dose                               | Sample #          | Extraction Vol. Ratio | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|------------------------------------------|-------------------|-----------------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk                               | H2O Blk-1         | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                                          | H2O Blk-2         | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| Matrix Blk                               | Rabbit Sera Blk-1 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                                          | Rabbit Sera Blk-2 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| Matrix Blk                               | Monkey Sera Blk-1 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   | <LOQ            | NA                       |
|                                          | Monkey Sera Blk-2 | 1.0                   | 0.9582                     | 1                    | 0.00             | <LOQ (0.0248 ug/mL)                   |                 |                          |
| QC - 250 ppb                             | MKS07139-MS-1     | 1.0                   | NA                         | 1                    | 228              | 92%                                   | .88%            | 9%                       |
|                                          | MKS07139-MSD-1    | 1.0                   | NA                         | 1                    | 208              | 84%                                   |                 |                          |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.37                  | 0.9582                     | 1                    | 27.8             | 0.0719                                | 0.0738          | 3.47<br>0.00256          |
|                                          | I05720M           | 0.50                  | 0.9582                     | 1                    | 39.4             | 0.0756                                |                 |                          |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 0.49                  | 0.9582                     | 2                    | 452              | 1.77                                  | 1.18            | 69.8<br>0.827            |
|                                          | I05716M           | 0.36                  | 0.9582                     | 1                    | 225              | 0.600                                 |                 |                          |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

**POAA** = Perfluorooctanoate

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Sera                                                    |
| Method/Revision:                    | ETS-8-4.1 & ETS-8-5.1                                          |
| Analytical Equipment System Number: | Soup 020199, Amelia 062498                                     |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Date of Extraction/Analyst:         | 07/13/99 MCH                                                   |
| Date of Analysis/Analyst:           | 07/14/99, 07/21/99 GML/DRB                                     |
| Date of Data Reduction/Analyst:     | 07/15/99, 07/22/99, 11/06/00 GML/DRB/KJH                       |

**Sample Data**

**Week 40 MONKEY SERA**

| Group<br>Dose                            | Sample #          | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------|-------------------|---------------------------------------------|-----------------------|--------------------------------|
| Method Blk                               | H2O Blk-1         | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | H2O Blk-2         | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk                               | Rabbit Sera Blk-1 | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | Rabbit Sera Blk-2 | <LOQ (0.0248 ug/mL)                         |                       |                                |
| Matrix Blk                               | Monkey Sera Blk-1 | <LOQ (0.0248 ug/mL)                         | <LOQ                  | NA                             |
|                                          | Monkey Sera Blk-2 | <LOQ (0.0248 ug/mL)                         |                       |                                |
| QC - 250 ppb                             | MKS07139-MS-1     | 92%                                         | 88%                   | 9%                             |
|                                          | MKS07139-MSD-1    | 84%                                         |                       |                                |
| <b>Group 1</b><br>Control, 0.0 mg/kg/day | I05718M           | 0.0719                                      | 0.0738                | 3.47<br>0.00256                |
|                                          | I05720M           | 0.076                                       |                       |                                |
| <b>Group 3</b><br>10 mg/kg/day           | I05712M           | 1.77                                        | 1.18                  | 69.8<br>0.827                  |
|                                          | I05716M           | 0.600                                       |                       |                                |

Limit of Quantitation (LOQ): 0.0137 ug/mL

Correction factors not applicable for MS/MSD QC data

POAA = Perfluorooctanoate

Date Entered/By: 07/20/99, 07/23/99, 11/06/00 LAC

Date Verified/ By: 08/04/99 GML, 11/09/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

**Sera QC Summary - TOX026**

|            |                 | PFOA |    |
|------------|-----------------|------|----|
| 11/13/1998 | RBS11138-MS     | 109% | ** |
|            | RBS11138-MSD    | 100% | ** |
| 01/08/1999 | RBS01089-MS     | 100% |    |
|            | RBS01089-MSD    | 98%  |    |
| 04/29/1999 | I05709M-MS      | 79%  |    |
|            | I05714M-MS      | 108% |    |
| 05/12/1999 | RBS05129-MS-3   | 80%  |    |
|            | RBS05129-MSD-3  | 79%  |    |
|            | RBS05129-MS-4   | 92%  | ** |
|            | RBS05129-MSD-4  | 85%  | ** |
|            | RBS05129-MS-7   | 103% | ** |
|            | RBS05129-MSD-7  | 106% | ** |
|            | RBS05129-MS-8   | 103% |    |
|            | RBS05129-MSD-8  | 127% |    |
| 05/14/1999 | RBS05149-MS-11  | 116% |    |
|            | RBS05149-MSD-11 | 95%  |    |
|            | RBS05149-MS-12  | 99%  |    |
|            | RBS05149-MSD-12 | 80%  |    |
|            | RBS05149-MS-9   | 104% |    |
|            | RBS05149-MSD-9  | 109% |    |
|            | MKS05149-MS-3   | 83%  |    |
|            | MKS05149-MSD-3  | 91%  |    |
| 06/11/1999 | MKS06119-MS-1   | 106% |    |
|            | MKS06119-MSD-1  | 104% |    |
| 07/13/1999 | MKS07139-MS-1   | 92%  |    |
|            | MKS07139-MSD-1  | 84%  |    |

\*\*Bracketed by a CCV not within method criteria (31%).

NA = Not Applicable

NR = Not Reported

|                          |     |                      |
|--------------------------|-----|----------------------|
| Average of Monkey Spikes | 93% | Used in Final Report |
| Standard Deviation       | 11% |                      |
| Grubbs outliers?         | No  |                      |
| Number of monkey spikes  | 8   | Used in Final Report |

|                       |     |               |
|-----------------------|-----|---------------|
| Average of All Spikes | 97% | Data not used |
| Standard Deviation    | 12% |               |
| Grubbs outliers?      | No  |               |
| Number of spikes      | 26  | Data not used |

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Liver  
Method/Revision: FACT-M-1.1 & FACT-M-2.1  
Analytical Equipment System Number: Soup 020199  
Instrument Software/Version: MassLynx 3.2  
Date of Extraction/Analyst: 06/21/99 SAH  
Date of Analysis/Analyst: 06/23/99, 07/28/99, 10/05/99 DRB/IAS  
Date of Data Reduction/Analyst: 06/25/99, 07/29/99, 10/06/99, 11/02/00 DRB/IAS/KJH

FileNames

|                |                           |                      |
|----------------|---------------------------|----------------------|
| <b>Blks</b>    | <b>POAA</b>               | <b>Dilutions</b>     |
|                | S062399047-48 & 89-90     | 1/1                  |
| <b>Grp 1</b>   | 062399059-62              | 1/1                  |
| <b>Grp 2</b>   | 062399065-68              | 1/100                |
| <b>Grp 3</b>   | 062399071-74              | 1/50,100             |
| <b>Grp 4</b>   | 062399077-80,82 100599077 | 1/1,2,10,100,200,500 |
| <b>MS, MSD</b> | 062399085-86              | 1/1                  |

reanalyzed on 06/24/99 with confirmation of low recoveries

**Sample Data**

**MONKEY LIVER Week 27**

| Group Dose                                                       | Sample #           | Initial Wt. g | Total Mass of Liver g | POAA Std Correction Factor | POAA Conc. ng/g | POAA Dilution Factor | POAA Calc. Conc. ng/g | Concentration of POAA ug/g or % Rec. | Mean POAA ug/g | RSD Std. Dev. MS/MSD RPD |
|------------------------------------------------------------------|--------------------|---------------|-----------------------|----------------------------|-----------------|----------------------|-----------------------|--------------------------------------|----------------|--------------------------|
| Method Blk                                                       | H2O Blk-3          | 1.0000        | NA                    | 0.9582                     | 13.0            | 1                    | 12.4                  | <LOQ                                 |                |                          |
|                                                                  | H2O Blk-4          | 1.0000        | NA                    | 0.9582                     | 2.33            | 1                    | 2.23                  | <LOQ                                 | <LOQ           | NA                       |
| Matrix Blk                                                       | Rabbit Liver Blk-3 | 1.0000        | NA                    | 0.9582                     | 14.0            | 1                    | 13.5                  | <LOQ                                 |                |                          |
|                                                                  | Rabbit Liver Blk-4 | 1.0000        | NA                    | 0.9582                     | 16.3            | 1                    | 15.6                  | <LOQ                                 | <LOQ           | NA                       |
| QC - 250 ppb                                                     | I05709M-MS-3       | 1.0153        | NA                    | NA                         | 318             | 1                    | 218                   | 74%                                  |                |                          |
|                                                                  | I05709M-MSD-3      | 1.0153        | NA                    | NA                         | 282             | 1                    | 183                   | 62%                                  | 68%            | 18%                      |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day                       | I05709M Wk27       | 1.0153        | NA                    | 0.9582                     | 96.7            | 1                    | 91.3                  | 0.0913                               |                |                          |
|                                                                  | I05714M Wk27       | 0.9931        | NA                    | 0.9582                     | 56.0            | 1                    | 54.0                  | <LOQ                                 |                |                          |
|                                                                  | I05715M Wk27       | 1.0073        | NA                    | 0.9582                     | 238             | 1                    | 226                   | 0.226                                |                | 62.4                     |
|                                                                  | I05725M Wk27       | 1.0006        | NA                    | 0.9582                     | 43.9            | 1                    | 42.0                  | <LOQ                                 | 0.117          | 0.0730                   |
| <b>Group 2</b><br>Mid Dose<br>3.0 mg/kg/day<br>5721M replacement | I05702M Wk27       | 1.0104        | NA                    | 0.9582                     | 160             | 100                  | 15206                 | 15.2                                 |                |                          |
|                                                                  | I05706M Wk27       | 1.0002        | NA                    | 0.9582                     | 193             | 100                  | 18531                 | 18.5                                 |                |                          |
|                                                                  | I05717M Wk27       | 0.9931        | NA                    | 0.9582                     | 117             | 100                  | 11307                 | 11.3                                 |                | 19.7                     |
|                                                                  | I05723M            | 1.0163        | NA                    | 0.9582                     | 173             | 100                  | 16301                 | 16.3                                 | 15.3           | 3.02                     |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day                  | I05707M Wk27       | 1.0143        | NA                    | 0.9582                     | 232             | 100                  | 21917                 | 21.9                                 |                |                          |
|                                                                  | I05708M Wk27       | 1.0061        | NA                    | 0.9582                     | 132             | 50                   | 6294                  | 6.29                                 |                |                          |
|                                                                  | I05710M Wk27       | 1.0045        | NA                    | 0.9582                     | 92.9            | 100                  | 8864                  | 8.86                                 |                | 54.1                     |
|                                                                  | I05719M Wk27       | 1.0048        | NA                    | 0.9582                     | 197             | 100                  | 18753                 | 18.8                                 | 14.0           | 7.55                     |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day                      | I05703M Wk27       | 1.0036        | NA                    | 0.9582                     | 137             | 10                   | 1309                  | 1.31                                 |                |                          |
|                                                                  | I05704M Wk27       | 1.0036        | NA                    | 0.9582                     | 1679            | 10                   | 16026                 | 16.0                                 | *              |                          |
|                                                                  | I05711M Wk27       | 1.0039        | NA                    | 0.9582                     | 226             | 1                    | 216                   | 0.216                                |                |                          |
|                                                                  | I05713M Wk27       | 1.0143        | NA                    | 0.9582                     | 441             | 200                  | 83299                 | 83.3                                 |                |                          |
|                                                                  | I05722M Wk27       | 1.0117        | NA                    | 0.9582                     | 746             | 2                    | 1414                  | 1.41                                 |                | 148                      |
|                                                                  | I05724M Moribund   | 1.0164        | NA                    | 0.9582                     | 327             | 500                  | 154369                | 154                                  | 42.8           | 63.3                     |

POAA = Perfluorooctanoate

\*Overall dilution = 1:100. 1 uL of the 1:10 dilution was injected on 6/23/99. LAC 11/03/00

Limit of Quantitation (LOQ) = 75.5 ppb

Correction Factors not applicable to MS/MSD QC data

Date Entered/By: 06/24/99, 06/25/99, 07/29/99, 10/07/99, 11/03/00 LAC

Date Verified/ By: 08/03/99 GML, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

|                                     |                                                                |                  |                 |
|-------------------------------------|----------------------------------------------------------------|------------------|-----------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |                  |                 |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |                  |                 |
| Matrix:                             | Monkey Liver                                                   | Filename:        | See Attachments |
| Method/Revision:                    | FACT-M-1.1 & FACT-M-2.1                                        | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Soup 020199                                                    | Slope:           | See Attachments |
| Instrument Software/Version:        | MassLynx 3.2                                                   | Y-Intercept:     | See Attachments |
| Date of Extraction/Analyst:         | 06/21/99 SAH                                                   |                  |                 |
| Date of Analysis/Analyst:           | 06/23/99, 07/28/99, 10/05/99 DRB/IAS                           |                  |                 |
| Date of Data Reduction/Analyst:     | 06/25/99, 07/29/99, 10/06/99, 11/02/00 DRB/IAS/KJH             |                  |                 |

**Sample Data**

**MONKEY LIVER Week 27**

| Group<br>Dose                                                    | Sample #           | POAA<br>Calc. Conc.<br>ng/g | Concentration<br>of POAA<br>ug/g or % Rec. | Mean<br>POAA<br>ug/g | RSD<br>Std. Dev.<br>MS/MSD RPD |
|------------------------------------------------------------------|--------------------|-----------------------------|--------------------------------------------|----------------------|--------------------------------|
| Method Blk                                                       | H2O Blk-3          | 12.4                        | <LOQ                                       |                      |                                |
|                                                                  | H2O Blk-4          | 2.23                        | <LOQ                                       | <LOQ                 | NA                             |
| Matrix Blk                                                       | Rabbit Liver Blk-3 | 13.5                        | <LOQ                                       |                      |                                |
|                                                                  | Rabbit Liver Blk-4 | 15.6                        | <LOQ                                       | <LOQ                 | NA                             |
| QC - 250 ppb                                                     | I05709M-MS-3       | 218                         | 74%                                        |                      |                                |
|                                                                  | I05709M-MSD-3      | 183                         | 62%                                        | 68%                  | 18%                            |
| <b>Group 1</b><br>Control<br>0.0 mg/kg/day                       | I05709M Wk27       | 91.3                        | 0.0913                                     |                      |                                |
|                                                                  | I05714M Wk27       | 54.0                        | <LOQ                                       |                      |                                |
|                                                                  | I05715M Wk27       | 226                         | 0.226                                      |                      | 62.4                           |
|                                                                  | I05725M Wk27       | 42.0                        | <LOQ                                       | 0.117                | 0.0730                         |
| <b>Group 2</b><br>Mid Dose<br>3.0 mg/kg/day<br>5721M replacement | I05702M Wk27       | 15206                       | 15.2                                       |                      |                                |
|                                                                  | I05706M Wk27       | 18531                       | 18.5                                       |                      |                                |
|                                                                  | I05717M Wk27       | 11307                       | 11.3                                       |                      | 19.7                           |
|                                                                  | I05723M            | 16301                       | 16.3                                       | 15.3                 | 3.02                           |
| <b>Group 3</b><br>Mid-High Dose<br>10 mg/kg/day                  | I05707M Wk27       | 21917                       | 21.9                                       |                      |                                |
|                                                                  | I05708M Wk27       | 6294                        | 6.29                                       |                      |                                |
|                                                                  | I05710M Wk27       | 8864                        | 8.86                                       |                      | 54.1                           |
|                                                                  | I05719M Wk27       | 18753                       | 18.8                                       | 14.0                 | 7.55                           |
| <b>Group 4</b><br>High Dose<br>30 mg/kg/day                      | I05703M Wk27       | 1309                        | 1.31                                       |                      |                                |
|                                                                  | I05704M Wk27       | 16026                       | 16.0                                       |                      |                                |
|                                                                  | I05711M Wk27       | 216                         | 0.216                                      |                      |                                |
|                                                                  | I05713M Wk27       | 83299                       | 83.3                                       |                      |                                |
|                                                                  | I05722M Wk27       | 1414                        | 1.41                                       |                      | 148                            |
|                                                                  | I05724M Moribund   | 154369                      | 154                                        | 42.8                 | 63.3                           |

POAA = Perfluorooctanoate

\*Overall dilution = 1:100. 1 uL of the 1:10 dilution was injected on 6/23/99. LAC 11/03/00

Limit of Quantitation (LOQ) = 75.5 ppb

Correction Factors not applicable to MS/MSD QC data

Date Entered/By: 06/24/99, 06/25/99, 07/29/99, 10/07/99, 11/03/00 LAC  
Date Verified/ By: 08/03/99 GML, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Liver  
Method/Revision: FACT-M-1.1 & FACT-M-2.1  
Analytical Equipment System Number: Soup020199  
Instrument Software/Version: MassLynx 3.2  
Date of Extraction/Analyst: 7/13/99 SEE  
Date of Analysis/Analyst: 07/13/99 DRB  
Date of Data Reduction/Analyst: 07/14/99, 11/02/00, 11/06/00 DRB/KJH

Filename: See List to Right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments

| FileNames      | POAA                | Dilutions |
|----------------|---------------------|-----------|
| <b>Blks</b>    | A071399003-4, 30-31 | 1/1       |
| <b>Grp 1</b>   | 071399019-20        | 1/1       |
| <b>Grp 2</b>   | 072899016           | 1/100     |
| <b>Grp 3</b>   | 071399023-24        | 1/1       |
| <b>MS, MSD</b> | 071399015-16        | 1/1       |
|                | Box 99-131          |           |

**Sample Data**

**MONKEY LIVER Week 20 and 40**

| Group<br>Dose                         | Sample #            | Initial Wt.<br>g | Total Mass<br>of Liver<br>g | POAA Std<br>Correction<br>Factor | POAA<br>Conc.<br>ng/g | POAA<br>Dilution<br>Factor | POAA<br>Calc. Conc.<br>ng/g | Concentration<br>of POAA<br>ug/g or % Rec. | Mean<br>POAA<br>ug/g | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------------|---------------------|------------------|-----------------------------|----------------------------------|-----------------------|----------------------------|-----------------------------|--------------------------------------------|----------------------|--------------------------------|
| Method Blk                            | H2O Blk-11          | 1.0000           | NA                          | 0.9582                           | 9.55                  | 1                          | 9.15                        | <LOQ                                       |                      |                                |
|                                       | H2O Blk-12          | 1.0000           | NA                          | 0.9582                           | 0.00                  | 1                          | 0.00                        | <LOQ                                       | <LOQ                 | NA                             |
| Matrix Blk                            | Rabbit Liver Blk-11 | 1.0000           | NA                          | 0.9582                           | 3.08                  | 1                          | 2.95                        | <LOQ                                       |                      |                                |
|                                       | Rabbit Liver Blk-12 | 1.0000           | NA                          | 0.9582                           | 2.95                  | 1                          | 2.83                        | <LOQ                                       | <LOQ                 | NA                             |
| QC - 250 ppb                          | I05718M-MS-11       | 0.9933           | NA                          | NA                               | 353                   | 1                          | 341                         | 113%                                       |                      |                                |
|                                       | I05718M-MSD-12      | 0.9933           | NA                          | NA                               | 345                   | 1                          | 334                         | 111%                                       | 112%                 | 2%                             |
| <b>Group 1, Wk40</b><br>0.0 mg/kg/day | I05718M             | 0.9933           | NA                          | 0.9582                           | 14.0                  | 1                          | 13.5                        | <LOQ                                       |                      |                                |
|                                       | I05720M             | 0.9997           | NA                          | 0.9582                           | 22.5                  | 1                          | 21.6                        | <LOQ                                       | <LOQ                 | NA                             |
| <b>Group 2, Wk20</b>                  | I05721M             | 1.0083           | NA                          | 0.9582                           | 193                   | 100                        | 18321                       | 18.3                                       | NA                   | NA                             |
| <b>Group 3, Wk40</b><br>10 mg/kg/day  | I05712M             | 1.0157           | NA                          | 0.9582                           | 154                   | 1                          | 146                         | 0.146                                      |                      | 38.6                           |
|                                       | I05716M             | 0.9965           | NA                          | 0.9582                           | 86.6                  | 1                          | 83.3                        | 0.0833                                     | 0.114                | 0.0441                         |

POAA = Perfluorooctanoate

Limit of Quantitation (LOQ) = 75.5 ppb

Correction Factors not applicable to MS/MSD QC data

Date Entered/By: 07/26/99, 11/02/00, 11/03/00, 11/06/00 LAC

Date Verified/ By: 08/03/99 GML, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Liver  
Method/Revision: FACT-M-1.1 & FACT-M-2.1  
Analytical Equipment System Number: Soup020199  
Instrument Software/Version: MassLynx 3.2  
Date of Extraction/Analyst: 7/13/99 SEE  
Date of Analysis/Analyst: 07/13/99 DRB  
Date of Data Reduction/Analyst: 07/14/99, 11/02/00, 11/06/00 DRB/KJH

Filename:  
R-Squared Valu  
Slope:  
Y-Intercept:

**Sample Data**

**MONKEY LIVER Week 20 and 40**

| Group Dose                     | Sample #            | POAA Calc. Conc. ng/g | Concentration of POAA ug/g or % Rec. | Mean POAA ug/g | RSD Std. Dev. MS/MSD RPD |
|--------------------------------|---------------------|-----------------------|--------------------------------------|----------------|--------------------------|
| Method Blk                     | H2O Blk-11          | 9.15                  | <LOQ                                 |                |                          |
|                                | H2O Blk-12          | 0.00                  | <LOQ                                 | <LOQ           | NA                       |
| Matrix Blk                     | Rabbit Liver Blk-11 | 2.95                  | <LOQ                                 |                |                          |
|                                | Rabbit Liver Blk-12 | 2.83                  | <LOQ                                 | <LOQ           | NA                       |
| QC - 250 ppb                   | I05718M-MS-11       | 341                   | 113%                                 |                |                          |
|                                | I05718M-MSD-12      | 334                   | 111%                                 | 112%           | 2%                       |
| Group 1, Wk40<br>0.0 mg/kg/day | I05718M             | 13.5                  | <LOQ                                 |                | 0.00                     |
|                                | I05720M             | 21.6                  | <LOQ                                 | <LOQ           | NA                       |
| Group 2, Wk20                  | I05721M             | 18321                 | 18.3                                 | NA             | NA                       |
| Group 3, Wk40<br>10 mg/kg/day  | I05712M             | 146                   | 0.146                                |                | 38.6                     |
|                                | I05716M             | 83.3                  | 0.0833                               | 0.114          | 0.0441                   |

POAA = Perfluorooctanoate

Limit of Quantitation (LOQ) = 75.5 ppb

Correction Factors not applicable to MS/MSD QC data

Date Entered/By: 07/26/99, 11/02/00, 11/03/00, 11/06/00 LAC

Date Verified/ By: 08/03/99 GML, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance# 6329-231**

**Liver QC Summary - TOX026**

|            |                | <b>PFOA</b> |
|------------|----------------|-------------|
| 06/21/1999 | I05709M-MS-3   | 74%         |
|            | I05709M-MSD-3  | 62%         |
| 07/13/1999 | I05718M-MS-11  | 113%        |
|            | I05718M-MSD-12 | 111%        |

NA = Not Applicable

NR = Not Reported

|                    |     |                      |
|--------------------|-----|----------------------|
| Average            | 90% | Used in Final Report |
| Standard Deviation | 26% |                      |
| Grubbs outliers?   | No  |                      |
| Number of spikes   | 4   | Used in Final Report |

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                      |               |                               |     |                  |                              |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|---------------|-------------------------------|-----|------------------|------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                       |               | File names                    |     |                  |                              |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                      |               | POAA                          |     | Dilutions        | POAA                         |
| Matrix:                             | Monkey Urine                                                                                                         | Blks 08/04/99 | 081399004-5, A000927018,32    | 1/1 | MS/MSDs 08/04/99 | A000927045-48, A001017020-21 |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                              | Blks 08/05/99 | A000927034, A001017017-18, 19 | 1/1 | MS/MSDs 08/05/99 | A000927049,52                |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                          | Blks 08/17/99 | A081999003,4, S082499003-4    | 1/1 | MS/MSDs 08/17/99 | 081999016-19                 |
| Instrument Software/Version:        | MassLynx 3.2, 3.4                                                                                                    | Blks 08/20/99 | 082399003-4, 0908006,8        | 1/1 | MS/MSDs 08/17/99 | A000927059-62, A001017024    |
| Filename:                           | See listing to the right                                                                                             | Blks 09/21/99 | 092299003-4, 092899059-60     | 1/1 | MS/MSDs 08/20/99 | 082399016-17                 |
| R-Squared Value:                    | See Attachments                                                                                                      | Box           | 99-160-1, 99-162-3, 99-166-7  |     | MS/MSDs 08/20/99 | A001017025-26                |
| Slope:                              | See Attachments                                                                                                      |               |                               |     | MS/MSDs 09/21/99 | 092299016-17                 |
| Y-Intercept:                        | See Attachments                                                                                                      |               |                               |     |                  |                              |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                     |               |                               |     |                  |                              |
| Dates of Analysis/Analyst:          | 08/13/99, 08/16/99, 08/19/99, 08/23/99, 09/08/99, 09/22/99, 09/28/99, 09/27/00, 10/17/00 PTF/DRB/SAH/GML/IAS/MMH     |               |                               |     |                  |                              |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/27/99, 09/29/99, 09/29/00, 10/23/00, 11/06/00 PTF/IAS/GML/DRB/MEE/MMH/KJH |               |                               |     |                  |                              |

**Sample Data**

**MONKEY URINE QC SAMPLES**

| Group Dose        | Sample #             | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------|----------------------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk        | MKU08049 H2O Blk-1   | 1               | 0.9582                     | 1                    | 18.6             | 0.0178                                |                 |                          |
|                   | MKU08049 H2O Blk-2   | 1               | 0.9582                     | 1                    | 18.4             | 0.0177                                | 0.0177          | 0.00703                  |
|                   | MKU08059 H2O Blk-1   | 1               | 0.9582                     | 1                    | 20.8             | 0.0199                                |                 |                          |
|                   | MKU08059 H2O Blk-2   | 1               | 0.9582                     | 1                    | 7.52             | <LOQ                                  | NA              | NA                       |
|                   | MKU08179 H2O Blk-1   | 1               | 0.9582                     | 1                    | 6.17             | <LOQ                                  |                 |                          |
|                   | MKU08179 H2O Blk-2   | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  | NA              | NA                       |
|                   | MKU08209 H2O Blk-1   | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                   | MKU08209 H2O Blk-2   | 1               | 0.9582                     | 1                    | 6.64             | <LOQ                                  | NA              | NA                       |
| Matrix Blk        | MKU09219 H2O Blk-1   | 1               | 0.9582                     | 1                    | 6.90             | <LOQ                                  |                 |                          |
|                   | MKU09219 H2O Blk-2   | 1               | 0.9582                     | 1                    | 0.270            | <LOQ                                  | NA              | NA                       |
|                   | MKU08049 Urine Blk-1 | 1               | 0.9582                     | 1                    | 5.13             | <LOQ                                  |                 |                          |
|                   | MKU08049 Urine Blk-2 | 1               | 0.9582                     | 1                    | 8.00             | <LOQ                                  | NA              | NA                       |
|                   | MKU08059 Urine Blk-1 | 1               | 0.9582                     | 1                    | 8.23             | <LOQ                                  |                 |                          |
|                   | MKU08059 Urine Blk-2 | 1               | 0.9582                     | 1                    | 33.0             | 0.0316                                | NA              | NA                       |
|                   | MKU08179 Urine Blk-1 | 1               | 0.9582                     | 1                    | 5.24             | <LOQ                                  |                 |                          |
|                   | MKU08179 Urine Blk-2 | 1               | 0.9582                     | 1                    | 31.2             | 0.0299                                | NA              | NA                       |
| QC-MS<br>61.6 ppb | MKU08209 Urine Blk-1 | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                   | MKU08209 Urine Blk-2 | 1               | 0.9582                     | 1                    | 14.4             | <LOQ                                  | NA              | NA                       |
|                   | MKU09219 Urine Blk-1 | 1               | 0.9582                     | 1                    | 2.88             | <LOQ                                  |                 |                          |
|                   | MKU09219 Urine Blk-2 | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  | <LOQ            | NA                       |
|                   | MKU08049 MS-1        | 1               | NA                         | 1                    | 100              | 133%                                  | *               |                          |
|                   | MKU08049 MSD-1       | 1               | NA                         | 1                    | 75.1             | 92%                                   | *               | 36%                      |
|                   | MKU08049 MS-2        | 1               | NA                         | 1                    | 77.1             | 95%                                   |                 |                          |
|                   | MKU08049 MSD-2       | 1               | NA                         | 1                    | 61.4             | 70%                                   | 82%             | 31%                      |
|                   | MKU08059 MS          | 1               | NA                         | 1                    | 70.5             | 91%                                   |                 |                          |
|                   | MKU08059 MSD         | 1               | NA                         | 1                    | 67.8             | 87%                                   | 89%             | 5%                       |
|                   | MKU08179 MS 1-1      | 1               | NA                         | 1                    | 75.0             | 92%                                   |                 |                          |
|                   | MKU08179 MSD 1-1     | 1               | NA                         | 1                    | 82.4             | 104%                                  | 98%             | 12%                      |
|                   | MKU08179 MS 1-2      | 1               | NA                         | 1                    | 83.1             | 105%                                  |                 |                          |
|                   | MKU08179 MSD 1-2     | 1               | NA                         | 1                    | 80.5             | 101%                                  | 103%            | 4%                       |
|                   | MKU08179 MS 1-3      | 1               | NA                         | 1                    | 63.8             | 74%                                   |                 |                          |
|                   | MKU08179 MSD 1-3     | 1               | NA                         | 1                    | 64.0             | 74%                                   | 74%             | 1%                       |
|                   | MKU08179 MS 1-4      | 1               | NA                         | 1                    | 63.0             | 73%                                   |                 |                          |
|                   | MKU08179 MSD 1-4     | 1               | NA                         | 1                    | 63.3             | 73%                                   | 73%             | 1%                       |
|                   | MKU08209 MS 1-1      | 1               | NA                         | 1                    | 47.5             | 66%                                   |                 |                          |
|                   | MKU08209 MSD 1-1     | 1               | NA                         | 1                    | 46.1             | 63%                                   | 64%             | 4%                       |
|                   | MKU08209 MS 1-2      | 1               | NA                         | 1                    | 62.5             | 90%                                   |                 |                          |
|                   | MKU08209 MSD 1-2     | 1               | NA                         | 1                    | 62.2             | 89%                                   | 90%             | 0%                       |
|                   | MKU09219 MS-1        | 1               | NA                         | 1                    | 58.3             | 92%                                   |                 |                          |
|                   | MKU09219 MSD-1       | 1               | NA                         | 1                    | 60.1             | 95%                                   | 94%             | 3%                       |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed

POAA = Perfluorooctanoate

\* Confirmed results. LAC 10/26/00

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/10/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 10/10/00, 10/26/00, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 10/26/00 KJH, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                      |
|-------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                       |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                      |
| Matrix:                             | Monkey Urine                                                                                                         |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                              |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                          |
| Instrument Software/Version:        | MassLynx 3.2, 3.4                                                                                                    |
| Filename:                           | See Attachments                                                                                                      |
| R-Squared Value:                    | See Attachments                                                                                                      |
| Slope:                              | See Attachments                                                                                                      |
| Y-Intercept:                        | See Attachments                                                                                                      |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                     |
| Dates of Analysis/Analyst:          | 08/13/99, 08/16/99, 08/19/99, 08/23/99, 09/08/99, 09/22/99, 09/28/99, 09/27/00, 10/17/00 PTF/DRB/SAH/GML/IAS/MMH     |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/27/99, 09/29/99, 09/29/00, 10/23/00, 11/06/00 PTF/IAS/GML/DRB/MEE/MMH/KJH |

**Sample Data**

**MONKEY URINE QC SAMPLES**

| Group Dose        | Sample #             | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|-------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| Method Blk        | MKU08049 H2O Blk-1   | 18.6             | 0.0178                                |                 |                          |
|                   | MKU08049 H2O Blk-2   | 18.4             | 0.0177                                | 0.0177          | 0.00703                  |
|                   | MKU08059 H2O Blk-1   | 20.8             | 0.0199                                |                 |                          |
|                   | MKU08059 H2O Blk-2   | 7.52             | <LOQ                                  | NA              | NA                       |
|                   | MKU08179 H2O Blk-1   | 6.17             | <LOQ                                  |                 |                          |
|                   | MKU08179 H2O Blk-2   | 0.00             | <LOQ                                  | NA              | NA                       |
|                   | MKU08209 H2O Blk-1   | 0.00             | <LOQ                                  |                 |                          |
|                   | MKU08209 H2O Blk-2   | 6.64             | <LOQ                                  | NA              | NA                       |
| Matrix Blk        | MKU09219 H2O Blk-1   | 6.90             | <LOQ                                  |                 |                          |
|                   | MKU09219 H2O Blk-2   | 0.270            | <LOQ                                  | NA              | NA                       |
|                   | MKU08049 Urine Blk-1 | 5.13             | <LOQ                                  |                 |                          |
|                   | MKU08049 Urine Blk-2 | 8.00             | <LOQ                                  | NA              | NA                       |
|                   | MKU08059 Urine Blk-1 | 8.23             | <LOQ                                  |                 |                          |
|                   | MKU08059 Urine Blk-2 | 33.0             | 0.0316                                | NA              | NA                       |
|                   | MKU08179 Urine Blk-1 | 5.2              | <LOQ                                  |                 |                          |
|                   | MKU08179 Urine Blk-2 | 31.2             | 0.0299                                | NA              | NA                       |
| QC-MS<br>61.6 ppb | MKU08209 Urine Blk-1 | 0.00             | <LOQ                                  |                 |                          |
|                   | MKU08209 Urine Blk-2 | 14.4             | <LOQ                                  | NA              | NA                       |
|                   | MKU09219 Urine Blk-1 | 2.88             | <LOQ                                  |                 |                          |
|                   | MKU09219 Urine Blk-2 | 0.00             | <LOQ                                  | <LOQ            | NA                       |
|                   | MKU08049 MS-1        | 100              | 133%                                  | *               |                          |
|                   | MKU08049 MSD-1       | 75.1             | 92%                                   | *               |                          |
|                   | MKU08049 MS-2        | 77.1             | 95%                                   |                 |                          |
|                   | MKU08049 MSD-2       | 61.4             | 70%                                   | 82%             | 31%                      |
|                   | MKU08059 MS          | 70.5             | 91%                                   |                 |                          |
|                   | MKU08059 MSD         | 67.8             | 87%                                   | 89%             | 5%                       |
|                   | MKU08179 MS 1-1      | 75.0             | 92%                                   |                 |                          |
|                   | MKU08179 MSD 1-1     | 82.4             | 104%                                  | 98%             | 12%                      |
|                   | MKU08179 MS 1-2      | 83.1             | 105%                                  |                 |                          |
|                   | MKU08179 MSD 1-2     | 80.5             | 101%                                  | 103%            | 4%                       |
|                   | MKU08179 MS 1-3      | 63.8             | 74%                                   |                 |                          |
|                   | MKU08179 MSD 1-3     | 64.0             | 74%                                   | 74%             | 1%                       |
|                   | MKU08179 MS 1-4      | 63.0             | 73%                                   |                 |                          |
|                   | MKU08179 MSD 1-4     | 63.3             | 73%                                   | 73%             | 1%                       |
|                   | MKU08209 MS 1-1      | 47.5             | 66%                                   |                 |                          |
|                   | MKU08209 MSD 1-1     | 46.1             | 63%                                   | 64%             | 4%                       |
|                   | MKU08209 MS 1-2      | 62.5             | 90%                                   |                 |                          |
|                   | MKU08209 MSD 1-2     | 62.2             | 89%                                   | 90%             | 0%                       |
|                   | MKU09219 MS-1        | 58.3             | 92%                                   |                 |                          |
|                   | MKU09219 MSD-1       | 60.1             | 95%                                   | 94%             | 3%                       |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed

POAA = Perfluorooctanoate

\* Confirmed results. LAC 10/26/00

C8F17COO-

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/10/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 10/10/00, 10/26/00, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 10/26/00 KJH, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                  |              |                                                  |
|-------------------------------------|------------------------------------------------------------------|--------------|--------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys   |              |                                                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                  |              |                                                  |
| Matrix:                             | Monkey Urine                                                     | FileNames    |                                                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                          |              | <b>POAA</b>                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                      | <b>Grp 1</b> | 081999022-23, 082399020-21, 092299026, 092799050 |
| Instrument Software/Version:        | MassLynx 3.2                                                     | <b>Grp 2</b> | 090999018-21                                     |
| Filename:                           | See listing to the right                                         | <b>Grp 3</b> | 090899049,78, 091099019-21, 091599150            |
| R-Squared Value:                    | See Attachments                                                  | <b>Grp 4</b> | 091599035, 122-126                               |
| Slope:                              | See Attachments                                                  | <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                     |
| Y-Intercept:                        | See Attachments                                                  |              |                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL |              |                                                  |
| Dates of Analysis/Analyst:          | 08/19/99, 09/08/99, 09/09/99, 09/15/99 GML/PTF/IAS/MMH           |              |                                                  |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/09/99, 09/13/99, 09/16/99, 11/06/00 GML/MMH/KJH     |              |                                                  |

**Dilutions**  
1/1  
1/1000  
1/500, 1/250, 1/1000, 1/2000  
1/2000, 1/8000

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 2**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 8.27             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 12.0             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 10.6             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 10.3             | <LOQ                                  |                 | NA                       |
|                                 | I05725M  | 0.9             | 0.9582                     | 1                    | 4.36             | <LOQ                                  | <LOQ            | NA                       |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 57.1             | 54.7                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 62.7             | 60.1                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 51.1             | 48.9                                  |                 | 51.9                     |
|                                 | I05723M  | 1               | 0.9582                     | 1000                 | 136              | 130                                   | 73.5            | 38.1                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 2000                 | 215              | 412                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 208              | 199                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 500                  | 257              | 123                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 250                  | 254              | 60.9                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 252              | 242                                   |                 | 56.2                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 300              | 288                                   | 221             | 124                      |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 2000                 | 274              | 525                                   |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 8000                 | 106              | 811                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 8000                 | 123              | 941                                   |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 8000                 | 110              | 846                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 8000                 | 128              | 983                                   |                 | 29.6                     |
|                                 | I05724M  | 1               | 0.9582                     | 8000                 | 176              | 1350                                  | 909             | 269                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                  |
|-------------------------------------|------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys   |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                  |
| Matrix:                             | Monkey Urine                                                     |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                          |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                      |
| Instrument Software/Version:        | MassLynx 3.2                                                     |
| Filename:                           | See Attachments                                                  |
| R-Squared Value:                    | See Attachments                                                  |
| Slope:                              | See Attachments                                                  |
| Y-Intercept:                        | See Attachments                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL |
| Dates of Analysis/Analyst:          | 08/19/99, 09/08/99, 09/09/99, 09/15/99 GML/PTF/IAS/MMH           |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/09/99, 09/13/99, 09/16/99, 11/06/00 GML/MMH/KJH     |

**Sample Data**

**MONKEY URINE Week 2**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 8.27                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 12.0                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 10.6                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 10.3                   | <LOQ                                        |                       | NA                             |
|                                 | I05725M  | 4.36                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 57.1                   | 54.7                                        |                       |                                |
|                                 | I05706M  | 62.7                   | 60.1                                        |                       |                                |
|                                 | I05717M  | 51.1                   | 48.9                                        |                       | 51.9                           |
|                                 | I05723M  | 136                    | 130                                         | 73.5                  | 38.1                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 215                    | 412                                         |                       |                                |
|                                 | I05708M  | 208                    | 199                                         |                       |                                |
|                                 | I05710M  | 257                    | 123                                         |                       |                                |
|                                 | I05712M  | 254                    | 60.9                                        |                       |                                |
|                                 | I05716M  | 252                    | 242                                         |                       | 56.2                           |
|                                 | I05719M  | 300                    | 288                                         | 221                   | 124                            |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 274                    | 525                                         |                       |                                |
|                                 | I05704M  | 106                    | 811                                         |                       |                                |
|                                 | I05711M  | 123                    | 941                                         |                       |                                |
|                                 | I05713M  | 110                    | 846                                         |                       |                                |
|                                 | I05722M  | 128                    | 983                                         |                       | 29.6                           |
|                                 | I05724M  | 176                    | 1350                                        | 909                   | 269                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

|                    |                                                                                        |
|--------------------|----------------------------------------------------------------------------------------|
| Date Entered/By:   | 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC |
| Date Verified/ By: | 09/25/00 LAC, 11/08/00 HOJ                                                             |

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                              |              |                                                       |
|-------------------------------------|----------------------------------------------------------------------------------------------|--------------|-------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                               |              |                                                       |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                              |              |                                                       |
| Matrix:                             | Monkey Urine                                                                                 | FileNames    |                                                       |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                      |              |                                                       |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                  | <b>Grp 1</b> | <b>POAA</b><br>081999024-25, 082399022-3,092799045,51 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                 | <b>Grp 2</b> | 090999022-28                                          |
| Filename:                           | See listing to the right                                                                     | <b>Grp 3</b> | 090899057,79, 091099022-27                            |
| R-Squared Value:                    | See Attachments                                                                              | <b>Grp 4</b> | 091599043-45, 129-130, 092799047                      |
| Slope:                              | See Attachments                                                                              | <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                          |
| Y-Intercept:                        | See Attachments                                                                              |              |                                                       |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                             |              |                                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS |              |                                                       |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/28/99, 11/06/00 GML/MMH/IAS/KJH         |              |                                                       |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 4**

| Group<br>Dose                   | Sample # | Extraction<br>Vol. | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|--------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1                  | 0.9582                           | 1                          | 10.6                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 1                  | 0.9582                           | 1                          | 4.17                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 1                  | 0.9582                           | 1                          | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 0.80               | 0.9582                           | 1                          | 22.5                   | 0.0270                                      |                       |                                |
|                                 | I05720M  | 1                  | 0.9582                           | 1                          | 11.8                   | <LOQ                                        |                       | NA                             |
|                                 | I05725M  | 1                  | 0.9582                           | 5                          | 175                    | 0.839                                       | 0.152 A               | 0.337                          |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1                  | 0.9582                           | 1000                       | 57.7                   | 55.3                                        |                       |                                |
|                                 | I05706M  | 1                  | 0.9582                           | 1000                       | 57.9                   | 55.5                                        |                       |                                |
|                                 | I05717M  | 1                  | 0.9582                           | 1000                       | 62.6                   | 60.0                                        |                       | 8.43                           |
|                                 | I05721M  | 1                  | 0.9582                           | 1000                       | 50.9                   | 48.7                                        | 54.9                  | 4.62                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1                  | 0.9582                           | 1000                       | 204                    | 195                                         |                       |                                |
|                                 | I05708M  | 1                  | 0.9582                           | 1000                       | 283                    | 271                                         |                       |                                |
|                                 | I05710M  | 1                  | 0.9582                           | 1000                       | 276                    | 264                                         |                       |                                |
|                                 | I05712M  | 1                  | 0.9582                           | 250                        | 149                    | 35.8                                        |                       |                                |
|                                 | I05716M  | 1                  | 0.9582                           | 500                        | 277                    | 133                                         |                       | 48.1                           |
|                                 | I05719M  | 1                  | 0.9582                           | 1000                       | 253                    | 242                                         | 190                   | 91.6                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1                  | 0.9582                           | 4000                       | 99.9                   | 383                                         |                       |                                |
|                                 | I05704M  | 1                  | 0.9582                           | 2000                       | 24.8                   | 47.6                                        |                       |                                |
|                                 | I05711M  | 1                  | 0.9582                           | 1000                       | 194                    | 186                                         |                       |                                |
|                                 | I05713M  | 1                  | 0.9582                           | 2000                       | 232                    | 444                                         |                       |                                |
|                                 | I05722M  | 1                  | 0.9582                           | 1000                       | 308                    | 295                                         |                       | 67.1                           |
|                                 | I05724M  | 1                  | 0.9582                           | 1000                       | 86.6                   | 83.0                                        | 240                   | 161                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                              |
|-------------------------------------|----------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                               |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                              |
| Matrix:                             | Monkey Urine                                                                                 |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                  |
| Instrument Software/Version:        | MassLynx 3.2                                                                                 |
| Filename:                           | See Attachments                                                                              |
| R-Squared Value:                    | See Attachments                                                                              |
| Slope:                              | See Attachments                                                                              |
| Y-Intercept:                        | See Attachments                                                                              |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                             |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/28/99, 11/06/00 GML/MMH/IAS/KJH         |

**Sample Data**

**MONKEY URINE Week 4**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 10.6                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 4.17                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 22.5                   | 0.0270                                      |                       |                                |
|                                 | I05720M  | 11.8                   | <LOQ                                        |                       | NA                             |
|                                 | I05725M  | 175                    | 0.839                                       | 0.152 A               | 0.337                          |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 57.7                   | 55.3                                        |                       |                                |
|                                 | I05706M  | 57.9                   | 55.5                                        |                       |                                |
|                                 | I05717M  | 62.6                   | 60.0                                        |                       | 8.43                           |
|                                 | I05721M  | 50.9                   | 48.7                                        | 54.9                  | 4.62                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 204                    | 195                                         |                       |                                |
|                                 | I05708M  | 283                    | 271                                         |                       |                                |
|                                 | I05710M  | 276                    | 264                                         |                       |                                |
|                                 | I05712M  | 149                    | 36                                          |                       |                                |
|                                 | I05716M  | 277                    | 133                                         |                       | 48.1                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05719M  | 253                    | 242                                         | 190                   | 91.6                           |
|                                 | I05703M  | 99.9                   | 383                                         |                       |                                |
|                                 | I05704M  | 24.8                   | 47.6                                        |                       |                                |
|                                 | I05711M  | 194                    | 186                                         |                       |                                |
|                                 | I05713M  | 232                    | 444                                         |                       |                                |
|                                 | I05722M  | 308                    | 295                                         |                       | 67.1                           |
|                                 | I05724M  | 86.6                   | 83.0                                        | 240                   | 161                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable POAA = Perfluorooctanoate

A = Below LOQ

C8F17COO-

Date Entered/By: 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                            |              |                                           |
|-------------------------------------|----------------------------------------------------------------------------|--------------|-------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys             |              |                                           |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                            |              |                                           |
| Matrix:                             | Monkey Urine                                                               | FileNames    |                                           |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                    |              | <b>POAA</b>                               |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                | <b>Grp 1</b> | 081999028-29, 082399026-27, 092799052, 65 |
| Instrument Software/Version:        | MassLynx 3.2                                                               | <b>Grp 2</b> | 090999028-34                              |
| Filename:                           | See listing to the right                                                   | <b>Grp 3</b> | 091599151-157, 160                        |
| R-Squared Value:                    | See Attachments                                                            | <b>Grp 4</b> | 091599051-52, 131-133                     |
| Slope:                              | See Attachments                                                            | <b>Box</b>   | 99-160-1, 99-162-3                        |
| Y-Intercept:                        | See Attachments                                                            |              |                                           |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL           |              |                                           |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/09/99, 09/15/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS   |              |                                           |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/28/99, 11/06/00 GML/MMH/IAS/KJH |              |                                           |

**Sample Data**

**MONKEY URINE Week 6**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 179              | 0.172                                 |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 4.66             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 26.5             | 0.0254                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 14.9             | <LOQ                                  |                 | 122                      |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 131              | 0.126                                 | 0.0587          | 0.0716                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 138              | 132                                   |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 58.0             | 55.6                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 55.5             | 53.2                                  |                 | 71.4                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 22.8             | 21.8                                  | 65.7            | 46.9                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 164              | 157                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 203              | 195                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 156              | 150                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 250                  | 227              | 54.4                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 122              | 117                                   |                 | 39.1                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 98.4             | 94.3                                  | 128             | 50.0                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 4000                 | 92.3             | 354                                   |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 4000                 | 50.3             | 193                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 4000                 | 111              | 427                                   |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 1000                 | 75.5             | 72.3                                  |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 1000                 | 328              | 314                                   |                 | 51.6                     |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 272             | 140                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 08/24/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/KJH

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                            |
|-------------------------------------|----------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys             |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                            |
| Matrix:                             | Monkey Urine                                                               |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                    |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                |
| Instrument Software/Version:        | MassLynx 3.2                                                               |
| Filename:                           | See Attachments                                                            |
| R-Squared Value:                    | See Attachments                                                            |
| Slope:                              | See Attachments                                                            |
| Y-Intercept:                        | See Attachments                                                            |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL           |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/09/99, 09/15/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS   |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/28/99, 11/06/00 GML/MMH/IAS/KJH |

**Sample Data**

**MONKEY URINE Week 6**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 179                    | 0.172                                       |                       |                                |
|                                 | I05714M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 4.66                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 26.5                   | 0.0254                                      |                       |                                |
|                                 | I05720M  | 14.9                   | <LOQ                                        |                       | 122                            |
|                                 | I05725M  | 131                    | 0.126                                       | 0.0587                | 0.0716                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 138                    | 132                                         |                       |                                |
|                                 | I05706M  | 58.0                   | 55.6                                        |                       |                                |
|                                 | I05717M  | 55.5                   | 53.2                                        |                       | 71.4                           |
|                                 | I05721M  | 22.8                   | 21.8                                        | 65.7                  | 46.9                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 164                    | 157                                         |                       |                                |
|                                 | I05708M  | 203                    | 195                                         |                       |                                |
|                                 | I05710M  | 156                    | 150                                         |                       |                                |
|                                 | I05712M  | 227                    | 54.4                                        |                       |                                |
|                                 | I05716M  | 122                    | 117                                         |                       | 39.1                           |
|                                 | I05719M  | 98.4                   | 94.3                                        | 128                   | 50.0                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 92.3                   | 354                                         |                       |                                |
|                                 | I05704M  | 50.3                   | 193                                         |                       |                                |
|                                 | I05711M  | 111                    | 427                                         |                       |                                |
|                                 | I05713M  | 75.5                   | 72.3                                        |                       |                                |
|                                 | I05722M  | 328                    | 314                                         |                       | 51.6                           |
|                                 | I05724M  | NA                     | NA                                          | 272                   | 140                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/23/99, 08/24/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/KJH  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                        |              |                                                  |
|-------------------------------------|--------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                         |              |                                                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                        |              |                                                  |
| Matrix:                             | Monkey Urine                                                                                           | FileNames    |                                                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                |              | <b>POAA</b>                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                            | <b>Grp 1</b> | 081999030-31, 082399028-29, 092299029, 092799053 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                           | <b>Grp 2</b> | 090999035-40                                     |
| Filename:                           | See listing to the right                                                                               | <b>Grp 3</b> | 090899074, 091099028-34                          |
| R-Squared Value:                    | See Attachments                                                                                        | <b>Grp 4</b> | 082499061, 0915099056, 136-138                   |
| Slope:                              | See Attachments                                                                                        | <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                     |
| Y-Intercept:                        | See Attachments                                                                                        |              |                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                       |              |                                                  |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS |              |                                                  |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 11/06/00 GML/MMH/MEE/IAS/KJH     |              |                                                  |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 8**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 13.5             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 0.790            | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 1.31             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 32.0             | 0.0307                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 15.2             | <LOQ                                  |                 | 58.3                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 8.61             | <LOQ                                  | 0.0161 A        | 0.00940                  |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 49.2             | 49.2                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 78.7             | 75.4                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 40.0             | 38.3                                  |                 | 43.2                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 28.7             | 27.5                                  | 47.6            | 20.6                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 240              | 230                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 230              | 221                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 311              | 298                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 400                  | 196              | 75.2                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 200              | 192                                   |                 | 35.5                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 230              | 220                                   | 206             | 73.1                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 2000                 | 87.0             | 167                                   |                 |                          |
|                                 | I05704M  | 0.95            | 0.9582                     | 1000                 | 279              | 282                                   |                 |                          |
|                                 | I05711M  | 0.80            | 0.9582                     | 10                   | 302              | 3.62                                  |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 1000                 | 268              | 257                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 2000                 | 101              | 193                                   |                 | 60.5                     |
|                                 | I05724M  | NA              | 0.9582                     | NA                   | NA               | NA                                    | 180             | 109                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 08/24/99, 08/26/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                        |
|-------------------------------------|--------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                         |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                        |
| Matrix:                             | Monkey Urine                                                                                           |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                            |
| Instrument Software/Version:        | MassLynx 3.2                                                                                           |
| Filename:                           | See Attachments                                                                                        |
| R-Squared Value:                    | See Attachments                                                                                        |
| Slope:                              | See Attachments                                                                                        |
| Y-Intercept:                        | See Attachments                                                                                        |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 11/06/00 GML/MMH/MEE/IAS/KJH     |

**Sample Data**

**MONKEY URINE Week 8**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 13.5                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 0.790                  | <LOQ                                        |                       |                                |
|                                 | I05715M  | 1.31                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 32.0                   | 0.0307                                      |                       |                                |
|                                 | I05720M  | 15.2                   | <LOQ                                        |                       | 58.3                           |
|                                 | I05725M  | 8.61                   | <LOQ                                        | 0.0161 A              | 0.00940                        |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 49.2                   | 49.2                                        |                       |                                |
|                                 | I05706M  | 78.7                   | 75.4                                        |                       |                                |
|                                 | I05717M  | 40.0                   | 38.3                                        |                       | 43.2                           |
|                                 | I05721M  | 28.7                   | 27.5                                        | 47.6                  | 20.6                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 240                    | 230                                         |                       |                                |
|                                 | I05708M  | 230                    | 221                                         |                       |                                |
|                                 | I05710M  | 311                    | 298                                         |                       |                                |
|                                 | I05712M  | 196                    | 75.2                                        |                       |                                |
|                                 | I05716M  | 200                    | 192                                         |                       | 35.5                           |
|                                 | I05719M  | 230                    | 220                                         | 206                   | 73.1                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 87.0                   | 167                                         |                       |                                |
|                                 | I05704M  | 279                    | 282                                         |                       |                                |
|                                 | I05711M  | 302                    | 3.62                                        |                       |                                |
|                                 | I05713M  | 268                    | 257                                         |                       |                                |
|                                 | I05722M  | 101                    | 193                                         |                       | 60.5                           |
|                                 | I05724M  | NA                     | NA                                          | 180                   | 109                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C8F17COO-

Date Entered/By: 08/23/99, 08/24/99, 08/26/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |              |                                                              |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |              |                                                              |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |              |                                                              |
| Matrix:                             | Monkey Urine                                                                                                                   | FileNames    |                                                              |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |              |                                                              |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    | <b>Grp 1</b> | <b>POAA</b> 081999034-35, 082399032-33, 092299030, 092799054 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   | <b>Grp 2</b> | 090999041-44                                                 |
| Filename:                           | See listing to the right                                                                                                       | <b>Grp 3</b> | 090899072-73,80, 091099035-39                                |
| R-Squared Value:                    | See Attachments                                                                                                                | <b>Grp 4</b> | 081399017, 091599059,63,140, 092399022                       |
| Slope:                              | See Attachments                                                                                                                | <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                                 |
| Y-Intercept:                        | See Attachments                                                                                                                |              |                                                              |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |              |                                                              |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS     |              |                                                              |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                              |

**Sample Data**  
Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 10**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 10.9             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 1.08             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 23.5             | 0.0225                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 11.8             | <LOQ                                  |                 | 64.1                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 37.5             | 0.0359                                | 0.0177 A        | 0.0114                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 34.7             | 33.3                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 66.5             | 63.7                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 45.5             | 43.6                                  |                 | 46.9                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 20.0             | 19.2                                  | 39.9            | 18.7                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 284              | 272                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 263              | 252                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 243              | 233                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 250                  | 188              | 45.1                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 500                  | 197              | 94.3                                  |                 | 52.7                     |
|                                 | I05719M  | 1               | 0.9582                     | 500                  | 322              | 154                                   | 175             | 92.3                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 4000                 | 295              | 1131                                  |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 1000                 | 342              | 328                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 20                   | 73.7             | 1.41                                  |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 2000                 | 57.0             | 109                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 1000                 | 235              | 225                                   |                 | 125                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 359             | 449                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC,, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS  
Date of Data Reduction/Analyst: 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH

**Sample Data**

**MONKEY URINE Week 10**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 10.9                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 1.08                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 23.5                   | 0.0225                                      |                       |                                |
|                                 | I05720M  | 11.8                   | <LOQ                                        |                       | 64.1                           |
|                                 | I05725M  | 37.5                   | 0.0359                                      | 0.0177 A              | 0.0114                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 34.7                   | 33.3                                        |                       |                                |
|                                 | I05706M  | 66.5                   | 63.7                                        |                       |                                |
|                                 | I05717M  | 45.5                   | 43.6                                        |                       | 46.9                           |
|                                 | I05721M  | 20.0                   | 19.2                                        | 39.9                  | 18.7                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 284                    | 272                                         |                       |                                |
|                                 | I05708M  | 263                    | 252                                         |                       |                                |
|                                 | I05710M  | 243                    | 233                                         |                       |                                |
|                                 | I05712M  | 188                    | 45.1                                        |                       |                                |
|                                 | I05716M  | 197                    | 94.3                                        |                       | 52.7                           |
|                                 | I05719M  | 322                    | 154                                         | 175                   | 92.3                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 295                    | 1131                                        |                       |                                |
|                                 | I05704M  | 342                    | 328                                         |                       |                                |
|                                 | I05711M  | 73.7                   | 1.41                                        |                       |                                |
|                                 | I05713M  | 57.0                   | 109                                         |                       |                                |
|                                 | I05722M  | 235                    | 225                                         |                       | 125                            |
|                                 | I05724M  | NA                     | NA                                          | 359                   | 449                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC., 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |              |                                                  |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |              |                                                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |              |                                                  |
| Matrix:                             | Monkey Urine                                                                                                                   | FileNames    |                                                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |              | <b>POAA</b>                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    | <b>Grp 1</b> | 081999036-37, 082399034-35, 092299033, 092799057 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   | <b>Grp 2</b> | 090999047-50                                     |
| Filename:                           | See listing to the right                                                                                                       | <b>Grp 3</b> | 082499016-21                                     |
| R-Squared Value:                    | See Attachments                                                                                                                | <b>Grp 4</b> | 081399018-19, 091599143-145                      |
| Slope:                              | See Attachments                                                                                                                | <b>Box</b>   | 99-160-1, 99-162-3                               |
| Y-Intercept:                        | See Attachments                                                                                                                |              |                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |              |                                                  |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/15/99, 09/22/99, 09/27/99 GML/SAH/MMH/IAS                                 |              |                                                  |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                  |
|                                     | Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.            |              |                                                  |

**Sample Data**

**MONKEY URINE Week 12**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 14.7             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 1.49             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 1.96             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 18.9             | 0.0181                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 19.2             | 0.0184                                |                 | 46.1                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 15.6             | <LOQ                                  | 0.0141 A        | 0.00648                  |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 68.2             | 65.3                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 65.6             | 62.8                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 40.5             | 38.8                                  |                 | 38.9                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 27.8             | 26.6                                  | 48.4            | 18.8                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 177              | 170                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 150              | 144                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 381              | 365                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 1000                 | 270              | 259                                   |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 148              | 142                                   |                 | 46.0                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 135              | 130                                   | 201             | 92.7                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 2000                 | 91.3             | 175                                   |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 2000                 | 88.2             | 169                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 20                   | 37.8             | 0.725                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 2000                 | 127              | 243                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 20                   | 70.6             | 1.35                                  |                 | 93.8                     |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 118             | 111                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/26/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/15/99, 09/22/99, 09/27/99 GML/SAH/MMH/IAS  
Date of Data Reduction/Analyst: 08/18/99, 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH

**Sample Data**

**MONKEY URINE Week 12**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 14.7                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 1.49                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 1.96                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 18.9                   | 0.0181                                      |                       |                                |
|                                 | I05720M  | 19.2                   | 0.0184                                      |                       | 46.1                           |
|                                 | I05725M  | 15.6                   | <LOQ                                        | 0.0141 A              | 0.00648                        |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 68.2                   | 65.3                                        |                       |                                |
|                                 | I05706M  | 65.6                   | 62.8                                        |                       |                                |
|                                 | I05717M  | 40.5                   | 38.8                                        |                       | 38.9                           |
|                                 | I05721M  | 27.8                   | 26.6                                        | 48.4                  | 18.8                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 177                    | 170                                         |                       |                                |
|                                 | I05708M  | 150                    | 144                                         |                       |                                |
|                                 | I05710M  | 381                    | 365                                         |                       |                                |
|                                 | I05712M  | 270                    | 259                                         |                       |                                |
|                                 | I05716M  | 148                    | 142                                         |                       | 46.0                           |
|                                 | I05719M  | 135                    | 130                                         | 201                   | 92.7                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 91.3                   | 175                                         |                       |                                |
|                                 | I05704M  | 88.2                   | 169                                         |                       |                                |
|                                 | I05711M  | 37.8                   | 0.725                                       |                       |                                |
|                                 | I05713M  | 127                    | 243                                         |                       |                                |
|                                 | I05722M  | 70.6                   | 1.35                                        |                       | 93.8                           |
|                                 | I05724M  | NA                     | NA                                          | 118                   | 111                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/26/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                                                                                                     |                                                                                                        |           |                                                 |
|---------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------|-------------------------------------------------|
| Study:                                                                                                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                         |           |                                                 |
| Product Number(Test Substance):                                                                                     | T-6889.2 (POAA)                                                                                        |           |                                                 |
| Matrix:                                                                                                             | Monkey Urine                                                                                           | FileNames |                                                 |
| Method/Revision:                                                                                                    | ETS-8-96.0 & ETS-8-97.0                                                                                |           | POAA                                            |
| Analytical Equipment System Number:                                                                                 | Soup 020199, Madeline 040198, Amelia 062498                                                            | Grp 1     | 081999040, 082399052-3, 090899020, 092299034-35 |
| Instrument Software/Version:                                                                                        | MassLynx 3.2                                                                                           | Grp 2     | 090999051-56                                    |
| Filename:                                                                                                           | See listing to the right                                                                               | Grp 3     | 090899021-028                                   |
| R-Squared Value:                                                                                                    | See Attachments                                                                                        | Grp 4     | 081399023-25,71, 092399019                      |
| Slope:                                                                                                              | See Attachments                                                                                        | Box       | 99-160-1, 99-162-3, 99-166-7                    |
| Y-Intercept:                                                                                                        | See Attachments                                                                                        |           |                                                 |
| Dates of Extraction/Analyst:                                                                                        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                       |           |                                                 |
| Dates of Analysis/Analyst:                                                                                          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/22/99, 09/23/99 GML/PTF/IAS/MMH/MEE/IAS           |           |                                                 |
| Date of Data Reduction/Analyst:                                                                                     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/24/99, 09/27/99, 11/06/00 PTF/DRB/GML/MMH/MEE/KJH |           |                                                 |
| Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes. |                                                                                                        |           |                                                 |

**Sample Data**

**MONKEY URINE Week 14**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 0.15            | 0.9582                     | 1                    | 1.19             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 2.88             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 4.36             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 23.4             | 0.0224                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 250                  | 199              | 47.7                                  |                 | 245                      |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 6.52             | <LOQ                                  | 7.96            | 19.5                     |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 61.2             | 58.7                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 136              | 131                                   |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 42.2             | 40.4                                  |                 | 74.8                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 23.9             | 22.9                                  | 63.1            | 47.2                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 133              | 127                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 178              | 171                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 193              | 185                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 1000                 | 52.5             | 50.3                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 120              | 115                                   |                 | 37.7                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 191              | 183                                   | 139             | 52.2                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 10                   | 18.9             | 0.181                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 1000                 | 163              | 156                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 10                   | 6.86             | <LOQ                                  |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 500                  | 280              | 134                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 10                   | 116              | 1.11                                  |                 | 115                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 72.9            | 84.0                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                        |
|-------------------------------------|--------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                         |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                        |
| Matrix:                             | Monkey Urine                                                                                           |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                            |
| Instrument Software/Version:        | MassLynx 3.2                                                                                           |
| Filename:                           | See Attachments                                                                                        |
| R-Squared Value:                    | See Attachments                                                                                        |
| Slope:                              | See Attachments                                                                                        |
| Y-Intercept:                        | See Attachments                                                                                        |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/22/99, 09/23/99 GML/PTF/IAS/MMH/MEE/IAS           |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/24/99, 09/27/99, 11/06/00 PTF/DRB/GML/MMH/MEE/KJH |

**Sample Data**

**MONKEY URINE Week 14**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1.19                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 2.88                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 4.36                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 23.4                   | 0.0224                                      |                       |                                |
|                                 | I05720M  | 199                    | 47.7                                        |                       | 245                            |
|                                 | I05725M  | 6.52                   | <LOQ                                        | 7.96                  | 19.5                           |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 61.2                   | 58.7                                        |                       |                                |
|                                 | I05706M  | 136                    | 131                                         |                       |                                |
|                                 | I05717M  | 42.2                   | 40.4                                        |                       | 74.8                           |
|                                 | I05721M  | 23.9                   | 22.9                                        | 63.1                  | 47.2                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 133                    | 127                                         |                       |                                |
|                                 | I05708M  | 178                    | 171                                         |                       |                                |
|                                 | I05710M  | 193                    | 185                                         |                       |                                |
|                                 | I05712M  | 52.5                   | 50.3                                        |                       |                                |
|                                 | I05716M  | 120                    | 115                                         |                       | 37.7                           |
|                                 | I05719M  | 191                    | 183                                         | 139                   | 52.2                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 18.9                   | 0.181                                       |                       |                                |
|                                 | I05704M  | 163                    | 156                                         |                       |                                |
|                                 | I05711M  | 6.86                   | <LOQ                                        |                       |                                |
|                                 | I05713M  | 280                    | 134                                         |                       |                                |
|                                 | I05722M  | 116                    | 1.11                                        |                       | 115                            |
|                                 | I05724M  | NA                     | NA                                          | 72.9                  | 84.0                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

|                    |                                                                                                                      |
|--------------------|----------------------------------------------------------------------------------------------------------------------|
| Date Entered/By:   | 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC |
| Date Verified/ By: | 09/25/00 LAC, 11/08/00 HOJ                                                                                           |

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See listing to the right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS  
Date of Data Reduction/Analyst: 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH

**FileNames**

**POAA**

**Dilutions**

|              |                                                  |                      |
|--------------|--------------------------------------------------|----------------------|
| <b>Grp 1</b> | 081999042-43, 082399054-55, 092299036, 092799058 | 1/1                  |
| <b>Grp 2</b> | 090999057-62                                     | 1/1000               |
| <b>Grp 3</b> | 090899029-036                                    | 1/1000               |
| <b>Grp 4</b> | 081399029,31,72, 091599080, 092399018            | 1/1,1/10, 1/500, 1/5 |
| <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                     |                      |

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**Sample Data**

**MONKEY URINE Week 16**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 17.8             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 1.62             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 1.24             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 99.4             | 0.0952                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 38.2             | 0.0366                                |                 | 113                      |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 8.01             | <LOQ                                  | 0.0299          | 0.0339                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 39.1             | 37.4                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 65.1             | 62.4                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 76.2             | 73.1                                  |                 | 41.8                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 29.3             | 28.1                                  | 50.2            | 21.0                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 172              | 165                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 196              | 187                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 169              | 162                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 1000                 | 44.6             | 42.7                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 102              | 98.1                                  |                 | 40.9                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 188              | 181                                   | 139             | 57.0                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 10                   | 18.7             | 0.179                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 500                  | 283              | 136                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 5                    | 79.3             | 0.380                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 500                  | 234              | 112                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 10                   | 244              | 2.34                                  |                 | 135                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 50.2            | 67.9                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |
| Matrix:                             | Monkey Urine                                                                                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   |
| Filename:                           | See Attachments                                                                                                                |
| R-Squared Value:                    | See Attachments                                                                                                                |
| Slope:                              | See Attachments                                                                                                                |
| Y-Intercept:                        | See Attachments                                                                                                                |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS               |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |

**Sample Data**

**MONKEY URINE Week 16**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 17.8                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 1.62                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 1.24                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 99.4                   | 0.0952                                      |                       |                                |
|                                 | I05720M  | 38.2                   | 0.0366                                      |                       | 113                            |
|                                 | I05725M  | 8.01                   | <LOQ                                        | 0.0299                | 0.0339                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 39.1                   | 37.4                                        |                       |                                |
|                                 | I05706M  | 65.1                   | 62.4                                        |                       |                                |
|                                 | I05717M  | 76.2                   | 73.1                                        |                       | 41.8                           |
|                                 | I05721M  | 29.3                   | 28.1                                        | 50.2                  | 21.0                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 172                    | 165                                         |                       |                                |
|                                 | I05708M  | 196                    | 187                                         |                       |                                |
|                                 | I05710M  | 169                    | 162                                         |                       |                                |
|                                 | I05712M  | 44.6                   | 42.7                                        |                       |                                |
|                                 | I05716M  | 102.4                  | 98.1                                        |                       | 40.9                           |
|                                 | I05719M  | 188                    | 181                                         | 139                   | 57.0                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 18.7                   | 0.179                                       |                       |                                |
|                                 | I05704M  | 283                    | 136                                         |                       |                                |
|                                 | I05711M  | 79.3                   | 0.380                                       |                       |                                |
|                                 | I05713M  | 234                    | 112                                         |                       |                                |
|                                 | I05722M  | 244                    | 2.34                                        |                       | 135                            |
|                                 | I05724M  | NA                     | NA                                          | 50.2                  | 67.9                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable POAA = Perfluorooctanoate

C8F17COO-

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |              |                                                  |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |              |                                                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |              |                                                  |
| Matrix:                             | Monkey Urine                                                                                                                   | FileNames    |                                                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |              | <b>POAA</b>                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    | <b>Grp 1</b> | 081999060-61, 082399058-59, 092299037, 092799059 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   | <b>Grp 2</b> | 090999063-65, 091599118                          |
| Filename:                           | See listing to the right                                                                                                       | <b>Grp 3</b> | 090899037-044                                    |
| R-Squared Value:                    | See Attachments                                                                                                                | <b>Grp 4</b> | 081399035,38, 091599081,87, 092399017            |
| Slope:                              | See Attachments                                                                                                                | <b>Box</b>   | 99-160-1, 99-162-3, 99-166-7                     |
| Y-Intercept:                        | See Attachments                                                                                                                |              |                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |              |                                                  |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS               |              |                                                  |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                  |

**Sample Data**  
Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 18**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 11.0             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 14.3             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 7.25             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 0.9             | 0.9582                     | 1                    | 5.32             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 78.5             | 0.0752                                |                 | 96.8                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 4.78             | <LOQ                                  | 0.0256          | 0.0248                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 38.3             | 36.7                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 60.8             | 58.3                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 45.6             | 43.7                                  |                 | 51.3                     |
|                                 | I05721M  | 1               | 0.9582                     | 500                  | 25.0             | 12.0                                  | 37.7            | 19.3                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 1000                 | 164              | 157                                   |                 |                          |
|                                 | I05708M  | 1               | 0.9582                     | 1000                 | 238              | 228                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 203              | 194                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 1000                 | 78.9             | 75.6                                  |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1000                 | 213              | 204                                   |                 | 34.3                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 269              | 258                                   | 186             | 63.9                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 10                   | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 500                  | 355              | 170                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 1                    | 210              | 0.201                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 5                    | 84.8             | 0.406                                 |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 10                   | 174              | 1.67                                  |                 | 196                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 43.1            | 84.6                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |
| Matrix:                             | Monkey Urine                                                                                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   |
| Filename:                           | See Attachments                                                                                                                |
| R-Squared Value:                    | See Attachments                                                                                                                |
| Slope:                              | See Attachments                                                                                                                |
| Y-Intercept:                        | See Attachments                                                                                                                |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/08/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/PTF/IAS/MMH/MEE/IAS               |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/09/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |

**Sample Data**

**MONKEY URINE Week 18**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 11.0                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 14.3                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 7.25                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 5.32                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 78.5                   | 0.0752                                      |                       | 96.8                           |
|                                 | I05725M  | 4.78                   | <LOQ                                        | 0.0256                | 0.0248                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 38.3                   | 36.7                                        |                       |                                |
|                                 | I05706M  | 60.8                   | 58.3                                        |                       |                                |
|                                 | I05717M  | 45.6                   | 43.7                                        |                       | 51.3                           |
|                                 | I05721M  | 25.0                   | 12.0                                        | 37.7                  | 19.3                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 164                    | 157                                         |                       |                                |
|                                 | I05708M  | 238                    | 228                                         |                       |                                |
|                                 | I05710M  | 203                    | 194                                         |                       |                                |
|                                 | I05712M  | 78.9                   | 75.6                                        |                       |                                |
|                                 | I05716M  | 213                    | 204                                         |                       | 34.3                           |
|                                 | I05719M  | 269                    | 258                                         | 186                   | 63.9                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 0.0                    | <LOQ                                        |                       |                                |
|                                 | I05704M  | 355                    | 170                                         |                       |                                |
|                                 | I05711M  | 210                    | 0.201                                       |                       |                                |
|                                 | I05713M  | 84.8                   | 0.406                                       |                       |                                |
|                                 | I05722M  | 174                    | 1.67                                        |                       | 196                            |
|                                 | I05724M  | NA                     | NA                                          | 43.1                  | 84.6                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

|                    |                                                                                                  |
|--------------------|--------------------------------------------------------------------------------------------------|
| Date Entered/By:   | 08/20/99, 08/23/99, 08/24/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00 GML/LAC |
| Date Verified/ By: | 09/25/00 LAC, 11/08/00 HOJ                                                                       |

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |              |                                                  |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |              |                                                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |              |                                                  |
| Matrix:                             | Monkey Urine                                                                                                                   | FileNames    |                                                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |              | <b>POAA</b>                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    | <b>Grp 1</b> | 081999062-63, 082399060-61, 092299040, 092799060 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   | <b>Grp 2</b> | 090999069-72                                     |
| Filename:                           | See listing to the right                                                                                                       | <b>Grp 3</b> | 082499049-53, 092899073                          |
| R-Squared Value:                    | See Attachments                                                                                                                | <b>Grp 4</b> | 081399044,73, 09159982,89-90                     |
| Slope:                              | See Attachments                                                                                                                | <b>Box</b>   | 99-160-1, 99-162-3                               |
| Y-Intercept:                        | See Attachments                                                                                                                |              |                                                  |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |              |                                                  |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/09/99, 09/15/99, 09/22/99, 09/27/99, 09/28/99 GML/MMH/IAS/MEE                                 |              |                                                  |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 09/29/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                  |

**Sample Data**  
Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 20**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 17.7             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 7.61             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 13.4             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 22.3             | 0.0213                                |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 47.1             | 0.0451                                |                 | 61.3                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 5.20             | <LOQ                                  | 0.0211          | 0.0130                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 51.0             | 48.9                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 69.0             | 66.1                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 46.2             | 44.3                                  |                 | 18.5                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | 51.1             | 48.9                                  | 52.1            | 9.63                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 1               | 0.9582                     | 5000                 | 344              | 1648 *                                |                 |                          |
|                                 | I05708M+ | 1               | 0.9582                     | 1000                 | 378              | 362                                   |                 |                          |
|                                 | I05710M+ | 1               | 0.9582                     | 1000                 | 185              | 177                                   |                 |                          |
|                                 | I05712M+ | 1               | 0.9582                     | 1000                 | 61.9             | 59.3                                  |                 |                          |
|                                 | I05716M+ | 1               | 0.9582                     | 1000                 | 109              | 104                                   |                 | 93.9                     |
|                                 | I05719M+ | 1               | 0.9582                     | 1000                 | 18.8             | 18.0 **                               | 144             | 135                      |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 1                    | 379              | 0.363                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 500                  | 258              | 124                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 1                    | 140              | 0.134                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 500                  | 195              | 93.3                                  |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 20                   | 122              | 2.33                                  |                 | 136                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 44.0            | 59.9                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

\*\* Conc. was below the LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/25/00, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 09/29/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

\* This sample was not diluted 1/5000; a 2uL injection of D1000 was analyzed instead.

All other injections were 10 uL. This provided a 1/5 dilution of D1000 for a total of D5000.

A 1/4000 dilution of I05707M was also analyzed, but was just out of calibration range. GML/KJH 9/29/99

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |
| Matrix:                             | Monkey Urine                                                                                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   |
| Filename:                           | See Attachments                                                                                                                |
| R-Squared Value:                    | See Attachments                                                                                                                |
| Slope:                              | See Attachments                                                                                                                |
| Y-Intercept:                        | See Attachments                                                                                                                |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 09/09/99, 09/15/99, 09/22/99, 09/27/99, 09/28/99 GML/MMH/IAS/MEE                                 |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/27/99, 09/28/99, 09/29/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |

**Sample Data**

**MONKEY URINE Week 20**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 17.7                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 7.61                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 13.4                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 22.3                   | 0.0213                                      |                       |                                |
|                                 | I05720M  | 47.1                   | 0.0451                                      |                       | 61.3                           |
|                                 | I05725M  | 5.20                   | <LOQ                                        | 0.0211                | 0.0130                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 51.0                   | 48.9                                        |                       |                                |
|                                 | I05706M  | 69.0                   | 66.1                                        |                       |                                |
|                                 | I05717M  | 46.2                   | 44.3                                        |                       | 18.5                           |
|                                 | I05721M  | 51.1                   | 48.9                                        | 52.1                  | 9.63                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M  | 344                    | 1648 *                                      |                       |                                |
|                                 | I05708M+ | 378                    | 362                                         |                       |                                |
|                                 | I05710M+ | 185                    | 177                                         |                       |                                |
|                                 | I05712M+ | 61.9                   | 59.3                                        |                       |                                |
|                                 | I05716M+ | 109                    | 104                                         |                       | 93.9                           |
|                                 | I05719M+ | 18.8                   | 18.0 **                                     | 144                   | 135                            |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 379                    | 0.363                                       |                       |                                |
|                                 | I05704M  | 258                    | 124                                         |                       |                                |
|                                 | I05711M  | 140                    | 0.134                                       |                       |                                |
|                                 | I05713M  | 195                    | 93.3                                        |                       |                                |
|                                 | I05722M  | 122                    | 2.33                                        |                       | 136                            |
|                                 | I05724M  | NA                     | NA                                          | 44.0                  | 59.9                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

\*\* Conc. was below the LOQ

C8F17COO-

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/25/00, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 09/29/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

\* This sample was not diluted 1/5000; a 2uL injection of D1000 was analyzed instead.

All other injections were 10 uL. This provided a 1/5 dilution of D1000 for a total of D5000.

A 1/4000 dilution of I05707M was also analyzed, but was just out of calibration range. GML/KJH 9/29/99

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                |              |                                                                |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|--------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                 |              |                                                                |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                |              |                                                                |
| Matrix:                             | Monkey Urine                                                                                                                   | FileNames    |                                                                |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                        |              |                                                                |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                    | <b>Grp 1</b> | <b>POAA</b><br>081999066-67, 082399064-5, 092299041, 092799061 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                   | <b>Grp 2</b> | 090999075-77, 091599119                                        |
| Filename:                           | See listing to the right                                                                                                       | <b>Grp 3</b> | 082499056-58,60-61, 091099040                                  |
| R-Squared Value:                    | See Attachments                                                                                                                | <b>Grp 4</b> | 091599093-94, 092399016,24-25                                  |
| Slope:                              | See Attachments                                                                                                                | <b>Box</b>   | 99-160-1, 99-162-3                                             |
| Y-Intercept:                        | See Attachments                                                                                                                |              |                                                                |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                               |              |                                                                |
| Dates of Analysis/Analyst:          | 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/SAH/PTF/MMH/IAS/MEE                         |              |                                                                |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                                |

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**Sample Data**

**MONKEY URINE Week 22**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 35.9             | 0.0344                                |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 29.9             | 0.0287                                |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 7.09             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 14.8             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 21.7             | 0.0208                                |                 | 29.8                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 8.21             | <LOQ                                  | 0.0231          | 0.00688                  |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 46.7             | 44.7                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 500                  | 112              | 53.7                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 197              | 189                                   |                 | 84.4                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | NA               | NA                                    | 95.8            | 80.8                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 1               | 0.9582                     | 1000                 | 60.6             | 58.0                                  |                 |                          |
|                                 | I05708M+ | 1               | 0.9582                     | 1000                 | 175              | 168                                   |                 |                          |
|                                 | I05710M+ | 1               | 0.9582                     | 1000                 | 246              | 235                                   |                 |                          |
|                                 | I05712M  | 1               | 0.9582                     | 1000                 | 90.7             | 86.9                                  |                 |                          |
|                                 | I05716M+ | 1               | 0.9582                     | 1000                 | 150              | 144                                   |                 | 49.7                     |
|                                 | I05719M+ | 1               | 0.9582                     | 1000                 | 266              | 255                                   | 158             | 78.4                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 1                    | 367              | 0.352                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 1000                 | 266              | 255                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 1                    | 348              | 0.334                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 1000                 | 247              | 237                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 20                   | 45.3             | 0.868                                 |                 | 136                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 98.5            | 134                      |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/SAH/PTF/MMH/IAS/MEE  
Date of Data Reduction/Analyst: 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH

**Sample Data**

**MONKEY URINE Week 22**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 35.9                   | 0.0344                                      |                       |                                |
|                                 | I05714M  | 29.9                   | 0.0287                                      |                       |                                |
|                                 | I05715M  | 7.09                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 14.8                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 21.7                   | 0.0208                                      |                       | 29.8                           |
|                                 | I05725M  | 8.21                   | <LOQ                                        | 0.0231                | 0.00688                        |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 46.7                   | 44.7                                        |                       |                                |
|                                 | I05706M  | 112                    | 53.7                                        |                       |                                |
|                                 | I05717M  | 197                    | 189                                         |                       | 84.4                           |
|                                 | I05721M  | NA                     | NA                                          | 95.8                  | 80.8                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 60.6                   | 58.0                                        |                       |                                |
|                                 | I05708M+ | 175                    | 168                                         |                       |                                |
|                                 | I05710M+ | 246                    | 235                                         |                       |                                |
|                                 | I05712M  | 90.7                   | 86.9                                        |                       |                                |
|                                 | I05716M+ | 150                    | 144                                         |                       | 49.7                           |
|                                 | I05719M+ | 266                    | 255                                         | 158                   | 78.4                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 367                    | 0.352                                       |                       |                                |
|                                 | I05704M  | 266                    | 255                                         |                       |                                |
|                                 | I05711M  | 348                    | 0.334                                       |                       |                                |
|                                 | I05713M  | 247                    | 237                                         |                       |                                |
|                                 | I05722M  | 45.3                   | 0.868                                       |                       | 136                            |
|                                 | I05724M  | NA                     | NA                                          | 98.5                  | 134                            |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable POAA = Perfluorooctanoate

C8F17COO-

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                     |              |                                                      |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------|--------------|------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                      |              |                                                      |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                     |              |                                                      |
| Matrix:                             | Monkey Urine                                                                                                        | FileNames    |                                                      |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                             |              |                                                      |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                         | <b>Grp 1</b> | <b>POAA</b> 081999068-69, 082399066-67, 092299042-43 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                        | <b>Grp 2</b> | 090999078-82                                         |
| Filename:                           | See listing to the right                                                                                            | <b>Grp 3</b> | 082499064-65,67-69, 091099041                        |
| R-Squared Value:                    | See Attachments                                                                                                     | <b>Grp 4</b> | 081399055,65, 091599083,95-96                        |
| Slope:                              | See Attachments                                                                                                     | <b>Box</b>   | 99-160-1, 99-162-3                                   |
| Y-Intercept:                        | See Attachments                                                                                                     |              |                                                      |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                                    |              |                                                      |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99 GML/SAH/PTF/MMH/IAS                  |              |                                                      |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/27/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/KJH              |              |                                                      |
|                                     | Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes. |              |                                                      |

**Sample Data**

**MONKEY URINE Week 24**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 0.4             | 0.9582                     | 1                    | 7.42             | <LOQ                                  |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 6.32             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 4.53             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 3.10             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 22.2             | 0.0213                                |                 | 60.1                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 5.39             | <LOQ                                  | 0.0125 A        | 0.00749                  |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 38.4             | 36.8                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 55.6             | 53.3                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 50.9             | 48.7                                  |                 | 18.4                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | NA               | NA                                    | 46.3            | 8.52                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 1               | 0.9582                     | 1000                 | 192              | 184                                   |                 |                          |
|                                 | I05708M+ | 1               | 0.9582                     | 1000                 | 226              | 216                                   |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 222              | 212                                   |                 |                          |
|                                 | I05712M+ | 1               | 0.9582                     | 1000                 | 56.3             | 54.0                                  |                 |                          |
|                                 | I05716M+ | 1               | 0.9582                     | 1000                 | 117              | 112                                   |                 | 40.3                     |
|                                 | I05719M+ | 1               | 0.9582                     | 1000                 | 170              | 163                                   | 157             | 63.3                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 1                    | 319              | 0.306                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 500                  | 324              | 155                                   |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 1                    | 112              | 0.107                                 |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 500                  | 259              | 124                                   |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 10                   | 25.9             | 0.248                                 |                 | 138                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 56.0            | 77.2                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                        |
|-------------------------------------|--------------------------------------------------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                         |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                        |
| Matrix:                             | Monkey Urine                                                                                           |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                            |
| Instrument Software/Version:        | MassLynx 3.2                                                                                           |
| Filename:                           | See Attachments                                                                                        |
| R-Squared Value:                    | See Attachments                                                                                        |
| Slope:                              | See Attachments                                                                                        |
| Y-Intercept:                        | See Attachments                                                                                        |
| Dates of Extraction/Analyst:        | 08/04/99, 08/05/99, 08/17/99, 08/20/99, 09/21/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99 GML/SAH/PTF/MMH/IAS     |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 09/13/99, 09/16/99, 09/27/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/KJH |

**Sample Data**

**MONKEY URINE Week 24**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 7.42                   | <LOQ                                        |                       |                                |
|                                 | I05714M  | 6.32                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 4.53                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 3.10                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 22.2                   | 0.0213                                      |                       | 60.1                           |
|                                 | I05725M  | 5.39                   | <LOQ                                        | 0.0125 A              | 0.00749                        |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 38.4                   | 36.8                                        |                       |                                |
|                                 | I05706M  | 55.6                   | 53.3                                        |                       |                                |
|                                 | I05717M  | 50.9                   | 48.7                                        |                       | 18.4                           |
|                                 | I05721M  | NA                     | NA                                          | 46.3                  | 8.52                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 192                    | 184                                         |                       |                                |
|                                 | I05708M+ | 226                    | 216                                         |                       |                                |
|                                 | I05710M  | 222                    | 212                                         |                       |                                |
|                                 | I05712M+ | 56.3                   | 54.0                                        |                       |                                |
|                                 | I05716M+ | 117                    | 112                                         |                       | 40.3                           |
|                                 | I05719M+ | 170                    | 163                                         | 157                   | 63.3                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 319                    | 0.306                                       |                       |                                |
|                                 | I05704M  | 324                    | 155                                         |                       |                                |
|                                 | I05711M  | 112                    | 0.107                                       |                       |                                |
|                                 | I05713M  | 259                    | 124                                         |                       |                                |
|                                 | I05722M  | 25.9                   | 0.248                                       |                       | 138                            |
|                                 | I05724M  | NA                     | NA                                          | 56.0                  | 77.2                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable POAA = Perfluorooctanoate

A = Below LOQ

C8F17COO-

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/20/99, 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/28/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                                          |              |                                                              |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                                           |              |                                                              |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                                          |              |                                                              |
| Matrix:                             | Monkey Urine                                                                                                                             | FileNames    |                                                              |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                                                  |              |                                                              |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                                              | <b>Grp 1</b> | <b>POAA</b> 081999072-73, 082399070-71, 092299044, 092799064 |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                                             | <b>Grp 2</b> | 090999083-85                                                 |
| Filename:                           | See listing to the right                                                                                                                 | <b>Grp 3</b> | 082499072-75, 091099042, 092799046                           |
| R-Squared Value:                    | See Attachments                                                                                                                          | <b>Grp 4</b> | 081399066, 091599099-100, 092399015,23                       |
| Slope:                              | See Attachments                                                                                                                          | <b>Box</b>   | 99-160-1, 99-162-3                                           |
| Y-Intercept:                        | See Attachments                                                                                                                          |              |                                                              |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                                                                                                 |              |                                                              |
| Dates of Analysis/Analyst:          | 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/SAH/PTF/MMH/IAS/MEE               |              |                                                              |
| Date of Data Reduction/Analyst:     | 08/18/99, 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH |              |                                                              |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 26**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 1               | 0.9582                     | 1                    | 21.7             | 0.0208                                |                 |                          |
|                                 | I05714M  | 1               | 0.9582                     | 1                    | 10.0             | <LOQ                                  |                 |                          |
|                                 | I05715M  | 1               | 0.9582                     | 1                    | 7.19             | <LOQ                                  |                 |                          |
|                                 | I05718M  | 1               | 0.9582                     | 1                    | 8.53             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 83.2             | 0.0797                                |                 | 98.7                     |
|                                 | I05725M  | 1               | 0.9582                     | 1                    | 4.29             | <LOQ                                  | 0.0268          | 0.0265                   |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 1               | 0.9582                     | 1000                 | 38.6             | 37.0                                  |                 |                          |
|                                 | I05706M  | 1               | 0.9582                     | 1000                 | 55.9             | 53.6                                  |                 |                          |
|                                 | I05717M  | 1               | 0.9582                     | 1000                 | 67.0             | 64.2                                  |                 | 26.6                     |
|                                 | I05721M  | 1               | 0.9582                     | 1000                 | NA               | NA                                    | 51.6            | 13.7                     |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 1               | 0.9582                     | 1000                 | 268              | 256                                   |                 |                          |
|                                 | I05708M+ | 1               | 0.9582                     | 1000                 | 69.6             | 66.6                                  |                 |                          |
|                                 | I05710M  | 1               | 0.9582                     | 1000                 | 53.0             | 50.8                                  |                 |                          |
|                                 | I05712M+ | 1               | 0.9582                     | 1000                 | 75.5             | 72.3                                  |                 |                          |
|                                 | I05716M+ | 1               | 0.9582                     | 1000                 | 107              | 103                                   |                 | 68.8                     |
|                                 | I05719M  | 1               | 0.9582                     | 1000                 | 112              | 107                                   | 109             | 75.2                     |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 1               | 0.9582                     | 1                    | 233              | 0.223                                 |                 |                          |
|                                 | I05704M  | 1               | 0.9582                     | 250                  | 241              | 57.7                                  |                 |                          |
|                                 | I05711M  | 1               | 0.9582                     | 1                    | 71.2             | 0.0682                                |                 |                          |
|                                 | I05713M  | 1               | 0.9582                     | 250                  | 158              | 37.8                                  |                 |                          |
|                                 | I05722M  | 1               | 0.9582                     | 1                    | 225              | 0.215                                 |                 | 141                      |
|                                 | I05724M  | 1               | 0.9582                     | NA                   | NA               | NA                                    | 19.2            | 27.0                     |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/13/99, 08/19/99, 08/23/99, 08/24/99, 09/09/99, 09/10/99, 09/15/99, 09/22/99, 09/23/99, 09/27/99 GML/SAH/PTF/MMH/IAS/MEE  
Date of Data Reduction/Analyst: 08/18/99, 08/20/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/24/99, 09/27/99, 09/28/99, 11/06/00, 11/13/00 PTF/DRB/GML/MMH/MEE/IAS/KJH

**Sample Data**

**MONKEY URINE Week 26**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05709M  | 21.7                   | 0.0208                                      |                       |                                |
|                                 | I05714M  | 10.0                   | <LOQ                                        |                       |                                |
|                                 | I05715M  | 7.19                   | <LOQ                                        |                       |                                |
|                                 | I05718M  | 8.53                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 83.2                   | 0.0797                                      |                       | 98.7                           |
|                                 | I05725M  | 4.29                   | <LOQ                                        | 0.0268                | 0.0265                         |
| <b>Group 2</b><br>3.0 mg/kg/day | I05702M  | 38.6                   | 37.0                                        |                       |                                |
|                                 | I05706M  | 55.9                   | 53.6                                        |                       |                                |
|                                 | I05717M  | 67.0                   | 64.2                                        |                       | 26.6                           |
|                                 | I05721M  | NA                     | NA                                          | 51.6                  | 13.7                           |
| <b>Group 3</b><br>10 mg/kg/day  | I05707M+ | 268                    | 256                                         |                       |                                |
|                                 | I05708M+ | 69.6                   | 66.6                                        |                       |                                |
|                                 | I05710M  | 53.0                   | 50.8                                        |                       |                                |
|                                 | I05712M+ | 75.5                   | 72.3                                        |                       |                                |
|                                 | I05716M+ | 107                    | 103                                         |                       | 68.8                           |
|                                 | I05719M  | 112                    | 107                                         | 109                   | 75.2                           |
| <b>Group 4</b><br>30 mg/kg/day  | I05703M  | 233                    | 0.223                                       |                       |                                |
|                                 | I05704M  | 241                    | 57.7                                        |                       |                                |
|                                 | I05711M  | 71.2                   | 0.0682                                      |                       |                                |
|                                 | I05713M  | 158                    | 37.8                                        |                       |                                |
|                                 | I05722M  | 225                    | 0.215                                       |                       | 141                            |
|                                 | I05724M  | NA                     | NA                                          | 19.2                  | 27.0                           |

Limit of Quantitation (LOQ): 0.0182 ug/mL NA = Not Analyzed/Not Applicable POAA = Perfluorooctanoate

C8F17COO-

+ CCVs analyzed prior to these samples were not within +/- 30% criteria. LAC 11/16/00

Date Entered/By: 08/23/99, 08/24/99, 08/25/99, 09/13/99, 09/16/99, 09/17/99, 09/27/99, 09/28/99, 11/06/00, 11/07/00, 11/14/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ, 11/15/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See listing to the right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 08/24/99 GML/SAH/PTF  
Date of Data Reduction/Analyst: 08/20/99, 08/25/99, 11/06/00 GML/PTF/KJH

**FileNames**

|              | <b>POAA</b>  | <b>Dilutions</b> |
|--------------|--------------|------------------|
| <b>Grp 1</b> | 081999074-75 | 1/1              |
| <b>Grp 3</b> | 082499052-53 | 1/10             |
| <b>Box</b>   | 99-160-1     |                  |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 28**

| Group<br>Dose                   | Sample # | Extraction<br>Vol. | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|--------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1                  | 0.9582                           | 1                          | 228                    | 0.218                                       |                       |                                |
|                                 | I05720M  | 1                  | 0.9582                           | 1                          | 11.9                   | <LOQ                                        | 0.118                 | 0.142                          |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1                  | 0.9582                           | 10                         | 32.8                   | 0.314                                       |                       |                                |
|                                 | I05716M  | 1                  | 0.9582                           | 10                         | 35.5                   | 0.340                                       | 0.327                 | 0.0182                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 08/26/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 08/24/99 GML/SAH/PTF                                 |
| Date of Data Reduction/Analyst:     | 08/20/99, 08/25/99, 11/06/00 GML/PTF/KJH                       |

**Sample Data**

**MONKEY URINE Week 28**

| Group<br>Dose            | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|--------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Group 1<br>0.0 mg/kg/day | I05718M  | 228                    | 0.218                                       |                       |                                |
|                          | I05720M  | 11.9                   | <LOQ                                        | 0.118                 | 0.142                          |
| Group 3<br>10 mg/kg/day  | I05712M  | 32.8                   | 0.314                                       |                       |                                |
|                          | I05716M  | 35.5                   | 0.340                                       | 0.327                 | 0.0182                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

|                    |                                      |
|--------------------|--------------------------------------|
| Date Entered/By:   | 08/23/99, 08/26/99, 11/07/00 GML/LAC |
| Date Verified/ By: | 09/25/00 LAC, 11/08/00 HOJ           |

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |              |                              |
|-------------------------------------|----------------------------------------------------------------|--------------|------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |              |                              |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |              |                              |
| Matrix:                             | Monkey Urine                                                   |              |                              |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        | FileNames    |                              |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |              |                              |
| Instrument Software/Version:        | MassLynx 3.2                                                   | <b>Grp 1</b> | <b>POAA</b> 081999078-79 1/1 |
| Filename:                           | See listing to the right                                       | <b>Grp 3</b> | 091599018, 159 1/1&10        |
| R-Squared Value:                    | See Attachments                                                | <b>Box</b>   | 99-160-1                     |
| Slope:                              | See Attachments                                                |              |                              |
| Y-Intercept:                        | See Attachments                                                |              |                              |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |              |                              |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |              |                              |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |              |                              |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 30**

| Group<br>Dose                   | Sample # | Extraction<br>Vol. | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|--------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1                  | 0.9582                           | 1                          | 2.73                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 1                  | 0.9582                           | 1                          | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1                  | 0.9582                           | 1                          | 290                    | 0.278                                       |                       |                                |
|                                 | I05716M  | 1                  | 0.9582                           | 10                         | 46.5                   | 0.445                                       | 0.361                 | 0.118                          |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 09/16/99, 09/17/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: 8/23/99 GML, 8/24/99 GML, 9/ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See Attachments  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 09/15/99 GML  
Date of Data Reduction/Analyst: 08/20/99, 09/16/99, 11/06/00 GML/KJH

**Sample Data**

**MONKEY URINE Week 30**

| Group<br>Dose            | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|--------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| Group 1<br>0.0 mg/kg/day | I05718M  | 2.73                   | <LOQ                                        |                       |                                |
|                          | I05720M  | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| Group 3<br>10 mg/kg/day  | I05712M  | 290                    | 0.278                                       |                       |                                |
|                          | I05716M  | 46.5                   | 0.445                                       | 0.361                 | 0.118                          |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/23/99, 09/16/99, 09/17/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                     |              |                  |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------|--------------|------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                      |              |                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                     |              |                  |
| Matrix:                             | Monkey Urine                                                                                                        |              |                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                             | FileNames    |                  |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                         | <b>POAA</b>  | <b>Dilutions</b> |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                        | <b>Grp 1</b> | 081999080-81     |
| Filename:                           | See listing to the right                                                                                            | <b>Grp 3</b> | 091599019-20     |
| R-Squared Value:                    | See Attachments                                                                                                     | <b>Box</b>   | 99-160-1         |
| Slope:                              | See Attachments                                                                                                     |              |                  |
| Y-Intercept:                        | See Attachments                                                                                                     |              |                  |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                                                                            |              |                  |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                                                                              |              |                  |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                                                                                |              |                  |
| <b>Sample Data</b>                  | Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes. |              |                  |

**MONKEY URINE Week 32**

| Group<br>Dose                   | Sample # | Extraction<br>Vol. | POAA Std<br>Correction<br>Factor | POAA<br>Dilution<br>Factor | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|--------------------|----------------------------------|----------------------------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1                  | 0.9582                           | 1                          | 4.68                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 1                  | 0.9582                           | 1                          | 5.14                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1                  | 0.9582                           | 1                          | 164                    | 0.157                                       |                       |                                |
|                                 | I05716M  | 1                  | 0.9582                           | 1                          | 74.3                   | 0.0712                                      | 0.114                 | 0.0608                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL      NA = Not Analyzed/Not Applicable      POAA = Perfluorooctanoate

**C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>**

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |

**Sample Data**

**MONKEY URINE Week 32**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 4.68                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 5.14                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 164                    | 0.157                                       |                       |                                |
|                                 | I05716M  | 74.3                   | 0.0712                                      | 0.114                 | 0.0608                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

|                    |                                      |
|--------------------|--------------------------------------|
| Date Entered/By:   | 08/23/99, 09/16/99, 11/07/00 GML/LAC |
| Date Verified/ By: | 09/25/00 LAC, 11/08/00 HOJ           |

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See listing to the right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 09/15/99 GML  
Date of Data Reduction/Analyst: 08/20/99, 09/16/99, 11/06/00 GML/KJH

**FileNames**

|              | <b>POAA</b>  | <b>Dilutions</b> |
|--------------|--------------|------------------|
| <b>Grp 1</b> | 081999084-85 | 1/1              |
| <b>Grp 3</b> | 091599021-22 | 1/1              |
| <b>Box</b>   | 99-160-1     |                  |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 34**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  | <LOQ            | NA                       |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1               | 0.9582                     | 1                    | 104              | 0.0993                                |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1                    | 149              | 0.142                                 | 0.121           | 0.0305                   |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

$C_8F_{17}COO^-$

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |

**Sample Data**

**MONKEY URINE Week 34**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 104                    | 0.0993                                      |                       |                                |
|                                 | I05716M  | 149                    | 0.142                                       | 0.121                 | 0.0305                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See listing to the right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 09/15/99 GML  
Date of Data Reduction/Analyst: 08/20/99, 09/16/99, 11/06/00 GML/KJH

FileNames

|              | POAA         | Dilutions |
|--------------|--------------|-----------|
| <b>Grp 1</b> | 081999086-87 | 1/1       |
| <b>Grp 3</b> | 091599025-26 | 1/1       |
| <b>Box</b>   | 99-160-1     |           |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 36**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 18.4             | 0.0176                                | 0.0117 A        | 0.00841                  |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1               | 0.9582                     | 1                    | 40.1             | 0.0384                                |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1                    | 64.6             | 0.0619                                | 0.0502          | 0.0166                   |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |

**Sample Data**

**MONKEY URINE Week 36**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 18.4                   | 0.0176                                      | 0.0117 A              | 0.00841                        |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 40.1                   | 0.0384                                      |                       |                                |
|                                 | I05716M  | 64.6                   | 0.0619                                      | 0.0502                | 0.0166                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

A = Below LOQ

C8F17COO-

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                                                                     |              |                  |
|-------------------------------------|---------------------------------------------------------------------------------------------------------------------|--------------|------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys                                                      |              |                  |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                                                                     |              |                  |
| Matrix:                             | Monkey Urine                                                                                                        |              |                  |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                                                                             | FileNames    |                  |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                                                                         | <b>POAA</b>  | <b>Dilutions</b> |
| Instrument Software/Version:        | MassLynx 3.2                                                                                                        | <b>Grp 1</b> | 081999090-91     |
| Filename:                           | See listing to the right                                                                                            | <b>Grp 3</b> | 091599027-28     |
| R-Squared Value:                    | See Attachments                                                                                                     | <b>Box</b>   | 99-160-1         |
| Slope:                              | See Attachments                                                                                                     |              |                  |
| Y-Intercept:                        | See Attachments                                                                                                     |              |                  |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                                                                            |              |                  |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                                                                              |              |                  |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                                                                                |              |                  |
| <b>Sample Data</b>                  | Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes. |              |                  |

**MONKEY URINE Week 38**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. MS/MSD RPD |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|--------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |                          |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  | <LOQ            | NA                       |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1               | 0.9582                     | 1                    | 37.2             | 0.0357                                |                 |                          |
|                                 | I05716M  | 1               | 0.9582                     | 1                    | 22.1             | 0.0212                                | 0.0284          | 0.0102                   |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |

**Sample Data**

**MONKEY URINE Week 38**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 37.2                   | 0.0357                                      |                       |                                |
|                                 | I05716M  | 22.1                   | 0.0212                                      | 0.0284                | 0.0102                         |

Limit of Quantitation (LOQ): 0.0182 ug/mL

**POAA = Perfluorooctanoic Acid Anion**  
**C<sub>8</sub>F<sub>17</sub>COO<sup>-</sup>**

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

Study: 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys  
Product Number(Test Substance): T-6889.2 (POAA)  
Matrix: Monkey Urine  
Method/Revision: ETS-8-96.0 & ETS-8-97.0  
Analytical Equipment System Number: Soup 020199, Madeline 040198, Amelia 062498  
Instrument Software/Version: MassLynx 3.2  
Filename: See listing to the right  
R-Squared Value: See Attachments  
Slope: See Attachments  
Y-Intercept: See Attachments  
Dates of Extraction/Analyst: 08/17/99 SEE/RWW/MCH/SAL  
Dates of Analysis/Analyst: 08/19/99, 09/15/99 GML  
Date of Data Reduction/Analyst: 08/20/99, 09/16/99, 11/06/00 GML/KJH

|              | POAA         | Dilutions |
|--------------|--------------|-----------|
| <b>Grp 1</b> | 081999092-93 | 1/1       |
| <b>Grp 3</b> | 091599031-32 | 1/1       |
| <b>Box</b>   | 99-160-1     |           |

**Sample Data**

Ext. Vol. = 1 since curves are extracted at the same ratio as the specimens, 2 mL initial and 0.5 mL final volumes.

**MONKEY URINE Week 40**

| Group Dose                      | Sample # | Extraction Vol. | POAA Std Correction Factor | POAA Dilution Factor | POAA Conc. ng/mL | Concentration of POAA ug/mL or % Rec. | Mean POAA ug/mL | RSD Std. Dev. |
|---------------------------------|----------|-----------------|----------------------------|----------------------|------------------|---------------------------------------|-----------------|---------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  |                 |               |
|                                 | I05720M  | 1               | 0.9582                     | 1                    | 0.00             | <LOQ                                  | <LOQ            | NA            |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 1               | 0.9582                     | 1                    | 34.0             | 0.0325                                |                 |               |
|                                 | I05716M  | 1               | 0.9582                     | 1                    | 17.7             | <LOQ                                  | 0.0254          | 0.0101        |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate

$C_8F_{17}COO^-$

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC

Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

|                                     |                                                                |
|-------------------------------------|----------------------------------------------------------------|
| Study:                              | 6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys |
| Product Number(Test Substance):     | T-6889.2 (POAA)                                                |
| Matrix:                             | Monkey Urine                                                   |
| Method/Revision:                    | ETS-8-96.0 & ETS-8-97.0                                        |
| Analytical Equipment System Number: | Soup 020199, Madeline 040198, Amelia 062498                    |
| Instrument Software/Version:        | MassLynx 3.2                                                   |
| Filename:                           | See Attachments                                                |
| R-Squared Value:                    | See Attachments                                                |
| Slope:                              | See Attachments                                                |
| Y-Intercept:                        | See Attachments                                                |
| Dates of Extraction/Analyst:        | 08/17/99 SEE/RWW/MCH/SAL                                       |
| Dates of Analysis/Analyst:          | 08/19/99, 09/15/99 GML                                         |
| Date of Data Reduction/Analyst:     | 08/20/99, 09/16/99, 11/06/00 GML/KJH                           |

**Sample Data**

**MONKEY URINE Week 40**

| Group<br>Dose                   | Sample # | POAA<br>Conc.<br>ng/mL | Concentration<br>of POAA<br>ug/mL or % Rec. | Mean<br>POAA<br>ug/mL | RSD<br>Std. Dev.<br>MS/MSD RPD |
|---------------------------------|----------|------------------------|---------------------------------------------|-----------------------|--------------------------------|
| <b>Group 1</b><br>0.0 mg/kg/day | I05718M  | 0.00                   | <LOQ                                        |                       |                                |
|                                 | I05720M  | 0.00                   | <LOQ                                        | <LOQ                  | NA                             |
| <b>Group 3</b><br>10 mg/kg/day  | I05712M  | 34.0                   | 0.0325                                      |                       |                                |
|                                 | I05716M  | 17.7                   | <LOQ                                        | 0.0254                | 0.010                          |

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

POAA = Perfluorooctanoate  
C8F17COO-

Date Entered/By: 08/23/99, 09/16/99, 11/07/00 GML/LAC  
Date Verified/ By: 09/25/00 LAC, 11/08/00 HOJ

**FACT-TOX-026**  
**Covance 6329-231**

**Urine QC Summary - TOX026**

|            |                  | PFOS   |
|------------|------------------|--------|
| 08/04/1999 | MKU08049 MS-1    | 133% * |
|            | MKU08049 MSD-1   | 92%    |
|            | MKU08049 MS-2    | 95%    |
|            | MKU08049 MSD-2   | 70%    |
| 08/05/1999 | MKU08059 MS      | 91%    |
|            | MKU08059 MSD     | 87%    |
| 08/17/1999 | MKU08179 MS 1-1  | 92%    |
|            | MKU08179 MSD 1-1 | 104%   |
|            | MKU08179 MS 1-2  | 105%   |
|            | MKU08179 MSD 1-2 | 101%   |
|            | MKU08179 MS 1-3  | 74%    |
|            | MKU08179 MSD 1-3 | 74%    |
|            | MKU08179 MS 1-4  | 73%    |
|            | MKU08179 MSD 1-4 | 73%    |
| 08/20/1999 | MKU08209 MS 1-1  | 66%    |
|            | MKU08209 MSD 1-1 | 63%    |
|            | MKU08209 MS 1-2  | 90%    |
|            | MKU08209 MSD 1-2 | 89%    |
| 09/12/1999 | MKU09219 MS-1    | 92%    |
|            | MKU09219 MSD-1   | 95%    |

NA = Not Applicable

NR = Not Reported

\*outlier by Grubbs test at 0.05 confidence level

|                          |     |                      |
|--------------------------|-----|----------------------|
| Average                  | 88% | Used in Final Report |
| Standard Deviation       | 17% |                      |
| Grubbs outlier included? | Yes |                      |
| Number of spikes         | 20  |                      |

|                          |     |               |
|--------------------------|-----|---------------|
| Average                  | 86% | Data not used |
| Standard Deviation       | 13% |               |
| Grubbs outlier included? | No  |               |
| Number of spikes         | 19  |               |

## Appendix G: Example Calculations

### Formula Used for Sera Analyses in Study FACT TOX-026

$$\text{AR (ng/mL)} \times \text{DF} \times \text{SC} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = \text{Reported Concentration } \mu\text{g/mL}$$

### Calculation Used for Group 2, Week 27, Animal ID 105702M

$$500 \text{ ng/mL} \times 100 \times 0.9582 \times \frac{1 \text{ mL}}{1 \text{ mL}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = 47.9 \mu\text{g/mL}$$

AR— Analytical result from MassLynx summary

DF— Dilution factor

SC—POAA salt correction constant (0.9582)

FV—Final extract volume (1.0 mL unless otherwise noted)

EV—Volume of sera extracted

### Formula Used for Liver Analyses in Study FACT TOX-026

$$\text{AR (ng/g)} \times \frac{\partial \text{ curve}^{(1)}}{\partial \text{ sample}} \times \text{SC} \times \text{DF} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = [\text{POAA}] \text{ sample } (\mu\text{g/g})$$

<sup>(1)</sup>  $\partial \text{ curve}$  is assumed to be:  $\frac{1 \text{ g liver}}{5 \text{ mL H}_2\text{O}}$

### Calculation Used for Group 2, Week 27, Animal ID 105702M

$$160 \text{ ng/g} \times \frac{1 \text{ g/ 5 mL}}{1.0104 \text{ g/ 5 mL}} \times 0.9582 \times 100 \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = 15.2 \mu\text{g/g}$$

AR— Analytical result from MassLynx summary

$\partial \text{ curve}$ —Density of the liver standard curve, assumed to be 1g liver/ 5 ml water

$\partial \text{ sample}$ —Density of the liver sample (g sample/ 5 mL H<sub>2</sub>O)

SC—POAA salt correction constant (0.9582)

DF— Dilution factor

**Formula Used for Urine Analyses in Study FACT TOX-026**

$$\text{AR (ng/mL)} \times \text{DF} \times \text{SC} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = \text{Reported Concentration } \mu\text{g/mL}$$

**Calculation Used for Group 2, Week 26, Animal ID 105702M**

$$38.6 \text{ ng/mL} \times 1000 \times 0.9582 \times \frac{1 \text{ mL}}{1 \text{ mL}} \times \frac{1.0 \mu\text{g}}{1000 \text{ ng}} = 37.0 \mu\text{g/mL}$$

AR—Analytical result from MassLynx summary

DF—Dilution factor

SC—POAA salt correction constant (0.9582)

ER—Extraction ratio (equal to 1.0 unless otherwise noted)

FV—Final extract volume (1.0 mL unless otherwise noted)

EV—Volume of urine extracted

---

## **Appendix H: Contract Lab Report**

This appendix includes the following contract laboratory report:

*Centre Analytical Laboratories, 26–Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys, (110 pages)*

# **ANALYTICAL REPORT**

## **STUDY TITLE**

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

## **DATA REQUIREMENTS**

Analytical Method Requirements

## **STUDY DIRECTOR**

Paul Lieder, 3M

## **PRINCIPAL INVESTIGATOR**

Emily R. Stauffer, Centre

## **ANALYTICAL REPORT COMPLETION DATE**

April 2, 2001

## **PERFORMING LABORATORY / TESTING FACILITY**

Centre Analytical Laboratories, Inc. (Centre)  
3048 Research Drive  
State College, PA 16801  
Phone: 814-231-8032

## **STUDY SPONSOR**

3M Toxicology Services – Medical Department  
3M Center, Building 220-2E-02  
P.O. Box 33220  
St. Paul, MN 55133-3220

## **PROJECT IDENTIFICATION**

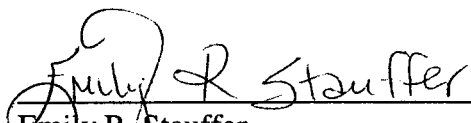
Sponsor Protocol Number: FACT-TOX-026  
Centre Study Number: 023-011  
Total Pages: 110

First Copy of Original  
RSD  
4/5/01  
Date  
Initial

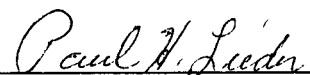
## **GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**

Centre Study Number 023-011, entitled "26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys," conducted for 3M Toxicology Services – Medical Department, was performed in compliance with US EPA Good Laboratory Practice Standards (40 CFR 792) by Centre Analytical Laboratories, Inc. with the following exceptions:

1. The reference substances (analytical standards) used in this study (PFOA/POAA and THPFOS) have not been characterized under GLP's §160.105 (a).
2. In Set 102000AD (extracted 10/20/00 and analyzed 10/23/00), dilutions were performed on samples and not documented on the sample extraction and analysis tracking sheet as required by §792.130 (e).

  
\_\_\_\_\_  
Emily R. Stauffer  
Principal Investigator  
Centre Analytical Laboratories, Inc.

4/2/01  
Date

  
\_\_\_\_\_  
Paul Lieder, Ph.D., DABT  
Study Director  
3M Toxicology Services

5/4/01  
Date

## QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-011 entitled, "26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys". All phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

| <u>Phase</u>           | <u>Date Inspected</u> | <u>Date Reported to Centre Management</u> | <u>Date Reported to Study Director and Sponsor Management</u> |
|------------------------|-----------------------|-------------------------------------------|---------------------------------------------------------------|
| 1. Protocol Review     | 10/5/00               | 11/2/00                                   | 11/17/00                                                      |
| 2. Extraction          | 10/12/00              | 11/2/00                                   | 11/17/00                                                      |
| 3. Raw Data Review     | 11/2-20/00            | 12/21/00                                  | 12/28/00                                                      |
| 4. Draft Report Review | 12/14,15,18/00        | 12/21/00                                  | 12/28/00                                                      |
| 5. Final Report Review | 3/15/01               | 3/19/01                                   | 3/19/01                                                       |

Naomi Lovallo  
Naomi Lovallo  
Sr. Quality Assurance Auditor

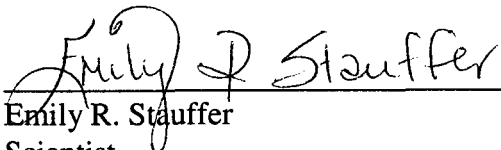
4/2/01  
Date

**CERTIFICATION OF AUTHENTICITY**

This report, for Centre Study Number 023-011 entitled, "26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys", is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.  
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State College, PA 16801  
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## **STUDY IDENTIFICATION**

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

SPONSOR PROTOCOL NUMBER: FACT-TOX-026

TYPE OF STUDY: Toxicity

TEST SYSTEM: Cynomolgus monkeys

TEST MATERIAL: Ammonium perfluorooctanoate (APFO/POAA)

SPONSOR: 3M Toxicology Services – Medical Department  
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|                  |                              |          |
|------------------|------------------------------|----------|
| ANALYTICAL PHASE | Study Initiation Date:       | 11/12/98 |
| TIMETABLE:       | Analytical Start Date:       | 09/27/00 |
|                  | Analytical Termination Date: | 10/26/00 |

## **PROJECT PERSONNEL**

The Study Director for this project was Paul Lieder at 3M Toxicology Services and the Principal Investigator for this project at Centre Analytical Laboratories, Inc. was Emily Stauffer. The following personnel from Centre Analytical Laboratories, Inc., were associated with various analytical phases of the study:

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## **1.0 SUMMARY**

The purpose of this study was to analyze residues of perfluorooctanoate (APFO/POAA) in the feces of cynomolgus monkeys as specified in 3M Protocol FACT-TOX-026. The analytical method used for this study was entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2" (Centre method number: 00M-023-003, revision 2). Besides APFO/POAA (referred to as POAA hereafter), all of the samples were analyzed for residues of PFOS, PFOSA, PFOSAA, M570, M556, EtFOSE-OH, and PFOSEA. However, as requested by the Principal Analytical Investigator at 3M, only the results for POAA will be detailed in this report.

The limit of quantification for POAA in monkey feces was 10 ppb.

Residues ranging from non-detected levels to 242 µg/g were found in the monkey feces samples.

Fortification recoveries ranged from 56-170% with an average of 117% and relative standard deviation of 19%.

## **2.0 OBJECTIVE**

The objective of this study was to determine levels of perfluorooctanoate (APFO/POAA) in specimens of feces of monkeys using the analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2."

## **3.0 INTRODUCTION**

The study was initiated on November 12, 1998, when the study director signed the protocol FACT-TOX-026. The complete protocol and amendments can be found in **Appendix A**. The analytical start date was September 27, 2000, and the analytical termination date was October 26, 2000.

This report details the results of the residues of POAA detected in monkey feces, using the analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (revision 2)." Complete details of the analytical methodology can be found in **Appendix B**.

## **4.0 TEST SYSTEM**

The 300 monkey feces samples analyzed in this study were received frozen on dry ice from 3M Environmental Laboratory St. Paul, MN on August 29, 2000 and stored frozen

upon receipt. The samples were then logged in on August 30, 2000 by Centre personnel and transferred to different frozen storage locale.

The control rat feces used for standards and blanks was purchased from Lampire Biological Laboratories, Inc., Pipersville, PA and received at Centre chilled on blue ice on February 29, 2000 and October 10, 2000, logged in by Centre personnel and placed in frozen storage ( $<-10^{\circ}\text{C}$ ).

Sample login and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc. and a true copy of the storage records can be found in the raw data package associated with this study.

## **5.0 REFERENCE MATERIAL**

The analytical standard POAA (as the ammonium salt) was received at Centre on July 6, 2000 and the surrogate standard THPFOS was received on July 17, 2000 from 3M Environmental Technology and Services. Characterization of the reference material POAA will be the responsibility of the sponsor.

The available information for the reference materials is listed below. The reference materials were stored at room temperature.

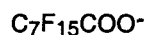
| <u>Compound</u> | <u>Centre Control No.</u> | <u>Batch No.</u> | <u>Purity (%)</u> | <u>Expiration Date</u> |
|-----------------|---------------------------|------------------|-------------------|------------------------|
| POAA            | 00-023-048                | 332              | TBD               | 07/06/01               |
| THPFOS          | 00-023-053                | 53406            | TBD               | 01/01/10               |

Molecular structures of POAA and THPFOS are given below.

### **POAA**

Chemical Name = Perfluorooctanoate

Molecular weight = 413

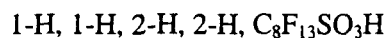


**Note:** The neutral molecule and standard form which POAA (anion) is derived from is ammonium perfluorooctanoate [ $\text{C}_7\text{F}_{15}\text{COONH}_4$ ], molecular weight 431.

### **THPFOS**

Chemical Name: 4-H, perfluorooctane sulfonic acid

Molecular weight: 428



## **6.0 EXPERIMENTAL DESIGN**

Samples were extracted according to sampling day. Each set of samples contained two matrix blanks, two matrix blanks spiked with the surrogate standard only (zero blank), two matrix samples fortified with known amounts of POAA and with the surrogate standard, and 12-22 samples fortified with the surrogate standard.

The extracts were analyzed by LC/MS/MS. Samples with residues outside the linear range of the calibration curve were diluted and re-analyzed.

## **7.0 DESCRIPTION OF ANALYTICAL METHOD**

Analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" was used for this study.

### **7.1 Extraction Procedure**

One gram  $\pm$  0.05 of sample was weighed into 20 mL polyethylene scintillation vials and fortified (if necessary) using disposable micropipettes. Ten mL of acetonitrile was added to the vial, capped tightly, and placed on a wrist-action shaker for ~ 30 min. The samples were filtered through a glass acrodisc filter. The filtered extract was passed through a conditioned carbon SPE column and collected. The columns were then eluted with ~10 mL acetonitrile followed by ~20 mL 90:10 acetonitrile:2% ascorbic acid in methanol. The combined extracts were evaporated down to almost dryness with a rotary evaporator and then re-constituted with methanol, making final volume 2 mL. The samples were analyzed using electrospray LC/MS/MS.

### **7.2 Preparation of Standards and Fortification Solutions**

Standard solutions were prepared on August 15, 2000, September 18, 2000 and September 26-27, 2000 as specified in Centre Analytical Laboratories' analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)." Individual stock standard solution of EtFOSE-OH, PFOSAA, M570, M556, PFOSEA, PFOS, PFOSA, and POAA was prepared at a concentration of 100  $\mu$ g/mL by dissolving 10 mg of the standard (corrected for purity and salt content where appropriate) in methanol. From this solution, a mixed 10  $\mu$ g/mL fortification standard solution was prepared by taking 10 mL of the each stock and bringing the volume up to 100 mL with methanol. Also, a stock standard of THPFOS was prepared at 100  $\mu$ g/mL by dissolving 10 mg of the standard in methanol. An individual 10  $\mu$ g/mL solution of THPFOS was prepared in the same fashion described above.

A mixed 2.5 µg/mL fortification standard was prepared by taking 25 mL of the 10 µg/mL mixed fortification solution and bringing the volume up to 100 mL with methanol. An individual fortification solution of THPFOS was prepared in the same manner. A 0.5 µg/mL mixed standard was prepared by taking 20 mL of the 2.5 µg/mL mixed standard and bringing to 100 mL with methanol. To make the 0.1 µg/mL mixed fortification standard, 20 mL of the 0.5 µg/mL mixed standard was brought to 100 mL with methanol.

Calibration standards were extracted according to the same procedure as the samples. Standards were fortified prior to extraction according to the following table:

| Conc. of Mixed Fortification Solution (µg/ml) | Fortification Volume (µL) | Weight of Control Sample (g) ± 0.05 | Fort. Level of Extracted Calibration Standard (ppb) | Vol. of Surrogate Standard* added (µL) |
|-----------------------------------------------|---------------------------|-------------------------------------|-----------------------------------------------------|----------------------------------------|
| 0.1                                           | 100                       | 1.0                                 | 10                                                  | 200                                    |
| 0.1                                           | 200                       | 1.0                                 | 20                                                  | 200                                    |
| 0.5                                           | 100                       | 1.0                                 | 50                                                  | 200                                    |
| 0.5                                           | 200                       | 1.0                                 | 100                                                 | 200                                    |
| 2.5                                           | 100                       | 1.0                                 | 250                                                 | 200                                    |
| 2.5                                           | 200                       | 1.0                                 | 500                                                 | 200                                    |

\* 2.5 µg/ml THPFOS fortification solution.

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation and extraction can be found in the raw data associated with this report.

### 7.3 Chromatography

Quantification of POAA and THPFOS was accomplished by LC/MS/MS analysis using electrospray LC/MS/MS. The retention times of POAA and THPFOS were ~ 5.0 min. and ~ 5.0 min., respectively, with no significant interfering peaks in the control matrices corresponding to either of the analyte retention times.

### 7.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run was equivalent to 5 ng/mL of POAA and 250 ng/mL of THPFOS in the monkey feces matrix.

## 7.5 Description of Instrument and Operating Conditions

A Micromass Quattro Ultima LC/MS/MS coupled to a Hewlett Packard HPLC system was used. Data acquisition and processing were performed using Masslynx 3.4 software.

Detailed operating conditions are listed below:

Instrument: Micromass Quattro Ultima

### ELECTROSPRAY ION SOURCE:

|                   |                           |
|-------------------|---------------------------|
| Capillary: 3.0 kV | Hexapole 2: 0.3 V         |
| Hexapole 1: 0.1 V | Source Block Temp.: 100°C |
| Aperture 1: 0.2 V | Desolvation Temp.: 350°C  |

### ANALYZER:

|                  |                   |
|------------------|-------------------|
| LM Res 1: 10.5 V | LM Res 2: 13.0 V  |
| HM Res 1: 10.5 V | HM Res 2: 13.0 V  |
| IEnergy 1: 1.0 V | IEnergy 2: 2.0 V  |
| Entrance: -2 V   | Multiplier: 650 V |
| Exit: 2 V        |                   |

### GAS FLOWS AND PRESSURE:

Desolvation N<sub>2</sub> Flow Rate: ~650 L/hr  
Nebuliser N<sub>2</sub> Flow Rate: ~150 L/hr  
Gas Cell Pressure: ~0.003 mbar

Computer: COMPAQ Professional Workstation AP200

Software: Microsoft Windows NT: Version 4 Build 1381: Service Pack 5  
Micromass Limited: Masslynx 3.4 Build 004

HPLC Equipment: Hewlett Packard (HP) Series 1100  
HP Binary (or Quaternary) 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  
Column Temperature: 35°C  
Mobile Phase (A) : 2 mM Ammonium Acetate in Type I Water  
Mobile Phase (B) : Methanol

### Gradient:

| Time (min) | % A  | % B   | Flow Rate (mL/min) |
|------------|------|-------|--------------------|
| 0.0        | 60.0 | 40.0  | 0.3                |
| 0.4        | 60.0 | 40.0  | 0.3                |
| 1.0        | 10.0 | 90.0  | 0.3                |
| 7.0        | 10.0 | 90.0  | 0.3                |
| 7.5        | 0.0  | 100.0 | 0.3                |
| 10.5       | 0.0  | 100.0 | 0.4                |
| 11.0       | 60.0 | 40.0  | 0.4                |
| 14.5       | 60.0 | 40.0  | 0.4                |
| 15.0       | 60.0 | 40.0  | 0.3                |

Injected Volume: 10 µL

Ions monitored :

| <u>Analyte</u> | <u>Transition Monitored</u> | <u>Dwell (secs)</u> | <u>Coll Energy (eV)</u> | <u>Cone (V)</u> |
|----------------|-----------------------------|---------------------|-------------------------|-----------------|
| POAA           | 413 → 369                   | 0.1                 | 10                      | 30              |
| THPFOS         | 427 → 80                    | 0.1                 | 35                      | 34              |

## 7.6 Quantitation and Example Calculation

Ten microliters of sample or extracted calibration standard was injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x weighted linear regression) by Masslynx software using at least six concentrations of standards. The surrogate standard, THPFOS, was only used to monitor the efficiency of the extraction procedure and was not used for quantitation of POAA. The residue concentration in monkey feces was determined using the following equations:

Equations 1 and 2 were used to calculate the amount of analyte found (in ppb, based on peak area) using the standard curve generated by the Masslynx software program.

Equation 1:

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

Equation 2:

$$\text{Analyte found (}\mu\text{g/g)} = \frac{\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF} \times 1(\mu\text{g})}{\text{sample wt. (g)} \times 1000(\text{ng})}$$

where DF = dilution factor.

For samples fortified with known amounts of analytes prior to extraction, use Equation 3 to calculate the percent recovery.

Equation 3:

Recovery (%) =

$$\frac{((\text{anal. found (ng/mL)} - (\text{anal. found in corresponding sample (ng/mL)/DF}) \times \text{FV (mL)} \times \text{DF}) \times 100}{\text{amount added (ng)}}$$

An example of a calculation using an actual sample analyzed with extracted standards follows:

Monkey feces sample Centre ID 0008434 Spk A (Set: 100200A), fortified with 50 ng of POAA.

Where:

|                       |   |         |
|-----------------------|---|---------|
| peak area             | = | 53080   |
| intercept             | = | 3141.11 |
| slope                 | = | 1928.40 |
| dilution factor       | = | 1       |
| ng added (fort level) | = | 50      |

$$\begin{aligned}\text{final vol.} &= 2 \text{ mL} \\ \text{sample wt.} &= 0.97 \text{ g}\end{aligned}$$

**From equation 1:**

$$\begin{aligned}\text{Analyte found (ng/mL)} &= \frac{[53080 - 3141.11]}{1928.40} \\ &= 25.9 \text{ ng/mL}\end{aligned}$$

**From equation 2:**

$$\begin{aligned}\text{Analyte found (}\mu\text{g/g)} &= \frac{[25.9 \text{ ng/mL} \times 2 \text{ mL} \times 1 \times 1 \mu\text{g}]}{0.97 \text{ g} \times 1000 \text{ ng}} \\ &= 0.0534 \mu\text{g/g}\end{aligned}$$

**From equation 3:**

$$\begin{aligned}\% \text{ Recovery} &= \frac{(25.9 \text{ ng/mL} \times 2 \text{ mL} \times 1) \times 100}{50 \text{ ng}} \\ &= 104\%\end{aligned}$$

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the RAW DATA.

The amount of surrogate standard THPFOS found was calculated using the following equation:

$$\text{Analyte found (ng/mL):} \quad \frac{\text{peak area}}{\text{mean response factor}}$$

Other statistical methods used in analyzing this data were:

$$\text{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}} \quad \text{Mean} = \bar{x} = \frac{\sum_{i=1}^n x_i}{n}$$

*Relative Standard Deviation (RSD or Coefficient of Variation (CV)) =*

$$\frac{\text{Standard Deviation} \times 100\%}{\text{Mean}}$$

## **8.0 RESULTS AND DISCUSSION**

Although, the majority of the control samples did not contain significant interferences, a couple of samples did contain residues that were comparable to control group levels. A summary of residues found in all of the matrix blanks and matrix zero blanks is detailed in **Table I**.

Fortification recoveries ranged from 56-170% with an average of 117% and relative standard deviation of 19% (n = 30). A summary of all of the fortification recoveries can be found in **Table II**.

Residues of perfluorooctanoate ranging from non-detected levels to 242 µg/g were found in the monkey feces samples. The residues found in all of the samples plus the averages and standard deviations for each group at each interval are detailed in **Tables III-XVII**.

It was established that ion suppression of the surrogate standard occurred in samples that contained high residues of perfluorooctanoate. Samples were diluted prior to the initial analysis to address the ion suppression of the surrogate standard.

Assuming that matrix spike studies form a suitable indication of endogenous analyte recovery, the data detailed in this report can be considered accurate to within one standard deviation of the average fortified sample recovery. The average fortified sample recovery was 117% with a standard deviation of 22%.

Typical calibration curves and chromatograms representing standards, controls, fortifications, and samples are depicted in **Figures 1-7**.

## **9.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA**

There are no circumstances that may have affected the quality or integrity of the data presented in this report.

## **10.0 RETENTION OF DATA AND SAMPLES**

When the final report is complete, all original study-specific paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 40 CFR 792. Retained samples of reference substances are archived by the sponsor.

# **11.0 TABLES**

**Table I. Summary of POAA residues in Matrix Blanks and Matrix Zero Blanks**

ND = Not Detected and NQ = Not Quantifiable

| Sponsor ID | Centre ID               | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|------------|-------------------------|------------|-----------------|---------------|----------------------|
| na         | 0001345-48 Blank A      | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| na         | 0001345-48 Blank B      | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| na         | 0001345-48 Zero Blank C | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| na         | 0001345-48 Zero Blank D | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| na         | 0001345-48 Blank A      | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| na         | 0001345-48 Blank B      | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| na         | 0001345-48 Zero Blank C | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| na         | 0001345-48 Zero Blank D | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| na         | 0001344 Blank A         | 100900A    | 10/9/00         | 10/10/00      | 0.0453               |
| na         | 0001344 Blank B         | 100900A    | 10/9/00         | 10/10/00      | 0.0212               |
| na         | 0001344 Zero Blank C    | 100900A    | 10/9/00         | 10/10/00      | 0.0913               |
| na         | 0001344 Zero Blank D    | 100900A    | 10/9/00         | 10/10/00      | 0.0259               |
| na         | 0001344 Blank A         | 101000A    | 10/10/00        | 10/10-11/00   | 0.108                |
| na         | 0001344 Blank B         | 101000A    | 10/10/00        | 10/10-11/00   | NQ                   |
| na         | 0001344 Zero Blank C    | 101000A    | 10/10/00        | 10/10-11/00   | NQ                   |
| na         | 0001344 Zero Blank D    | 101000A    | 10/10/00        | 10/10-11/00   | 0.0129               |
| na         | 0009684 Blank A         | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| na         | 0009684 Blank B         | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| na         | 0009684 Zero Blank C    | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| na         | 0009684 Zero Blank D    | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| na         | 0009684 Blank A         | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| na         | 0009684 Blank B         | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| na         | 0009684 Zero Blank C    | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| na         | 0009684 Zero Blank D    | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| na         | 0009684 Blank A         | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| na         | 0009684 Blank B         | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| na         | 0009684 Zero Blank C    | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| na         | 0009684 Zero Blank D    | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| na         | 0009684 Blank A         | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| na         | 0009684 Blank B         | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| na         | 0009684 Zero Blank C    | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| na         | 0009684 Zero Blank D    | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| na         | 0009684 Blank A         | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| na         | 0009684 Blank B         | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| na         | 0009684 Zero Blank C    | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| na         | 0009684 Zero Blank D    | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |

**Table I (cont.) Summary of POAA residues in Matrix Blanks and Matrix Zero Blanks**

ND = Not Detected and NQ = Not Quantifiable

| Sponsor ID          | Centre ID            | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|---------------------|----------------------|------------|-----------------|---------------|----------------------|
| na                  | 0009684 Blank A      | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| na                  | 0009684 Blank B      | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| na                  | 0009684 Zero Blank C | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| na                  | 0009684 Zero Blank D | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| Na                  | 0009684 Blank A      | 101900A    | 10/19/00        | 10/20-21/00   | NQ                   |
| Na                  | 0009684 Blank B      | 101900A    | 10/19/00        | 10/20-21/00   | ND                   |
| Na                  | 0009684 Zero Blank C | 101900A    | 10/19/00        | 10/20-21/00   | NQ                   |
| Na                  | 0009684 Zero Blank D | 101900A    | 10/19/00        | 10/20-21/00   | NQ                   |
| Na                  | 0009684 Blank A      | 102000A    | 10/20/00        | 10/22-23/00   | ND                   |
| na                  | 0009684 Blank B      | 102000A    | 10/20/00        | 10/22-23/00   | ND                   |
| na                  | 0009684 Zero Blank C | 102000A    | 10/20/00        | 10/22-23/00   | ND                   |
| na                  | 0009684 Zero Blank D | 102000A    | 10/20/00        | 10/22-23/00   | ND                   |
| na                  | 0009684 Blank A      | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| na                  | 0009684 Blank B      | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| na                  | 0009684 Zero Blank C | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| na                  | 0009684 Zero Blank D | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| na                  | 0009684 Blank A      | 102400A    | 10/24/00        | 10/24-25/00   | ND                   |
| na                  | 0009684 Blank B      | 102400A    | 10/24/00        | 10/24-25/00   | ND                   |
| na                  | 0009684 Zero Blank C | 102400A    | 10/24/00        | 10/24-25/00   | ND                   |
| na                  | 0009684 Zero Blank D | 102400A    | 10/24/00        | 10/24-25/00   | ND                   |
| na                  | 0009684 Blank A      | 102500A    | 10/25/00        | 10/26/00      | ND                   |
| na                  | 0009684 Blank B      | 102500A    | 10/25/00        | 10/26/00      | ND                   |
| na                  | 0009684 Zero Blank C | 102500A    | 10/25/00        | 10/26/00      | ND                   |
| na                  | 0009684 Zero Blank D | 102500A    | 10/25/00        | 10/26/00      | ND                   |
| AVERAGE:            |                      |            |                 |               | 0.0113               |
| STANDARD DEVIATION: |                      |            |                 |               | 0.0175               |

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table II. Summary of POAA recoveries in Fortified Samples**

| Sponsor<br>ID                | Centre<br>ID  | Set<br>Number | Extraction<br>Date | Analysis<br>Date | Amount<br>Added (ng) | %<br>Recovery |
|------------------------------|---------------|---------------|--------------------|------------------|----------------------|---------------|
| I05709M Grp 1 Wk 2           | 0008434 Spk A | 100200A       | 10/2/00            | 10/3/00          | 50                   | 104           |
| I05714M Grp 1 Wk 2           | 0008435 Spk B | 100200A       | 10/2/00            | 10/3/00          | 250                  | 88            |
| I05720M Grp 1 Wk 4           | 0008460 Spk A | 100600A       | 10/6/00            | 10/6-7/00        | 50                   | 119           |
| I05725M Grp 1 Wk 4           | 0008461 Spk B | 100600AD      | 10/6/00            | 10/7/00          | 1000                 | 137           |
| I05720M Grp 1 Wk 6           | 0008482 Spk A | 100900A       | 10/9/00            | 10/10/00         | 50                   | 116           |
| I05725M Grp 1 Wk 6           | 0008483 Spk B | 100900A       | 10/9/00            | 10/10/00         | 2000                 | 139           |
| I05709M Grp 1 Wk 8           | 0008499 Spk A | 101000A       | 10/10/00           | 10/10-11/00      | 50                   | 105           |
| I05714M Grp 1 Wk 8           | 0008500 Spk B | 101000A       | 10/10/00           | 10/10-11/00      | 10000                | 133           |
| I05715M Grp 1 Wk 10          | 0008522 Spk A | 101100A       | 10/11/00           | 10/12-13/00      | 50                   | 98            |
| I05718M Grp 1 Wk 10          | 0008523 Spk B | 101100A       | 10/11/00           | 10/12-13/00      | 10000                | 128           |
| I05720M Grp 1 Wk 12          | 0008545 Spk A | 101200A       | 10/12/00           | 10/13-14/00      | 50                   | 87            |
| I05725M Grp 1 Wk 12          | 0008546 Spk B | 101200A       | 10/12/00           | 10/13-14/00      | 20000                | 115           |
| I05709M Grp 1 Wk 14          | 0008562 Spk A | 101300A       | 10/13/00           | 10/14-15/00      | 50                   | 72            |
| I05714M Grp 1 Wk 14          | 0008563 Spk B | 101300A       | 10/13/00           | 10/14-15/00      | 100,250              | 128           |
| I05715M Grp 1 Wk 16          | 0008585 Spk A | 101600AR      | 10/16/00           | 10/18-19/00      | 50                   | 114           |
| I05718M Grp 1 Wk 16          | 0008586 Spk B | 101600AR      | 10/16/00           | 10/18-19/00      | 100,250              | 128           |
| I05720M Grp 1 Wk 18          | 0008608 Spk A | 101700A       | 10/17/00           | 10/17-18/00      | 50                   | 127           |
| I05725M Grp 1 Wk 18          | 0008609 Spk B | 101700A       | 10/17/00           | 10/17-18/00      | 200,250              | 125           |
| I05709M Grp 1 Wk 20          | 0008625 Spk A | 101800A       | 10/18/00           | 10/19-20/00      | 50                   | 120           |
| I05714M Grp 1 Wk 20          | 0008626 Spk B | 101800A       | 10/18/00           | 10/19-20/00      | 200,250              | 141           |
| I05720M Grp 1 Wk 22          | 0008650 Spk A | 101900AD      | 10/19/00           | 10/22/00         | 50                   | 130           |
| I05725M Grp 1 Wk 22          | 0008651 Spk B | 101900A       | 10/19/00           | 10/20-21/00      | 200,250              | 96            |
| I05709M Grp 1 Wk 24          | 0008666 Spk A | 102000A       | 10/20/00           | 10/22-23/00      | 50                   | 108           |
| I05714M Grp 1 Wk 24          | 0008667 Spk B | 102000AD      | 10/20/00           | 10/23/00         | 200,250              | 170           |
| I05718M Grp 1 Wk 26          | 0008689 Spk A | 102300AR      | 10/23/00           | 10/25/00         | 50                   | 56            |
| I05720M Grp 1 Wk 26          | 0008690 Spk B | 102300AR      | 10/23/00           | 10/25/00         | 250                  | 129           |
| I05718M Grp 1 Wk 28          | 0008706 Spk A | 102400A       | 10/24/00           | 10/24-25/00      | 50                   | 123           |
| I05720M Grp 1 Wk 30          | 0008711 Spk B | 102300AD      | 10/24/00           | 10/26/00         | 100,250              | 119           |
| I05718M Grp 1 Wk 36          | 0008722 Spk A | 102500A       | 10/25/00           | 10/26/00         | 50                   | 128           |
| I05720M Grp 1 Wk 38          | 0008726 Spk B | 102300AD      | 10/25/00           | 10/26/00         | 10,000               | 125           |
| AVERAGE:                     |               |               |                    |                  |                      | 117           |
| STANDARD DEVIATION:          |               |               |                    |                  |                      | 22            |
| RELATIVE STANDARD DEVIATION: |               |               |                    |                  |                      | 19            |

**Table III. Summary of POAA residues in Week 2**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 2         | 0008434   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| I05714M Grp 1 Wk 2         | 0008435   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| I05715M Grp 1 Wk 2         | 0008436   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| I05718M Grp 1 Wk 2         | 0008437   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| I05720M Grp 1 Wk 2         | 0008438   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| I05725M Grp 1 Wk 2         | 0008439   | 100200A    | 10/2/00         | 10/3/00       | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>NQ</b>            |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>NQ</b>            |
| I05702M Grp 2 Wk 2         | 0008440   | 100200AD   | 10/2/00         | 10/4/00       | 14.0                 |
| I05706M Grp 2 Wk 2         | 0008441   | 100200AD   | 10/2/00         | 10/4/00       | 12.1                 |
| I05717M Grp 2 Wk 2         | 0008442   | 100200AD   | 10/2/00         | 10/4/00       | 1.67                 |
| I05723M Grp 2 Wk 2         | 0008443   | 100200AD   | 10/2/00         | 10/4/00       | 1.94                 |
| <b>Average:</b>            |           |            |                 |               | <b>7.43</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>6.54</b>          |
| I05707M Grp 3 Wk 2         | 0008444   | 100200ADD  | 10/2/00         | 10/5/00       | 31.7                 |
| I05708M Grp 3 Wk 2         | 0008445   | 100200AD   | 10/2/00         | 10/4/00       | 20.9                 |
| I05710M Grp 3 Wk 2         | 0008446   | 100200AD   | 10/2/00         | 10/4/00       | 18.2                 |
| I05712M Grp 3 Wk 2         | 0008447   | 100200AD   | 10/2/00         | 10/4/00       | 9.69                 |
| I05716M Grp 3 Wk 2         | 0008448   | 100200AD   | 10/2/00         | 10/4/00       | 5.41                 |
| I05719M Grp 3 Wk 2         | 0008449   | 100200AD   | 10/2/00         | 10/4/00       | 6.50                 |
| <b>Average:</b>            |           |            |                 |               | <b>15.4</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>10.2</b>          |
| I05703M Grp 4 Wk 2         | 0008450   | 100200AD   | 10/2/00         | 10/4/00       | 18.6                 |
| I05704M Grp 4 Wk 2         | 0008451   | 100200ADD  | 10/2/00         | 10/5/00       | 28.3                 |
| I05711M Grp 4 Wk 2         | 0008452   | 100200ADD  | 10/2/00         | 10/5/00       | 41.2                 |
| I05713M Grp 4 Wk 2         | 0008453   | 100200AD   | 10/2/00         | 10/4/00       | 17.4                 |
| I05722M Grp 4 Wk 2         | 0008454   | 100200ADD  | 10/2/00         | 10/5/00       | 28.2                 |
| I05724M Grp 4 Wk 2         | 0008455   | 100200ADD  | 10/2/00         | 10/5/00       | 206                  |
| <b>Average:</b>            |           |            |                 |               | <b>56.6</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>73.7</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table IV. Summary of POAA residues in Week 4**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 4         | 0008456   | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| I05714M Grp 1 Wk 4         | 0008457   | 100600A    | 10/6/00         | 10/6-7/00     | 0.0403               |
| I05715M Grp 1 Wk 4         | 0008458   | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| I05718M Grp 1 Wk 4         | 0008459   | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| I05720M Grp 1 Wk 4         | 0008460   | 100600A    | 10/6/00         | 10/6-7/00     | NQ                   |
| I05725M Grp 1 Wk 4         | 0008461   | 100600A    | 10/6/00         | 10/6-7/00     | 0.0481               |
| <b>Average:</b>            |           |            |                 |               | <b>0.0214</b>        |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.0178</b>        |
| I05702M Grp 2 Wk 4         | 0008462   | 100600A    | 10/6/00         | 10/6-7/00     | 1.48                 |
| I05706M Grp 2 Wk 4         | 0008463   | 100600AD   | 10/6/00         | 10/7/00       | 28.0                 |
| I05717M Grp 2 Wk 4         | 0008464   | 100600A    | 10/6/00         | 10/6-7/00     | 4.07                 |
| I05721M Grp 2 Wk 4         | 0008465   | 100600AD   | 10/6/00         | 10/7/00       | 8.22                 |
| <b>Average:</b>            |           |            |                 |               | <b>10.4</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>12.0</b>          |
| I05707M Grp 3 Wk 4         | 0008466   | 100600AD   | 10/6/00         | 10/7/00       | 30.8                 |
| I05708M Grp 3 Wk 4         | 0008467   | 100600AD   | 10/6/00         | 10/7/00       | 24.0                 |
| I05710M Grp 3 Wk 4         | 0008468   | 100600AD   | 10/6/00         | 10/7/00       | 12.2                 |
| I05712M Grp 3 Wk 4         | 0008469   | 100600AD   | 10/6/00         | 10/7/00       | 9.72                 |
| I05716M Grp 3 Wk 4         | 0008470   | 100600AD   | 10/6/00         | 10/7/00       | 26.3                 |
| I05719M Grp 3 Wk 4         | 0008471   | 100600AD   | 10/6/00         | 10/7/00       | 37.2                 |
| <b>Average:</b>            |           |            |                 |               | <b>23.4</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>10.6</b>          |
| I05703M Grp 4 Wk 4         | 0008472   | 100600AD   | 10/6/00         | 10/7/00       | 21.6                 |
| I05704M Grp 4 Wk 4         | 0008473   | 100600AD   | 10/6/00         | 10/7/00       | 17.9                 |
| I05711M Grp 4 Wk 4         | 0008474   | 100600AD   | 10/6/00         | 10/7/00       | 17.1                 |
| I05713M Grp 4 Wk 4         | 0008475   | 100600AD   | 10/6/00         | 10/7/00       | 33.8                 |
| I05722M Grp 4 Wk 4         | 0008476   | 100600AD   | 10/6/00         | 10/7/00       | 18.5                 |
| I05724M Grp 4 Wk 4         | 0008477   | 100600AD   | 10/6/00         | 10/7/00       | 23.2                 |
| <b>Average:</b>            |           |            |                 |               | <b>22.0</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>6.23</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table V. Summary of POAA residues in Week 6**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|---------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 6  | 0008478   | 100900A    | 10/9/00         | 10/10/00      | NQ                   |
| I05714M Grp 1 Wk 6  | 0008479   | 100900A    | 10/9/00         | 10/10/00      | NQ                   |
| I05715M Grp 1 Wk 6  | 0008480   | 100900A    | 10/9/00         | 10/10/00      | NQ                   |
| I05718M Grp 1 Wk 6  | 0008481   | 100900A    | 10/9/00         | 10/10/00      | 0.0147               |
| I05720M Grp 1 Wk 6  | 0008482   | 100900A    | 10/9/00         | 10/10/00      | NQ                   |
| I05725M Grp 1 Wk 6  | 0008483   | 100900A    | 10/9/00         | 10/10/00      | NQ                   |
| Average:            |           |            |                 |               | 0.0108               |
| Standard Deviation: |           |            |                 |               | 0.00192              |
| I05702M Grp 2 Wk 6  | 0008484   | 100900A    | 10/9/00         | 10/10/00      | 1.63                 |
| I05706M Grp 2 Wk 6  | 0008485   | 100900AD   | 10/9/00         | 10/11/00      | 31.8                 |
| I05717M Grp 2 Wk 6  | 0008486   | 100900A    | 10/9/00         | 10/10/00      | 2.35                 |
| I05721M Grp 2 Wk 6  | 0008487   | 100900AD   | 10/9/00         | 10/11/00      | 12.6                 |
| Average:            |           |            |                 |               | 12.1                 |
| Standard Deviation: |           |            |                 |               | 14.1                 |
| I05707M Grp 3 Wk 6  | 0008488   | 100900AD   | 10/9/00         | 10/11/00      | 18.3                 |
| I05708M Grp 3 Wk 6  | 0008489   | 100900AD   | 10/9/00         | 10/11/00      | 16.8                 |
| I05710M Grp 3 Wk 6  | 0008490   | 100900AD   | 10/9/00         | 10/11/00      | 30.0                 |
| I05712M Grp 3 Wk 6  | 0008491   | 100900AD   | 10/9/00         | 10/11/00      | 17.7                 |
| I05716M Grp 3 Wk 6  | 0008492   | 100900AD   | 10/9/00         | 10/11/00      | 37.4                 |
| I05719M Grp 3 Wk 6  | 0008493   | 100900AD   | 10/9/00         | 10/11/00      | 19.4                 |
| Average:            |           |            |                 |               | 23.3                 |
| Standard Deviation: |           |            |                 |               | 8.46                 |
| I05703M Grp 4 Wk 6  | 0008494   | 100900ADD  | 10/9/00         | 10/12/00      | 242                  |
| I05704M Grp 4 Wk 6  | 0008495   | 100900AD   | 10/9/00         | 10/11/00      | 68.6                 |
| I05711M Grp 4 Wk 6  | 0008496   | 100900AD   | 10/9/00         | 10/11/00      | 75.7                 |
| I05713M Grp 4 Wk 6  | 0008497   | 100900AD   | 10/9/00         | 10/11/00      | 9.17                 |
| I05722M Grp 4 Wk 6  | 0008498   | 100900AD   | 10/9/00         | 10/11/00      | 110                  |
| Average:            |           |            |                 |               | 101                  |
| Standard Deviation: |           |            |                 |               | 86.7                 |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table VI. Summary of POAA residues in Week 8**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 8         | 0008499   | 101000A    | 10/10/00        | 10/10-11/00   | 0.0214               |
| I05714M Grp 1 Wk 8         | 0008500   | 101000A    | 10/10/00        | 10/10-11/00   | 0.157                |
| I05715M Grp 1 Wk 8         | 0008501   | 101000A    | 10/10/00        | 10/10-11/00   | 0.0102               |
| I05718M Grp 1 Wk 8         | 0008502   | 101000A    | 10/10/00        | 10/10-11/00   | 0.0159               |
| I05720M Grp 1 Wk 8         | 0008503   | 101000A    | 10/10/00        | 10/10-11/00   | 0.0104               |
| I05725M Grp 1 Wk 8         | 0008504   | 101000A    | 10/10/00        | 10/10-11/00   | 0.254                |
| <b>Average:</b>            |           |            |                 |               | <b>0.0782</b>        |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.103</b>         |
| I05702M Grp 2 Wk 8         | 0008505   | 101000A    | 10/10/00        | 10/10-11/00   | 3.24                 |
| I05706M Grp 2 Wk 8         | 0008506   | 101000AD   | 10/10/00        | 10/11-12/00   | 22.8                 |
| I05717M Grp 2 Wk 8         | 0008507   | 101000A    | 10/10/00        | 10/10-11/00   | 3.41                 |
| I05721M Grp 2 Wk 8         | 0008508   | 101000AD   | 10/10/00        | 10/11-12/00   | 8.37                 |
| <b>Average:</b>            |           |            |                 |               | <b>9.46</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>9.21</b>          |
| I05707M Grp 3 Wk 8         | 0008509   | 101000AD   | 10/10/00        | 10/11-12/00   | 51.7                 |
| I05708M Grp 3 Wk 8         | 0008510   | 101000AD   | 10/10/00        | 10/11-12/00   | 17.8                 |
| I05710M Grp 3 Wk 8         | 0008511   | 101000AD   | 10/10/00        | 10/11-12/00   | 76.3                 |
| I05712M Grp 3 Wk 8         | 0008512   | 101000AD   | 10/10/00        | 10/11-12/00   | 7.64                 |
| I05716M Grp 3 Wk 8         | 0008513   | 101000AD   | 10/10/00        | 10/11-12/00   | 40.8                 |
| I05719M Grp 3 Wk 8         | 0008514   | 101000AD   | 10/10/00        | 10/11-12/00   | 51.8                 |
| <b>Average:</b>            |           |            |                 |               | <b>41.0</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>25.0</b>          |
| I05703M Grp 4 Wk 8         | 0008515   | 101000AD   | 10/10/00        | 10/11-12/00   | 50.2                 |
| I05704M Grp 4 Wk 8         | 0008516   | 101000AD   | 10/10/00        | 10/11-12/00   | 21.0                 |
| I05711M Grp 4 Wk 8         | 0008517   | 101000A    | 10/10/00        | 10/10-11/00   | 1.69                 |
| I05713M Grp 4 Wk 8         | 0008518   | 101000AD   | 10/10/00        | 10/11-12/00   | 21.2                 |
| I05722M Grp 4 Wk 8         | 0008519   | 101000AD   | 10/10/00        | 10/11-12/00   | 89.4                 |
| <b>Average:</b>            |           |            |                 |               | <b>36.7</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>34.2</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND and NQ, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as < 0.0100.

**Table VII. Summary of POAA residues in Week 10**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|---------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 10 | 0008520   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| I05714M Grp 1 Wk 10 | 0008521   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| I05715M Grp 1 Wk 10 | 0008522   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| I05718M Grp 1 Wk 10 | 0008523   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| I05720M Grp 1 Wk 10 | 0008524   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| I05725M Grp 1 Wk 10 | 0008525   | 101100A    | 10/11/00        | 10/12-13/00   | NQ                   |
| Average:            |           |            |                 |               | NQ                   |
| Standard Deviation: |           |            |                 |               | NQ                   |
| I05702M Grp 2 Wk 10 | 0008526   | 101100A    | 10/11/00        | 10/12-13/00   | 1.67                 |
| I05706M Grp 2 Wk 10 | 0008527   | 101100A    | 10/11/00        | 10/12-13/00   | 2.64                 |
| I05717M Grp 2 Wk 10 | 0008528   | 101100A    | 10/11/00        | 10/12-13/00   | 2.09                 |
| I05721M Grp 2 Wk 10 | 0008529   | 101100AD   | 10/11/00        | 10/13/00      | 9.45                 |
| Average:            |           |            |                 |               | 3.96                 |
| Standard Deviation: |           |            |                 |               | 3.68                 |
| I05707M Grp 3 Wk 10 | 0008530   | 101100AD   | 10/11/00        | 10/13/00      | 31.0                 |
| I05708M Grp 3 Wk 10 | 0008531   | 101100AD   | 10/11/00        | 10/13/00      | 18.7                 |
| I05710M Grp 3 Wk 10 | 0008532   | 101100AD   | 10/11/00        | 10/13/00      | 44.1                 |
| I05712M Grp 3 Wk 10 | 0008533   | 101100AD   | 10/11/00        | 10/13/00      | 10.1                 |
| I05716M Grp 3 Wk 10 | 0008534   | 101100AD   | 10/11/00        | 10/13/00      | 5.39                 |
| I05719M Grp 3 Wk 10 | 0008535   | 101100AD   | 10/11/00        | 10/13/00      | 46.9                 |
| Average:            |           |            |                 |               | 26.0                 |
| Standard Deviation: |           |            |                 |               | 17.4                 |
| I05703M Grp 4 Wk 10 | 0008536   | 101100AD   | 10/11/00        | 10/13/00      | 32.6                 |
| I05704M Grp 4 Wk 10 | 0008537   | 101100AD   | 10/11/00        | 10/13/00      | 45.5                 |
| I05711M Grp 4 Wk 10 | 0008538   | 101100A    | 10/11/00        | 10/12-13/00   | 0.635                |
| I05713M Grp 4 Wk 10 | 0008539   | 101100AD   | 10/11/00        | 10/13/00      | 80.3                 |
| I05722M Grp 4 Wk 10 | 0008540   | 101100AD   | 10/11/00        | 10/13/00      | 81.1                 |
| Average:            |           |            |                 |               | 48.0                 |
| Standard Deviation: |           |            |                 |               | 34.0                 |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table VIII. Summary of POAA residues in Week 12**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 12        | 0008541   | 101200A    | 10/12/00        | 10/13-14/00   | 0.0209               |
| I05714M Grp 1 Wk 12        | 0008542   | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| I05715M Grp 1 Wk 12        | 0008543   | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| I05718M Grp 1 Wk 12        | 0008544   | 101200A    | 10/12/00        | 10/13-14/00   | 0.232                |
| I05720M Grp 1 Wk 12        | 0008545   | 101200A    | 10/12/00        | 10/13-14/00   | NQ                   |
| I05725M Grp 1 Wk 12        | 0008546   | 101200A    | 10/12/00        | 10/13-14/00   | 0.0160               |
| <b>Average:</b>            |           |            |                 |               | <b>0.0498</b>        |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.0894</b>        |
| I05702M Grp 2 Wk 12        | 0008547   | 101200AD   | 10/12/00        | 10/14/00      | 13.6                 |
| I05706M Grp 2 Wk 12        | 0008548   | 101200A    | 10/12/00        | 10/13-14/00   | 3.21                 |
| I05717M Grp 2 Wk 12        | 0008549   | 101200A    | 10/12/00        | 10/13-14/00   | 1.68                 |
| I05721M Grp 2 Wk 12        | 0008550   | 101200AD   | 10/12/00        | 10/14/00      | 10.1                 |
| <b>Average:</b>            |           |            |                 |               | <b>7.15</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>5.65</b>          |
| I05707M Grp 3 Wk 12        | 0008551   | 101200AD   | 10/12/00        | 10/14/00      | 19.9                 |
| I05708M Grp 3 Wk 12        | 0008552   | 101200AD   | 10/12/00        | 10/14/00      | 9.68                 |
| I05710M Grp 3 Wk 12        | 0008553   | 101200A    | 10/12/00        | 10/13-14/00   | 4.54                 |
| I05712M Grp 3 Wk 12        | 0008554   | 101200AD   | 10/12/00        | 10/14/00      | 8.86                 |
| I05716M Grp 3 Wk 12        | 0008555   | 101200AD   | 10/12/00        | 10/14/00      | 14.5                 |
| I05719M Grp 3 Wk 12        | 0008556   | 101200A    | 10/12/00        | 10/13-14/00   | 4.06                 |
| <b>Average:</b>            |           |            |                 |               | <b>10.3</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>6.07</b>          |
| I05703M Grp 4 Wk 12        | 0008557   | 101200AD   | 10/12/00        | 10/14/00      | 34.2                 |
| I05704M Grp 4 Wk 12        | 0008558   | 101200AD   | 10/12/00        | 10/14/00      | 105                  |
| I05711M Grp 4 Wk 12        | 0008559   | 101200A    | 10/12/00        | 10/13-14/00   | 0.508                |
| I05713M Grp 4 Wk 12        | 0008560   | 101200AD   | 10/12/00        | 10/14/00      | 16.3                 |
| I05722M Grp 4 Wk 12        | 0008561   | 101200A    | 10/12/00        | 10/13-14/00   | 3.87                 |
| <b>Average:</b>            |           |            |                 |               | <b>32.0</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>42.9</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table IX. Summary of POAA residues in Week 14**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 14        | 0008562   | 101300A    | 10/13/00        | 10/14-15/00   | 0.0276               |
| I05714M Grp 1 Wk 14        | 0008563   | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| I05715M Grp 1 Wk 14        | 0008564   | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| I05718M Grp 1 Wk 14        | 0008565   | 101300AD   | 10/13/00        | 10/15/00      | 0.768                |
| I05720M Grp 1 Wk 14        | 0008566   | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| I05725M Grp 1 Wk 14        | 0008567   | 101300A    | 10/13/00        | 10/14-15/00   | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>0.139</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.308</b>         |
| I05702M Grp 2 Wk 14        | 0008568   | 101300A    | 10/13/00        | 10/14-15/00   | 4.22                 |
| I05706M Grp 2 Wk 14        | 0008569   | 101300AD   | 10/13/00        | 10/15/00      | 9.25                 |
| I05717M Grp 2 Wk 14        | 0008570   | 101300AD   | 10/13/00        | 10/15/00      | 7.12                 |
| I05721M Grp 2 Wk 14        | 0008571   | 101300AD   | 10/13/00        | 10/15/00      | 9.42                 |
| <b>Average:</b>            |           |            |                 |               | <b>7.50</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>2.43</b>          |
| I05707M Grp 3 Wk 14        | 0008572   | 101300AD   | 10/13/00        | 10/15/00      | 82.2                 |
| I05708M Grp 3 Wk 14        | 0008573   | 101300AD   | 10/13/00        | 10/15/00      | 10.3                 |
| I05710M Grp 3 Wk 14        | 0008574   | 101300A    | 10/13/00        | 10/14-15/00   | 4.88                 |
| I05712M Grp 3 Wk 14        | 0008575   | 101300AD   | 10/13/00        | 10/15/00      | 8.30                 |
| I05716M Grp 3 Wk 14        | 0008576   | 101300AD   | 10/13/00        | 10/15/00      | 20.2                 |
| I05719M Grp 3 Wk 14        | 0008577   | 101300AD   | 10/13/00        | 10/15/00      | 37.6                 |
| <b>Average:</b>            |           |            |                 |               | <b>27.2</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>29.4</b>          |
| I05703M Grp 4 Wk 14        | 0008578   | 101300A    | 10/13/00        | 10/14-15/00   | 3.33                 |
| I05704M Grp 4 Wk 14        | 0008579   | 101300AD   | 10/13/00        | 10/15/00      | 55.9                 |
| I05711M Grp 4 Wk 14        | 0008580   | 101300A    | 10/13/00        | 10/14-15/00   | 0.363                |
| I05713M Grp 4 Wk 14        | 0008581   | 101300AD   | 10/13/00        | 10/15/00      | 37.0                 |
| I05722M Grp 4 Wk 14        | 0008582   | 101300A    | 10/13/00        | 10/14-15/00   | 2.91                 |
| <b>Average:</b>            |           |            |                 |               | <b>19.9</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>25.2</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table X. Summary of POAA residues in Week 16**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 16        | 0008583   | 101600AR   | 10/16/00        | 10/18-19/00   | 0.104                |
| I05714M Grp 1 Wk 16        | 0008584   | 101600AR   | 10/16/00        | 10/18-19/00   | 0.0159               |
| I05715M Grp 1 Wk 16        | 0008585   | 101600AR   | 10/16/00        | 10/18-19/00   | 0.193                |
| I05718M Grp 1 Wk 16        | 0008586   | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| I05720M Grp 1 Wk 16        | 0008587   | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| I05725M Grp 1 Wk 16        | 0008588   | 101600AR   | 10/16/00        | 10/18-19/00   | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>0.0572</b>        |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.0762</b>        |
| I05702M Grp 2 Wk 16        | 0008589   | 101600AD   | 10/16/00        | 10/19/00      | 6.83                 |
| I05706M Grp 2 Wk 16        | 0008590   | 101600AD   | 10/16/00        | 10/19/00      | 7.25                 |
| I05717M Grp 2 Wk 16        | 0008591   | 101600AR   | 10/16/00        | 10/18-19/00   | 3.52                 |
| I05721M Grp 2 Wk 16        | 0008592   | 101600AD   | 10/16/00        | 10/19/00      | 9.92                 |
| <b>Average:</b>            |           |            |                 |               | <b>6.88</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>2.62</b>          |
| I05707M Grp 3 Wk 16        | 0008593   | 101600AD   | 10/16/00        | 10/19/00      | 20.0                 |
| I05708M Grp 3 Wk 16        | 0008594   | 101600AR   | 10/16/00        | 10/18-19/00   | 5.04                 |
| I05710M Grp 3 Wk 16        | 0008595   | 101600AD   | 10/16/00        | 10/19/00      | 68.0                 |
| I05712M Grp 3 Wk 16        | 0008596   | 101600AD   | 10/16/00        | 10/19/00      | 13.2                 |
| I05716M Grp 3 Wk 16        | 0008597   | 101600AD   | 10/16/00        | 10/19/00      | 36.3                 |
| I05719M Grp 3 Wk 16        | 0008598   | 101600AD   | 10/16/00        | 10/19/00      | 45.7                 |
| <b>Average:</b>            |           |            |                 |               | <b>31.4</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>23.3</b>          |
| I05703M Grp 4 Wk 16        | 0008599   | 101600AR   | 10/16/00        | 10/18-19/00   | 2.61                 |
| I05704M Grp 4 Wk 16        | 0008600   | 101600AD   | 10/16/00        | 10/19/00      | 68.2                 |
| I05711M Grp 4 Wk 16        | 0008601   | 101600AR   | 10/16/00        | 10/18-19/00   | 0.327                |
| I05713M Grp 4 Wk 16        | 0008602   | 101600AD   | 10/16/00        | 10/19/00      | 17.8                 |
| I05722M Grp 4 Wk 16        | 0008603   | 101600AR   | 10/16/00        | 10/18-19/00   | 2.18                 |
| <b>Average:</b>            |           |            |                 |               | <b>18.2</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>28.8</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XI. Summary of POAA residues in Week 18**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 18        | 0008604   | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| I05714M Grp 1 Wk 18        | 0008605   | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| I05715M Grp 1 Wk 18        | 0008606   | 101700A    | 10/17/00        | 10/17-18/00   | 0.0153               |
| I05718M Grp 1 Wk 18        | 0008607   | 101700AD   | 10/17/00        | 10/19/00      | 1.49                 |
| I05720M Grp 1 Wk 18        | 0008608   | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| I05725M Grp 1 Wk 18        | 0008609   | 101700A    | 10/17/00        | 10/17-18/00   | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>0.258</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.604</b>         |
| I05702M Grp 2 Wk 18        | 0008610   | 101700A    | 10/17/00        | 10/17-18/00   | 1.62                 |
| I05706M Grp 2 Wk 18        | 0008611   | 101700A    | 10/17/00        | 10/17-18/00   | 1.76                 |
| I05717M Grp 2 Wk 18        | 0008612   | 101700AD   | 10/17/00        | 10/19/00      | 16.4                 |
| I05721M Grp 2 Wk 18        | 0008613   | 101700A    | 10/17/00        | 10/17-18/00   | 3.08                 |
| <b>Average:</b>            |           |            |                 |               | <b>5.72</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>7.15</b>          |
| I05707M Grp 3 Wk 18        | 0008614   | 101700AD   | 10/17/00        | 10/19/00      | 41.1                 |
| I05708M Grp 3 Wk 18        | 0008615   | 101700A    | 10/17/00        | 10/17-18/00   | 4.48                 |
| I05710M Grp 3 Wk 18        | 0008616   | 101700AD   | 10/17/00        | 10/19/00      | 22.9                 |
| I05712M Grp 3 Wk 18        | 0008617   | 101700AD   | 10/17/00        | 10/19/00      | 9.50                 |
| I05716M Grp 3 Wk 18        | 0008618   | 101700AD   | 10/17/00        | 10/19/00      | 16.3                 |
| I05719M Grp 3 Wk 18        | 0008619   | 101700AD   | 10/17/00        | 10/19/00      | 9.26                 |
| <b>Average:</b>            |           |            |                 |               | <b>17.3</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>13.3</b>          |
| I05703M Grp 4 Wk 18        | 0008620   | 101700A    | 10/17/00        | 10/17-18/00   | 0.895                |
| I05704M Grp 4 Wk 18        | 0008621   | 101700AD   | 10/17/00        | 10/19/00      | 38.1                 |
| I05711M Grp 4 Wk 18        | 0008622   | 101700A    | 10/17/00        | 10/17-18/00   | 0.159                |
| I05713M Grp 4 Wk 18        | 0008623   | 101700AD   | 10/17/00        | 10/19/00      | 70.9                 |
| I05722M Grp 4 Wk 18        | 0008624   | 101700A    | 10/17/00        | 10/17-18/00   | 0.609                |
| <b>Average:</b>            |           |            |                 |               | <b>22.1</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>31.7</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XII. Summary of POAA residues in Week 20**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 20        | 0008625   | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| I05714M Grp 1 Wk 20        | 0008626   | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| I05715M Grp 1 Wk 20        | 0008627   | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| I05718M Grp 1 Wk 20        | 0008628   | 101800AD   | 10/18/00        | 10/20/00      | 2.65                 |
| I05720M Grp 1 Wk 20        | 0008629   | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| I05725M Grp 1 Wk 20        | 0008630   | 101800A    | 10/18/00        | 10/19-20/00   | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>0.450</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>1.08</b>          |
| I05702M Grp 2 Wk 20        | 0008631   | 101800AD   | 10/18/00        | 10/20/00      | 6.91                 |
| I05706M Grp 2 Wk 20        | 0008632   | 101800A    | 10/18/00        | 10/19-20/00   | 4.33                 |
| I05717M Grp 2 Wk 20        | 0008633   | 101800A    | 10/18/00        | 10/19-20/00   | 2.41                 |
| I05721M Grp 2 Wk 20        | 0008634   | 101800AD   | 10/18/00        | 10/20/00      | 13.6                 |
| <b>Average:</b>            |           |            |                 |               | <b>6.81</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>4.89</b>          |
| I05707M Grp 3 Wk 20        | 0008635   | 101800AD   | 10/18/00        | 10/20/00      | 35.0                 |
| I05708M Grp 3 Wk 20        | 0008636   | 101800AD   | 10/18/00        | 10/20/00      | 24.9                 |
| I05710M Grp 3 Wk 20        | 0008637   | 101800AD   | 10/18/00        | 10/20/00      | 81.3                 |
| I05712M Grp 3 Wk 20        | 0008638   | 101800AD   | 10/18/00        | 10/20/00      | 25.5                 |
| I05716M Grp 3 Wk 20        | 0008639   | 101800AD   | 10/18/00        | 10/20/00      | 27.4                 |
| I05719M Grp 3 Wk 20        | 0008640   | 101800AD   | 10/18/00        | 10/20/00      | 120                  |
| <b>Average:</b>            |           |            |                 |               | <b>52.4</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>39.5</b>          |
| I05703M Grp 4 Wk 20        | 0008641   | 101800A    | 10/18/00        | 10/19-20/00   | 2.11                 |
| I05704M Grp 4 Wk 20        | 0008642   | 101800A    | 10/18/00        | 10/19-20/00   | 0.778                |
| I05711M Grp 4 Wk 20        | 0008643   | 101800AD   | 10/18/00        | 10/20/00      | 134                  |
| I05713M Grp 4 Wk 20        | 0008644   | 101800A    | 10/18/00        | 10/19-20/00   | 0.286                |
| I05722M Grp 4 Wk 20        | 0008645   | 101800AD   | 10/18/00        | 10/20/00      | 51.7                 |
| <b>Average:</b>            |           |            |                 |               | <b>37.8</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>58.1</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XIII. Summary of POAA residues in Week 22**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 22        | 0008646   | 101900A    | 10/19/00        | 10/20-21/00   | 0.0129               |
| I05714M Grp 1 Wk 22        | 0008647   | 101900A    | 10/19/00        | 10/20-21/00   | 0.0422               |
| I05715M Grp 1 Wk 22        | 0008648   | 101900A    | 10/19/00        | 10/20-21/00   | NQ                   |
| I05718M Grp 1 Wk 22        | 0008649   | 102000AD   | 10/19/00        | 10/23/00      | 90.8                 |
| I05720M Grp 1 Wk 22        | 0008650   | 101900A    | 10/19/00        | 10/20-21/00   | 0.0106               |
| I05725M Grp 1 Wk 22        | 0008651   | 101900AD   | 10/19/00        | 10/22/00      | 1.84                 |
| <b>Average:</b>            |           |            |                 |               | <b>15.5</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>36.9</b>          |
| I05702M Grp 2 Wk 22        | 0008652   | 101900AD   | 10/19/00        | 10/22/00      | 7.82                 |
| I05706M Grp 2 Wk 22        | 0008653   | 101900AD   | 10/19/00        | 10/22/00      | 17.1                 |
| I05717M Grp 2 Wk 22        | 0008654   | 101900AD   | 10/19/00        | 10/22/00      | 16.6                 |
| <b>Average:</b>            |           |            |                 |               | <b>13.8</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>5.22</b>          |
| I05707M Grp 3 Wk 22        | 0008655   | 101900AD   | 10/19/00        | 10/22/00      | 36.1                 |
| I05708M Grp 3 Wk 22        | 0008656   | 101900AD   | 10/19/00        | 10/22/00      | 69.4                 |
| I05710M Grp 3 Wk 22        | 0008657   | 101900AD   | 10/19/00        | 10/22/00      | 23.7                 |
| I05712M Grp 3 Wk 22        | 0008658   | 101900AD   | 10/19/00        | 10/22/00      | 13.7                 |
| I05716M Grp 3 Wk 22        | 0008659   | 101900AD   | 10/19/00        | 10/22/00      | 35.3                 |
| I05719M Grp 3 Wk 22        | 0008660   | 101900AD   | 10/19/00        | 10/22/00      | 58.9                 |
| <b>Average:</b>            |           |            |                 |               | <b>39.5</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>21.0</b>          |
| I05703M Grp 4 Wk 22        | 0008661   | 101900A    | 10/19/00        | 10/20-21/00   | 1.95                 |
| I05704M Grp 4 Wk 22        | 0008662   | 101900AD   | 10/19/00        | 10/22/00      | 83.9                 |
| I05711M Grp 4 Wk 22        | 0008663   | 101900A    | 10/19/00        | 10/20-21/00   | 1.30                 |
| I05713M Grp 4 Wk 22        | 0008664   | 101900AD   | 10/19/00        | 10/22/00      | 35.8                 |
| I05722M Grp 4 Wk 22        | 0008665   | 101900A    | 10/19/00        | 10/20-21/00   | 2.80                 |
| <b>Average:</b>            |           |            |                 |               | <b>25.2</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>36.0</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XIV. Summary of POAA residues in Week 24**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 24        | 0008666   | 102000A    | 10/20/00        | 10/22-23/00   | 0.0261               |
| I05714M Grp 1 Wk 24        | 0008667   | 102000AD   | 10/20/00        | 10/23/00      | 2.82                 |
| I05715M Grp 1 Wk 24        | 0008668   | 102000A    | 10/20/00        | 10/22-23/00   | NQ                   |
| I05718M Grp 1 Wk 24        | 0008669   | 102000A    | 10/20/00        | 10/22-23/00   | 0.0310               |
| I05720M Grp 1 Wk 24        | 0008670   | 102000A    | 10/20/00        | 10/22-23/00   | NQ                   |
| I05725M Grp 1 Wk 24        | 0008671   | 102000A    | 10/20/00        | 10/22-23/00   | 0.207                |
| <b>Average:</b>            |           |            |                 |               | <b>0.517</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>1.13</b>          |
| I05702M Grp 2 Wk 24        | 0008672   | 102000A    | 10/20/00        | 10/22-23/00   | 4.14                 |
| I05706M Grp 2 Wk 24        | 0008673   | 102000AD   | 10/20/00        | 10/23/00      | 12.40                |
| I05717M Grp 2 Wk 24        | 0008674   | 102000A    | 10/20/00        | 10/22-23/00   | 2.12                 |
| <b>Average:</b>            |           |            |                 |               | <b>6.22</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>5.45</b>          |
| I05707M Grp 3 Wk 24        | 0008675   | 102000AD   | 10/20/00        | 10/23/00      | 30.4                 |
| I05708M Grp 3 Wk 24        | 0008676   | 102000AD   | 10/20/00        | 10/23/00      | 53.7                 |
| I05710M Grp 3 Wk 24        | 0008677   | 102000AD   | 10/20/00        | 10/23/00      | 58.4                 |
| I05712M Grp 3 Wk 24        | 0008678   | 102000AD   | 10/20/00        | 10/23/00      | 10.0                 |
| I05716M Grp 3 Wk 24        | 0008679   | 102000AD   | 10/20/00        | 10/23/00      | 65.3                 |
| I05719M Grp 3 Wk 24        | 0008680   | 102000AD   | 10/20/00        | 10/23/00      | 25.3                 |
| <b>Average:</b>            |           |            |                 |               | <b>40.5</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>21.8</b>          |
| I05703M Grp 4 Wk 24        | 0008681   | 102000A    | 10/20/00        | 10/22-23/00   | 0.534                |
| I05704M Grp 4 Wk 24        | 0008682   | 102000AD   | 10/20/00        | 10/23/00      | 99.6                 |
| I05711M Grp 4 Wk 24        | 0008683   | 102000A    | 10/20/00        | 10/22-23/00   | 0.310                |
| I05713M Grp 4 Wk 24        | 0008684   | 102000AD   | 10/20/00        | 10/23/00      | 71.9                 |
| I05722M Grp 4 Wk 24        | 0008685   | 102000A    | 10/20/00        | 10/22-23/00   | 0.685                |
| <b>Average:</b>            |           |            |                 |               | <b>34.6</b>          |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>47.7</b>          |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XV. Summary of POAA residues in Week 26**

| Sponsor ID          | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|---------------------|-----------|------------|-----------------|---------------|----------------------|
| I05709M Grp 1 Wk 26 | 0008686   | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| I05714M Grp 1 Wk 26 | 0008687   | 102300AR   | 10/23/00        | 10/25/00      | 0.0317               |
| I05715M Grp 1 Wk 26 | 0008688   | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| I05718M Grp 1 Wk 26 | 0008689   | 102300AR   | 10/23/00        | 10/25/00      | 0.0227               |
| I05720M Grp 1 Wk 26 | 0008690   | 102300AR   | 10/23/00        | 10/25/00      | NQ                   |
| I05725M Grp 1 Wk 26 | 0008691   | 102300AR   | 10/23/00        | 10/25/00      | 0.0186               |
| Average:            |           |            |                 |               | 0.0172               |
| Standard Deviation: |           |            |                 |               | 0.00892              |
| I05702M Grp 2 Wk 26 | 0008692   | 102300AR   | 10/23/00        | 10/25/00      | 4.44                 |
| I05706M Grp 2 Wk 26 | 0008693   | 102300AR   | 10/23/00        | 10/25/00      | 2.48                 |
| I05717M Grp 2 Wk 26 | 0008694   | 102300AR   | 10/23/00        | 10/25/00      | 1.85                 |
| Average:            |           |            |                 |               | 2.92                 |
| Standard Deviation: |           |            |                 |               | 1.35                 |
| I05707M Grp 3 Wk 26 | 0008695   | 102300AD   | 10/23/00        | 10/26/00      | 108                  |
| I05708M Grp 3 Wk 26 | 0008696   | 102300AD   | 10/23/00        | 10/26/00      | 46.6                 |
| I05710M Grp 3 Wk 26 | 0008697   | 102300AD   | 10/23/00        | 10/26/00      | 10.7                 |
| I05712M Grp 3 Wk 26 | 0008698   | 102300AD   | 10/23/00        | 10/26/00      | 19.1                 |
| I05716M Grp 3 Wk 26 | 0008699   | 102300AD   | 10/23/00        | 10/26/00      | 15.9                 |
| I05719M Grp 3 Wk 26 | 0008700   | 102300AD   | 10/23/00        | 10/26/00      | 57.5                 |
| Average:            |           |            |                 |               | 43.0                 |
| Standard Deviation: |           |            |                 |               | 36.9                 |
| I05703M Grp 4 Wk 26 | 0008701   | 102300AR   | 10/23/00        | 10/25/00      | 0.238                |
| I05704M Grp 4 Wk 26 | 0008702   | 102300AR   | 10/23/00        | 10/25/00      | 3.77                 |
| I05711M Grp 4 Wk 26 | 0008703   | 102300AD   | 10/23/00        | 10/26/00      | NQ                   |
| I05713M Grp 4 Wk 26 | 0008704   | 102300AD   | 10/23/00        | 10/26/00      | 47.4                 |
| I05722M Grp 4 Wk 26 | 0008705   | 102300AR   | 10/23/00        | 10/25/00      | 0.107                |
| Average:            |           |            |                 |               | 10.3                 |
| Standard Deviation: |           |            |                 |               | 20.8                 |

ND = Not Detected

NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XVI. Summary of POAA residues in Week 28-34**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05718M Grp 1 Wk 28        | 0008706   | 102400A    | 10/24/00        | 10/24-25/00   | 0.0675               |
| I05720M Grp 1 Wk 28        | 0008707   | 102300AD   | 10/24/00        | 10/26/00      | 2.09                 |
| I05718M Grp 1 Wk 30        | 0008710   | 102400A    | 10/24/00        | 10/24-25/00   | NQ                   |
| I05720M Grp 1 Wk 30        | 0008711   | 102400A    | 10/24/00        | 10/24-25/00   | 0.0123               |
| I05718M Grp 1 Wk 32        | 0008714   | 102400A    | 10/24/00        | 10/24-25/00   | NQ                   |
| I05720M Grp 1 Wk 32        | 0008715   | 102400A    | 10/24/00        | 10/24-25/00   | 0.0260               |
| I05718M Grp 1 Wk 34        | 0008718   | 102400A    | 10/24/00        | 10/24-25/00   | NQ                   |
| I05720M Grp 1 Wk 34        | 0008719   | 102400A    | 10/24/00        | 10/24-25/00   | NQ                   |
| <b>Average:</b>            |           |            |                 |               | <b>0.279</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.732</b>         |
| I05712M Grp 3 Wk 28        | 0008708   | 102400A    | 10/24/00        | 10/24-25/00   | 1.23                 |
| I05716M Grp 3 Wk 28        | 0008709   | 102400A    | 10/24/00        | 10/24-25/00   | 0.381                |
| I05712M Grp 3 Wk 30        | 0008712   | 102400A    | 10/24/00        | 10/24-25/00   | 0.380                |
| I05716M Grp 3 Wk 30        | 0008713   | 102400A    | 10/24/00        | 10/24-25/00   | 0.498                |
| I05716M Grp 3 Wk 32        | 0008716   | 102400A    | 10/24/00        | 10/24-25/00   | 0.285                |
| I05716M Grp 3 Wk 32        | 0008717   | 102400A    | 10/24/00        | 10/24-25/00   | 0.0894               |
| I05716M Grp 3 Wk 34        | 0008720   | 102400A    | 10/24/00        | 10/24-25/00   | 0.130                |
| I05716M Grp 3 Wk 34        | 0008721   | 102400A    | 10/24/00        | 10/24-25/00   | 0.104                |
| <b>Average:</b>            |           |            |                 |               | <b>0.387</b>         |
| <b>Standard Deviation:</b> |           |            |                 |               | <b>0.372</b>         |

ND = Not Detected  
NQ = Not Quantifiable

For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

**Table XVII. Summary of POAA residues in Weeks 36-40**

| Sponsor ID                 | Centre ID | Set Number | Extraction Date | Analysis Date | Analyte Found (µg/g) |
|----------------------------|-----------|------------|-----------------|---------------|----------------------|
| I05718M Grp 1 Wk 36        | 0008722   | 102500A    | 10/25/00        | 10/26/00      | NQ                   |
| I05720M Grp 1 Wk 36        | 0008723   | 102500A    | 10/25/00        | 10/26/00      | 0.0216               |
| I05718M Grp 1 Wk 38        | 0008726   | 102500A    | 10/25/00        | 10/26/00      | ND                   |
| I05720M Grp 1 Wk 38        | 0008727   | 102500A    | 10/25/00        | 10/26/00      | NQ                   |
| I05718M Grp 1 Wk 40        | 0008730   | 102500A    | 10/25/00        | 10/26/00      | NQ                   |
| I05720M Grp 1 Wk 40        | 0008731   | 102500A    | 10/25/00        | 10/26/00      | NQ                   |
| <b>Average:</b>            |           |            |                 |               | 0.0103               |
| <b>Standard Deviation:</b> |           |            |                 |               | 0.00684              |
| I05712M Grp 3 Wk 36        | 0008724   | 102500A    | 10/25/00        | 10/26/00      | 0.0944               |
| I05716M Grp 3 Wk 36        | 0008725   | 102500A    | 10/25/00        | 10/26/00      | 0.0306               |
| I05712M Grp 3 Wk 38        | 0008728   | 102500A    | 10/25/00        | 10/26/00      | 0.0327               |
| I05716M Grp 3 Wk 38        | 0008729   | 102500A    | 10/25/00        | 10/26/00      | NQ                   |
| I05716M Grp 3 Wk 40        | 0008732   | 102500A    | 10/25/00        | 10/26/00      | 0.0103               |
| I05716M Grp 3 Wk 40        | 0008733   | 102500A    | 10/25/00        | 10/26/00      | 0.0235               |
| <b>Average:</b>            |           |            |                 |               | 0.0336               |
| <b>Standard Deviation:</b> |           |            |                 |               | 0.0313               |

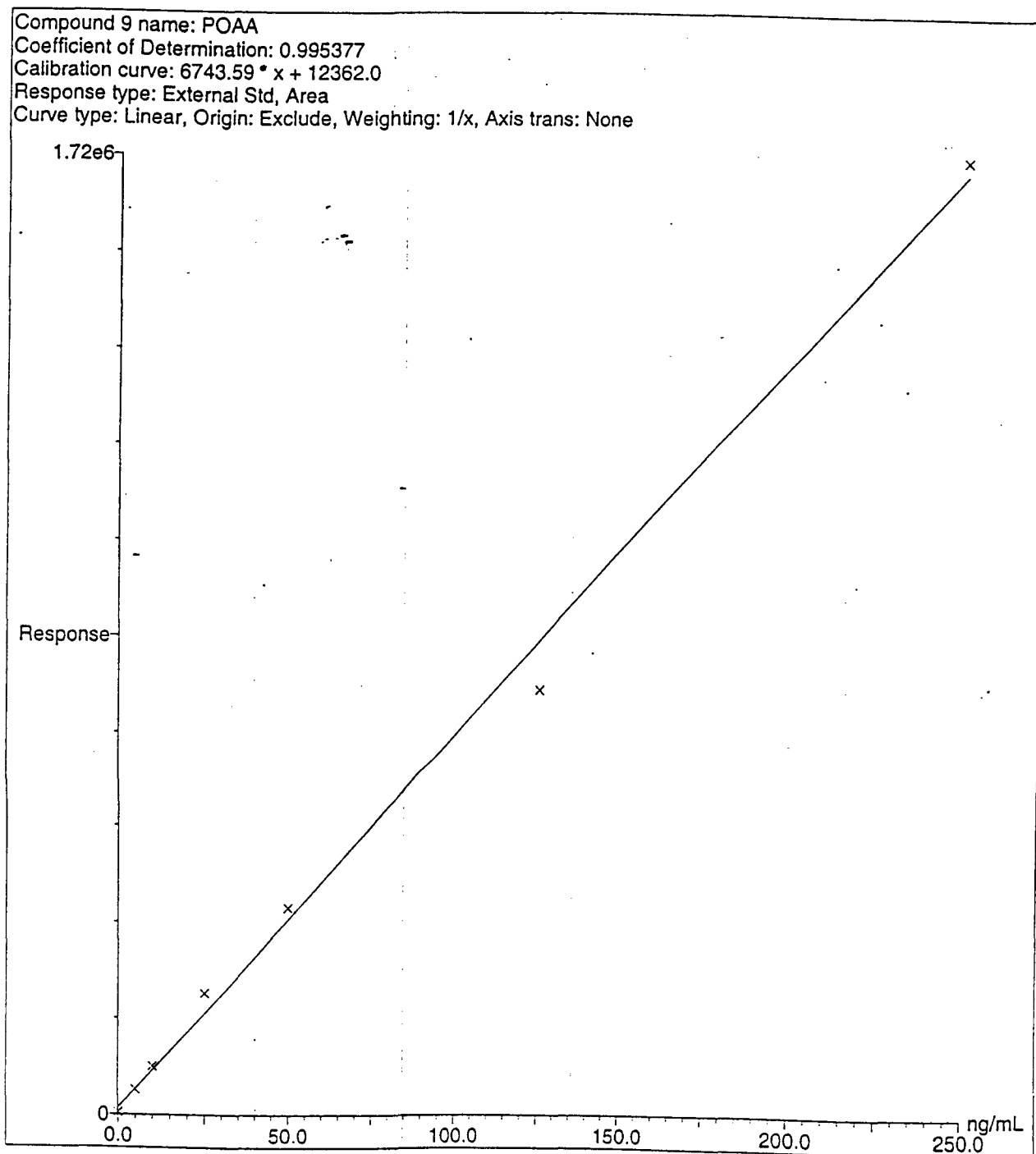
**ND = Not Detected**

**NQ = Not Quantifiable**

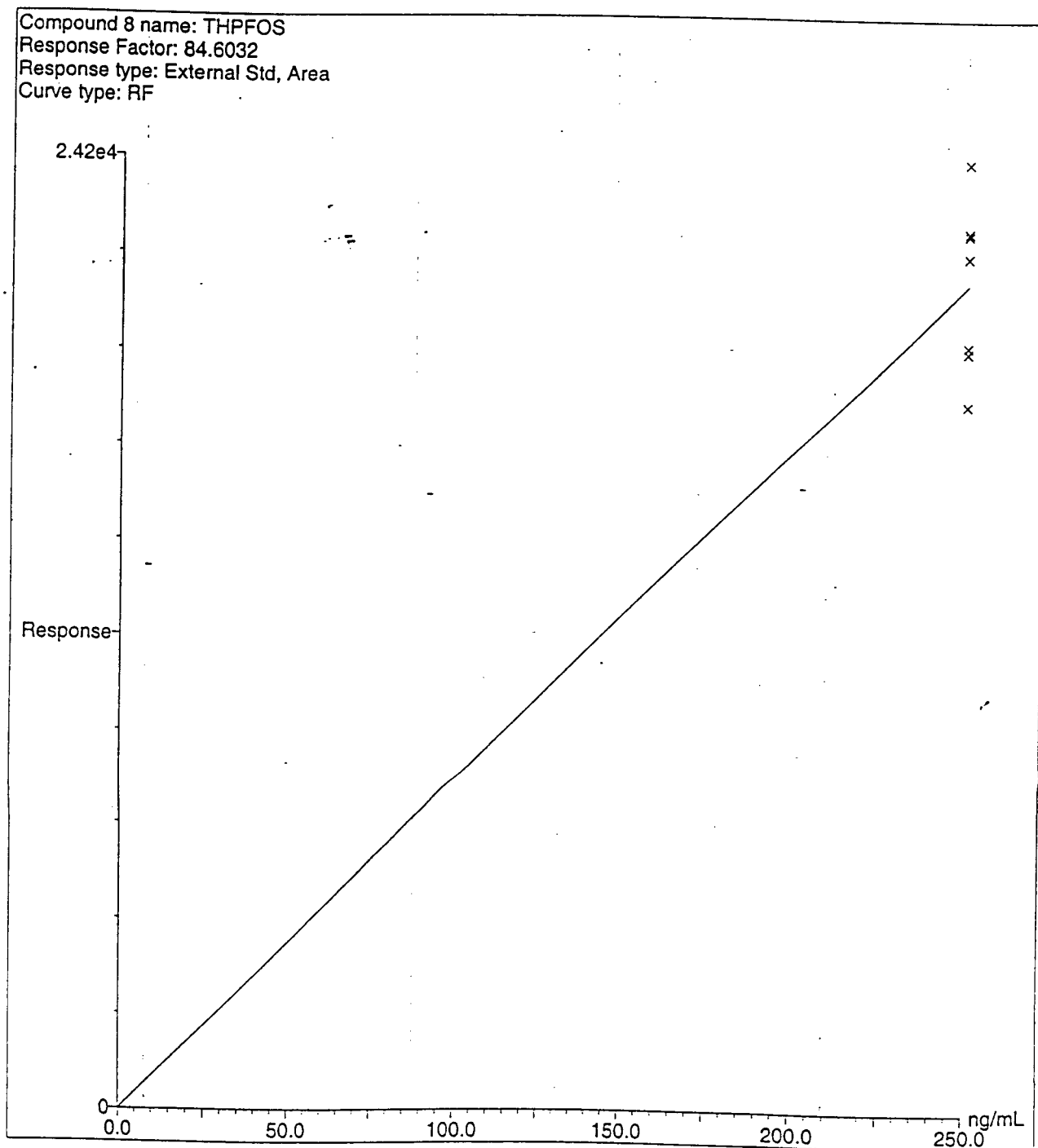
For residues recorded as ND, zero was used to calculate the average and standard deviation and 0.0100 was used for those recorded as NQ.

## **12.0 FIGURES**

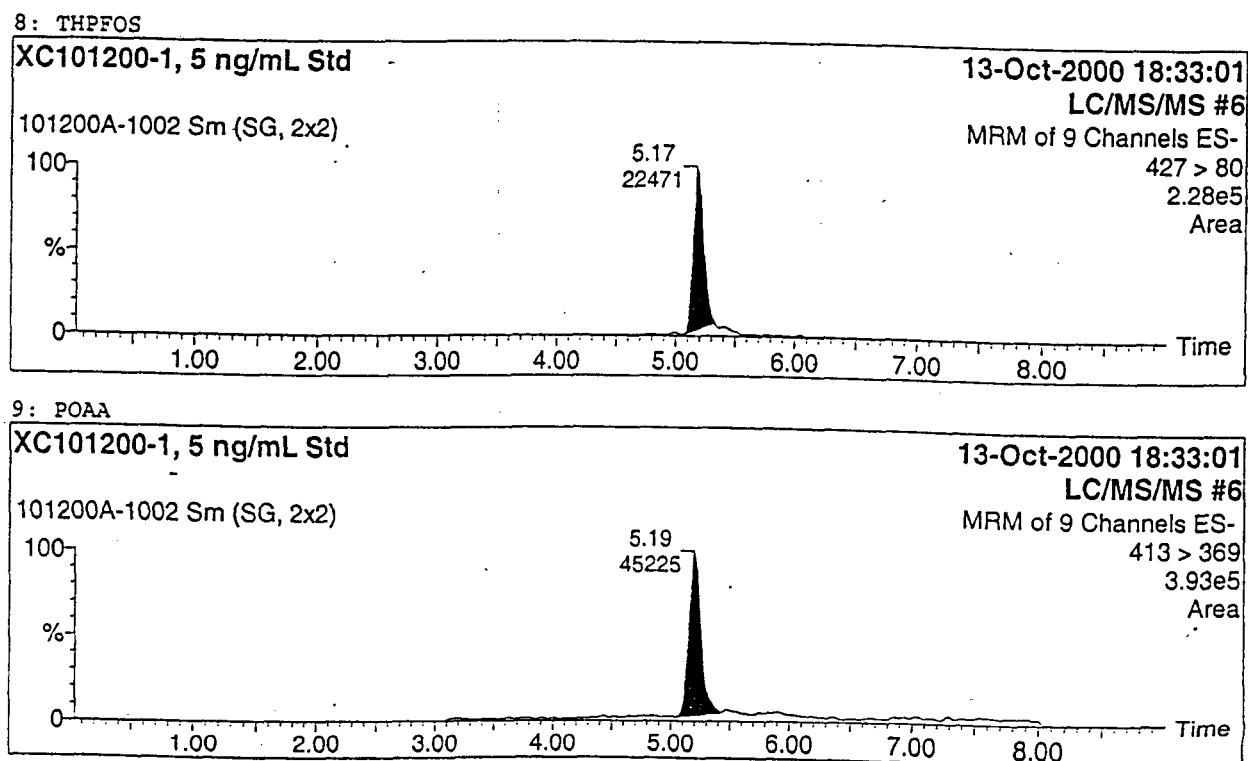
**Figure 1. Typical Calibration Curve for POAA**



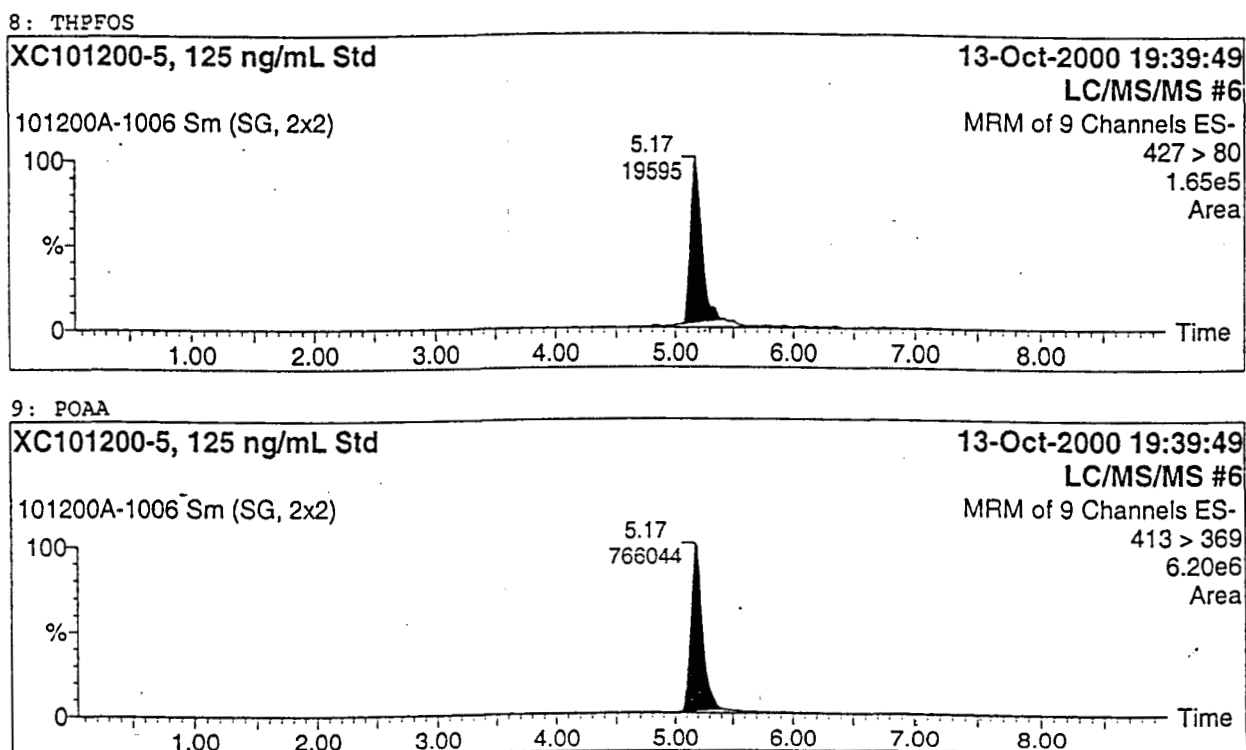
**Figure 2. Typical Mean Response Factor for THPFOS**



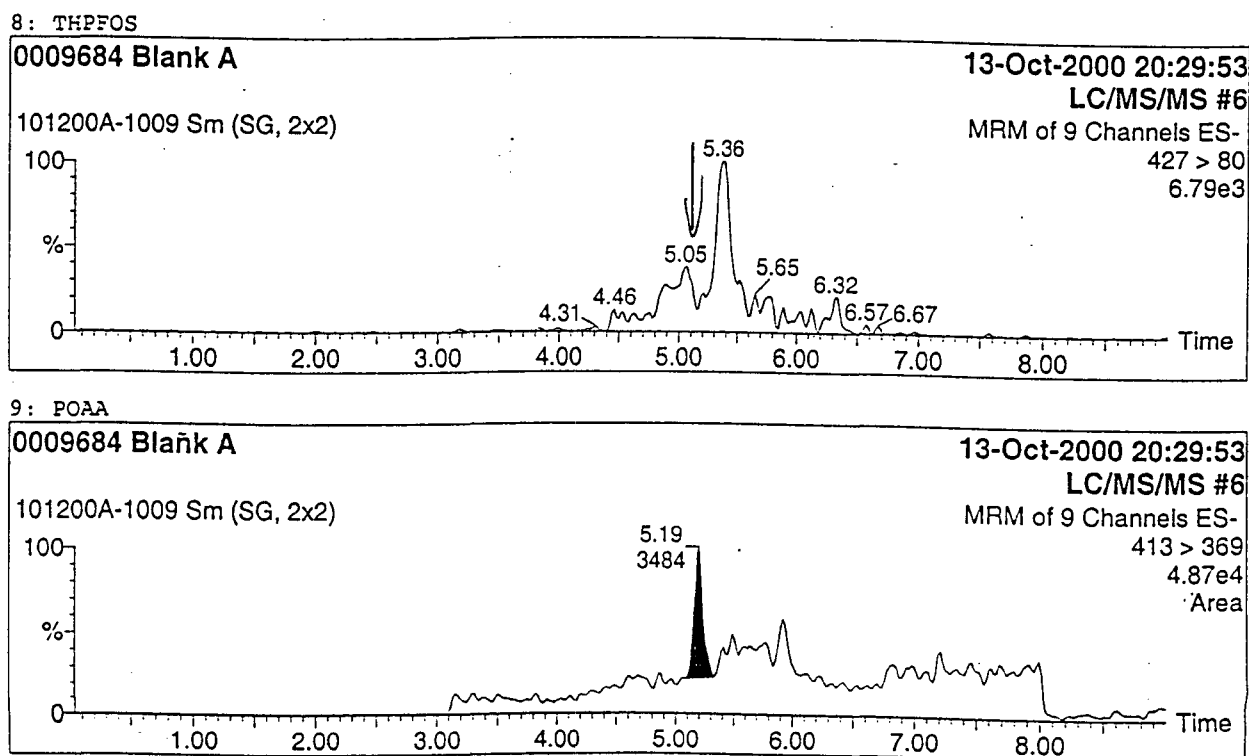
**Figure 3. Chromatogram Representing a 5 ng/mL extracted standard for POAA and 250 ng/mL extracted standard for THPFOS**



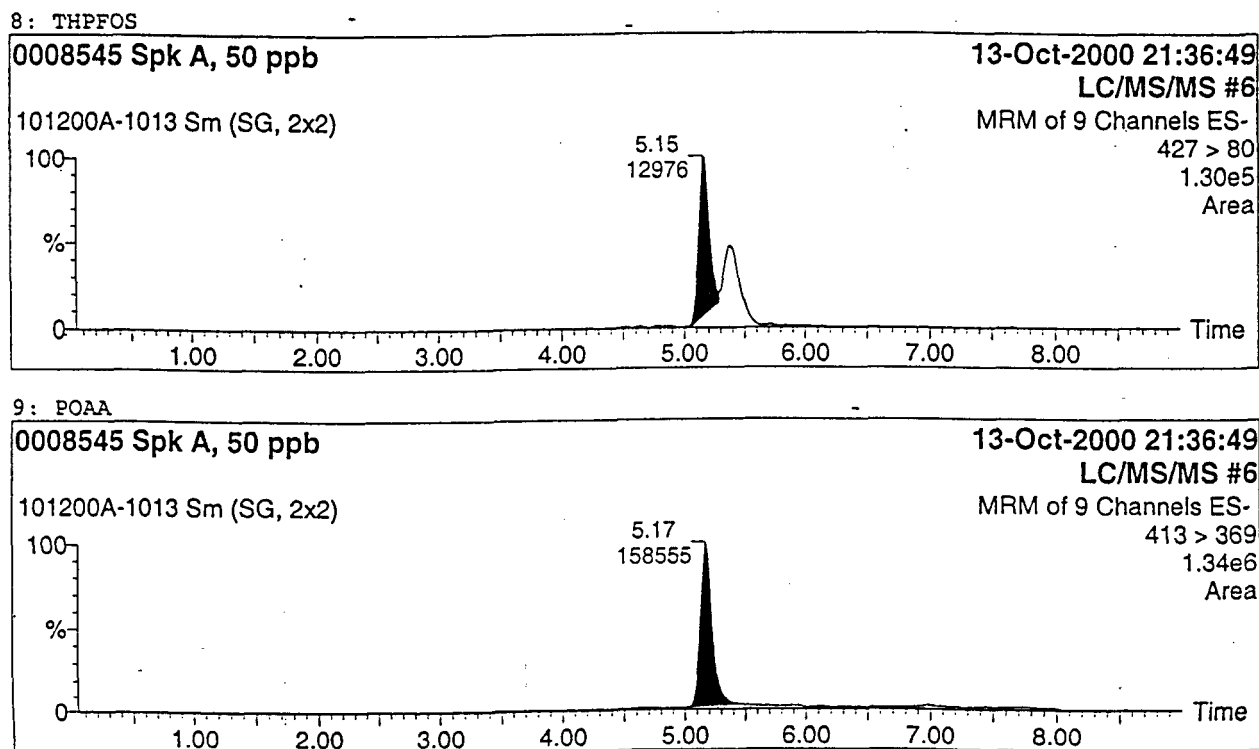
**Figure 4. Chromatogram Representing a 125 ng/mL extracted standard for POAA and 250 ng/mL extracted standard for THPFOS**



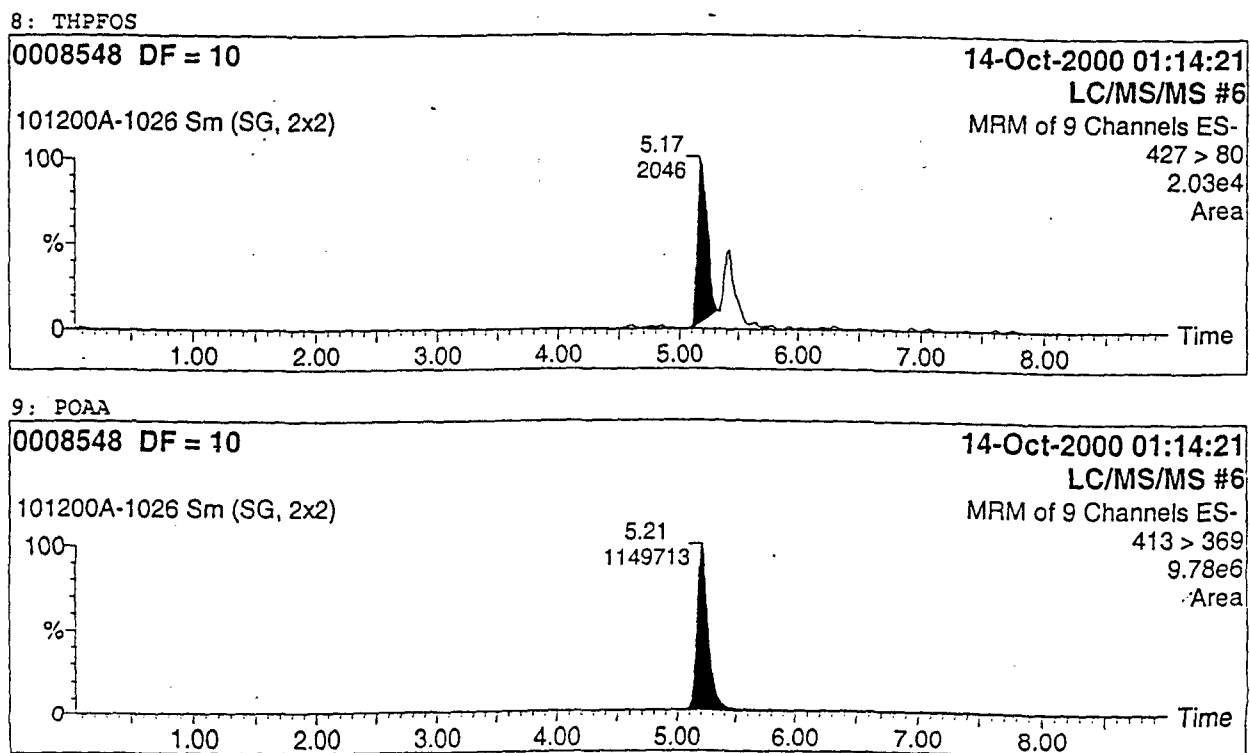
**Figure 5. Chromatogram Representing Control Monkey Feces for POAA and THPFOS**  
(Centre ID: 0009684 Blank A, Set: 101200A)



**Figure 6. Chromatogram Representing Control Monkey Feces  
Fortified with 50 ng/g of POAA and 500 ng/g of THPFOS  
(Centre ID: 0008545 Spk A, Set: 101200A)**



**Figure 7. Chromatogram of Monkey Feces Sample from Grp 2 in  
Week 12  
(Centre ID: 0008548, Set: 101200A)**



# **13.0 APPENDICES**

# **APPENDIX A**

**Study Protocol  
FACT-TOX-026  
(Centre Study No. 023-011)  
26-Week Capsule Toxicity Study with  
Ammonium Perfluorooctanoate  
(APFO/POAA) in Cynomolgus Monkeys  
and  
Amendments and Deviations**

Protocol #FACT-TOX-026

**Study Title**

**6-Month Capsule Toxicity Study with  
Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys**

**PROTOCOL**

**Author**

Lisa Clemen

**Date:**

October 12, 1998

**Performing Laboratory**

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**

FACT-TOX-026

Exact Copy of Original

LAC 09/26/98  
Initial Date

Protocol #FACT-TOX-026

Study Identification

6-Month Capsule Toxicity Study with  
Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

|                                                                                                       |                                                                                                                           |
|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| <b>Test Material</b>                                                                                  | Ammonium perfluorooctanoate<br>(APFO/POAA)                                                                                |
| <b>Sponsor</b>                                                                                        | 3M Toxicology Services - Medical Department<br>3M Center, Building 220-2E-02<br>P.O. Box 33220<br>St. Paul, MN 55133-3220 |
| <b>Sponsor Representative</b>                                                                         | Paul Lieder, Ph.D., DABT<br>3M Toxicology Services<br>Building 220-2E-02<br>651-737-2678                                  |
| <b>Study Director</b>                                                                                 | Kristen Hansen, Ph.D.<br>3M Environmental Technology and Safety<br>Services<br>Building 2-3E-09<br>651-778-6018           |
| <b>Study Location(s)</b><br><i>In vivo Testing Facility</i>                                           | Covance Laboratories, Inc.<br>3301 Kinsman Boulevard<br>Madison, Wisconsin 53704                                          |
| <i>Analytical Testing Laboratory</i>                                                                  | 3M Environmental Laboratory<br>Building 2-3E-09<br>935 Bush Avenue<br>St. Paul, MN 55106                                  |
| <b>Proposed Study Timetable</b><br><i>Analytical Start Date</i><br><i>Analytical Termination Date</i> | November 12, 1998<br>May 12, 1999                                                                                         |

3M Environmental Laboratory

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Protocol #FACT-TOX-026

**1. STUDY**

Six month capsule toxicity study with ammonium perfluorooctanoate (APFO/POAA) in cynomolgus monkeys.

**2. PURPOSE**

The analytical portion of this study is designed to determine levels of (APFO/POAA) in the liver and serum of cynomolgus monkeys. Based on these results additional tissues or fluids may be analyzed. The in-life portion of this study was conducted at Covance Laboratories, study #6329-231.

**3. REGULATORY COMPLIANCE**

This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practices Standards, 40 CFR 792. However, analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

**4. QUALITY ASSURANCE**

The 3M Environmental Laboratory Quality Assurance Unit will audit the protocol, study conduct, and final report in accordance with the Good Laboratory Practice Standards and 3M Environmental Laboratory Standard Operating Procedures.

**5. TEST MATERIAL**

**5.1 Identification** Ammonium perfluorooctanoate (APFO/POAA)

**5.2 Source** 3M Specialty Chemical Division

**5.3 Physical Description** Gelatin capsules

**5.4 Purity and Stability** Determined by the Sponsor

**5.5 Storage Conditions** Room temperature

**5.6 Reserve Samples** Responsibility of the Sponsor

**5.7 Disposition** Specimens will be retained per GLP regulation

**5.8 Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

Protocol #FACT-TOX-026

**6. CONTROL MATERIAL**

- 6.1 **Identification** Monkey liver and serum
- 6.2 **Source** Covance Laboratories, Inc.
- 6.3 **Physical Description** Liver and serum
- 6.4 **Purity and Stability** Not applicable
- 6.5 **Storage Conditions** Frozen at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$
- 6.6 **Reserve Samples** Not applicable
- 6.7 **Disposition** Biological tissues and fluids are retained per GLP regulation
- 6.8 **Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

**7. REFERENCE MATERIAL**

- 7.1 **Identification** Ammonium perfluorooctanoate (APFO/POAA), lot #377 or #245
- 7.2 **Source** 3M Specialty Chemicals
- 7.3 **Physical Description** White powder
- 7.4 **Purity and Stability** Responsibility of the Sponsor
- 7.5 **Storage Conditions** Room temperature
- 7.6 **Reserve Samples** Not applicable
- 7.7 **Disposition** Retained as per GLP regulation and 3M Environmental Laboratory policy
- 7.8 **Safety Precautions** Refer to MSDS for chemicals used. Wear appropriate laboratory attire, and follow adequate precautions for handling biological materials and preparing samples for analysis.

**8. TEST SYSTEM**

Cynomolgus monkeys were used as the test system, and were maintained and dosed as described in Covance protocol #6329-231. Group 1 control animals did not receive the test substance. Groups 2, 3, and 4 received the test substance daily for 26 weeks, in increasing concentration per group. Two animals each from Groups 1, 3, and 4 were designated as recovery animals and were allowed a 13 week recovery period after cessation of treatment.

Protocol #FACT-TOX-026

#### 9. SPECIMEN RECEIPT

The 3M Environmental Laboratory will receive samples of the following body tissues and fluids from the indicated points in the study:

| Body tissue/fluid               | Collected                                         | Shipped                                     | Expected # of samples                              |
|---------------------------------|---------------------------------------------------|---------------------------------------------|----------------------------------------------------|
| Serum – all animals             | 7 days post treatment, every two weeks thereafter | Packed on dry ice for shipping              | 264 from main study<br>78 additional from recovery |
| Urine, feces – recovery animals | After 6, 30, and 90 days recovery                 | Packed on dry ice for shipping              | 18 urine, 18 feces                                 |
| Liver – all animals             | At termination of the study                       | Shipped with final serum samples on dry ice | 22                                                 |

Total number of test animals: 16

Total number of control animals: 6

Samples sent to 3M Environmental Laboratories will be received and tracked according to applicable Standard Operating Procedures.

#### - 10. PREPARATORY METHODS

- 10.1 FACT-M-1.0, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.2 FACT-M-3.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry
- 10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written.

#### 11. ANALYTICAL METHODS

- 11.1 FACT-M-2.0, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.2 FACT-M-4.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry
- 11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written.

Protocol #FACT-TOX-026

## **12. DATA QUALITY OBJECTIVES**

The number of spikes/duplicates, use of surrogates, and information on other data quality indicators is included in the analytical methods. In addition, the following criteria will be met:

**12.1 Linearity**  $r^2 \geq 0.980$

**12.2 Limits of detection / quantitation**

**12.2.1** Method Detection Limit (MDL) for APFO/POAA

a. Serum: 5 ppb

b. Liver: 24 ppb

**12.2.2** Practical Quantitation Limit (PQL) – Equal to the lowest standard in the calibration curve

**12.3 Duplicate acceptable precision** < 30% for the method

**12.4 Spike acceptable recoveries** 70% - 130%

**12.5 Use of confirmatory methods** Indeterminate samples will be re-analyzed

**12.6 Demonstration of specificity** Chromatographic retention time, mass spectral daughter ion characterization

## **13. SUB-CONTRACTED ANALYSIS**

All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106.

## **14. STATISTICAL ANALYSIS**

Averages and standard deviations will be calculated. The statistical methods that will be used are described below:

**14.1 Data transformations and analysis** Data will be reported as the concentration (weight/weight or weight/vol) of APFO/POAA or metabolite per tissue or fluid, or of APFO/POAA or metabolite per unit of tissue or fluid.

**14.2 Statistical analysis** Statistics used may include regression analysis of concentrations over time, and standard deviations calculated for the concentrations within each dose group. If necessary, simple statistical tests, such as Student's t test, may be applied to evaluate statistical difference.

## **15. REPORT**

A report of the results of the study will be prepared by 3M Environmental Laboratory. The report will include, but not be limited to, the following, when applicable:

**15.1** Name and address of the facility performing the study

**15.2** Dates upon which the study was initiated and completed

Protocol #FACT-TOX-026

- 15.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards
- 15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol
- 15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor
- 15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor
- 15.7 A description of the methods used to conduct the test(s)
- 15.8 A description of the test system
- 15.9 A description of any circumstances that may have affected the quality or the integrity of the data
- 15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study
- 15.11 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses
- 15.12 Statistical methods used to evaluate the data, if applicable
- 15.13 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable
- 15.14 The location where raw data and the final report are to be stored
- 15.15 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

If it is necessary to make corrections or additions to a final report after it has been accepted, the changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

#### **16. LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT**

Original data, or copies thereof, will be available at 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

*Protocol #FACT-TOX-026*

**16.1** The following raw data and records will be retained in the study folder in the study/project archives according to 3M Environmental Laboratory Standard Operating Procedures:

- 16.1.1** Approved protocol and amendments
- 16.1.2** Study correspondence
- 16.1.3** Shipping records
- 16.1.4** Raw data
- 16.1.5** Approved final report (original signed copy)
- 16.1.6** Electronic copies of data

**16.2** The following supporting records will be retained separately from the study folder in the archives according to 3M Environmental Laboratory Standard Operating Procedures:

- 16.2.1** Training records
- 16.2.2** Calibration records
- 16.2.3** Instrument maintenance logs
- 16.2.4** Standard Operating Procedures, Equipment Procedures, and Methods

#### **17. SAMPLE RETENTION**

Specimens will be maintained in the laboratory specimen archives for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

#### **18. PROTOCOL AMENDMENTS AND DEVIATIONS**

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. All changes to the protocol will be indicated in the final report. Any other changes will be in the form of written deviations, signed by the Study Director and filed with the raw data.

#### **19. ATTACHMENTS**

- 19.1 Attachment A** Preparatory and analytical methods

Protocol #FACT-TOX-026

20. SIGNATURES

Paul H. Lieder 11/30/98  
Paul Lieder, Ph.D, DABT, Sponsor Representative Date

Kristen Hansen 11/12/98  
Kristen Hansen, Ph.D., 3M Environmental Laboratory Study Director Date

**Study Title**

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 1**

**Amendment Date:**

July 23, 1999

**Performing Laboratory**

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**

ET&SS FACT-TOX026  
LRN U2782

Exact Copy of Original  
luc 09/26/00  
Initial Date

3M Environmental Laboratory

Protocol FACT-TOX026  
Amendment No. 1

This amendment modifies the following portion(s) of the protocol:

1. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-3.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-2.0 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-4.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

**AMEND TO READ:** The extraction and analytical methods FACT-M-3.1 and FACT-M-4.1, respectively, were updated on 04/27/99 to:

ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** The methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix. The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

2. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-4.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

FACT-M-2.0 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

3M Environmental Laboratory

Protocol FACT-TOX026  
Amendment No. 1

**AMEND TO READ:** The extraction and analytical methods FACT-M-1.0 and FACT-M-2.0, respectively, were updated on 07/22/99 to:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** The methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE, POAA and Monester were removed from the standard mix, and M556 was added to the standard mix.

The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

3. **PROTOCOL READS:** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry" ETS-8-4.1

"Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum or Other Fluid for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-5.1 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry"

**AMEND TO READ:** Additional extraction and analytical methods, listed below, were added to the protocol:

ETS-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or other fluorochemical compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry"

ETS-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry"

**REASON:** These methods were developed and validated for urine extraction and analysis after the original protocol was written and approved.

3M Environmental Laboratory

Protocol FACT-TOX026  
Amendment No. 1

4. **PROTOCOL READS:** Section 2.0 lists serum and liver as the matrices of interest for determination of POAA.

**AMEND TO READ:** Urine will be included for determination of POAA.

**REASON:** The methods were developed and validated for extraction and analysis of urine after the original protocol was written and approved.

5. **PROTOCOL READS:** Section 6.0 lists monkey serum and liver as the control matrices received from Covance Laboratories.

**AMEND TO READ:** Control matrix monkey urine was obtained from Covance Laboratories and is maintained at a temperature of  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . All traceability information for this matrix will be included in the final report.

**REASON:** Addition of control urine matrix to the analytical protocol.

6. **PROTOCOL READS:** Section 12.2 lists monkey serum and liver method detection limits.

**AMEND TO READ:** Method detection limit for urine is 6 ppb.

**REASON:** Addition of method detection limit for urine matrix.

### Amendment Approval

Paul H. Lieder  
Paul Lieder, Ph.D., DABT, Sponsor Representative

August 3, 1999  
Date

Kris J. Hansen  
Kris J. Hansen, Ph.D., Study Director

8/4/99  
Date

3M Environmental Laboratory

**Study Title**

6-Month Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 2**

**Amendment Date:**

20 January 2000

**Performing Laboratory**

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106 -

**Laboratory Project Identification**

ET&SS LRN-U2782  
FACT TOX-026  
Covance Study: 6329-231  
3M Medical Department Study: T-6889.3

3M Environmental Laboratory

Exact Copy of Original  
1AC 09/26/00  
Initial Date

Protocol LRN-U2782  
Amendment Number 2

This amendment modifies the following portion(s) of the protocol:

1. **PROTOCOL READS:**

The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.

**AMEND TO READ:**

The role of study director for the present study was reassigned to Paul Lieder, Ph.D., as of 20 January 2000. The previous study director, Kristen Hansen, has been reassigned to the role of Principle Analytical Investigator.

**REASON:**

The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 40 CFR Part 792).

2. **PROTOCOL READS:**

The sponsor for the present study was identified as Paul Lieder.

**AMEND TO READ:**

The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.

**REASON:**

To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

3. **PROTOCOL READS:**

**17. Sample Retention:**

Specimens will be maintained in the laboratory specimen archives for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

**AMEND TO READ:**

**17. Specimen Retention:**

Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Any specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. Specimens analyzed at sub-contract laboratories will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

**REASON:**

To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

3M Environmental Laboratory

Protocol LRN-U2782  
Amendment Number 2

**4. PROTOCOL READS:**

Section 16 states that the following raw data and records will be retained in the study folder in the archives according to AMDT-S-8: Approved protocol and amendments; study correspondence; shipping records; raw data; approved final report (original signed copy); and electronic copies of data. Additionally, Section 16 states that supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following: Training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.

**AMEND TO READ:**

Section 16 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

**REASON:**

To direct subcontract laboratories in the disposition of the items listed above.

3M Environmental Laboratory

Protocol LRN-U2782  
Amendment Number 2

Amendment Approval

John T. Butenhoff February 10, 2000  
John Butenhoff, Ph.D., Sponsor Representative Date

Kristen Hansen 11-Feb-2000  
Kristen J. Hansen, Ph.D., Outgoing Study Director Date

Paul H. Lieder Feb 10, 2000  
Paul Lieder, Ph.D., Incoming Study Director Date

3M Environmental Laboratory

**Study Title**

6-Month Capsule Toxicity Study with Ammonium Perfluorooctanoate  
(APFO/POAA) in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 3**

**Amendment Date:**

20 April 2000

**Performing Laboratories**

**LIVER, SERUM, AND URINE ANALYSES**

3M Environmental Technology and Safety Services  
Fluorine Analytical Chemistry Team  
Building 2-3E-09, 935 Bush Avenue  
St. Paul, MN 55106

**FECES ANALYSES**

Centre Analytical Laboratories, Inc.  
3048 Research Drive  
State College, PA 16801

**Laboratory Project Identification**

ET&SS LRN-U2782  
FACT TOX-026  
Covance Study: 6329-231  
3M Medical Department Study: T-6889.3

Exact Copy of Original

LAC      04/26/00  
Initial      Date

3M Environmental Laboratory

Protocol LRN-U2782  
Amendment Number 3

This amendment modifies the following portion(s) of the protocol:

**1. PROTOCOL READS:**

The amended section 2.0 text states that this study is designed to determine levels of APFO/POAA in the liver, serum, and urine of cynomolgus monkeys.

**AMEND TO READ:**

This study is designed to determine levels of APFO/POAA in the liver, serum, feces, and urine of cynomolgus monkeys.

**REASON:**

The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

**2. PROTOCOL READS:**

The amended section 6.0 lists monkey liver, serum, and urine.

**AMEND TO READ:**

Add: monkey feces with a physical description of feces.

**REASON:**

Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

**3. PROTOCOL READS:**

The amended Section 12.2.1 items a., b., and c., list the Method Detection Limits for matrices analyzed in this study.

**AMEND TO READ:**

The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

**REASON:**

The method detection limits are specific to the 3M Environmental Laboratory. This statement was added to allow for sub-contracted analyses, revised methods, and added matrices.

**4. PROTOCOL READS:**

Section 13. lists the laboratories that will be conducting analyses for this study.

**AMEND TO READ:**

Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801

**REASON:**

Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

3M Environmental Laboratory

Protocol LRN-U2782  
Amendment Number 3

5. **PROTOCOL READS:**

Sections 10.3 and 11.3 state that if methods other than those listed are used in this study, an amendment will be written to include the new methods.

**AMEND TO READ:**

The feces extraction and analytical method used by Centre Analytical Laboratories will be; 00M-023-003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."

**REASON:**

The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Amendment Approval

*John L. Butenhoff*

*April 19, 2000*

John L. Butenhoff, Ph.D., Sponsor Representative

Date

*Paul H. Lieder*

*April 19, 2000*

Paul Lieder, Ph.D., Study Director

Date

3M Environmental Laboratory



*Study Title*

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 4

*Amendment Date:*  
August 2, 2000

*Performing Laboratories*  
Covance Laboratories Inc.  
3301 Kinsman Boulevard  
Madison, WI 53704

3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

*Laboratory Project Identification*  
FACT-TOX-026  
LIMS U2782  
Covance 6329-231  
3M Medical Department Study: T-6889.3

Exact Copy of Original  
LAC 10/07/00  
Initial Date

*Protocol TOX-026  
Amendment 4*

This amendment modifies the following portion(s) of the protocol:

1. *PROTOCOL READS:*

*STUDY TITLE:* "6-Month Capsule Toxicity [..]" (on the title page and page 2 of the protocol for the analytical phase of the study).

*AMEND TO READ:*

*STUDY TITLE:* "26-Week Capsule Toxicity [..]" (on the title page and page 2 of the protocol for the analytical phase of the study).

*REASON:*

To correct the title for the analytical phase of the study.

2. *PROTOCOL READS:*

DATA QUALITY OBJECTIVES (Section 12., page 6)

*AMEND TO READ:*

Add to this section: LOQ in feces of 10 ng/g

*REASON:*

To specify the analytical limits for feces analyses.

3. *PROTOCOL READS:* (page 2)

*STUDY DIRECTOR:* Peter J. Thomford and Paul Lieder

*TESTING FACILITY:* Covance Laboratories

*SPONSOR:* APME AdHoc APFO Toxicology Working Group and 3M Toxicology Services - Medical Department

*SPONSOR REPRESENTATIVE:* David Farrar and John Butenhoff

*AMEND TO READ:*

*STUDY DIRECTOR:* Paul Lieder

*TESTING FACILITY:* 3M Toxicology Services - Medical Department

*SPONSOR:* APME AdHoc APFO Toxicology Working Group (including 3M Toxicology Services - Medical Department)

*SPONSOR REPRESENTATIVE:* David Farrar (in-life) and John Butenhoff (analytical)

*REASON:*

To reassign the testing facility, in order to abide by the GLP requirement for one study director. To clarify the responsibilities of all parties included in this study.

*Protocol TOX-026  
Amendment 4*

4. **PROTOCOL READS:**

**PRINCIPAL ANALYTICAL INVESTIGATOR:** Kristen Hansen, Ph.D. (Amendment No 2 to TOX 026, Section 1.)

**AMEND TO READ:**

Add: **PRINCIPAL ANALYTICAL INVESTIGATORS:** Kristen Hansen, Ph.D. and Enaksha Wickremesinhe, Ph.D.

**REASON:**

To specify the PAI at Centre.

5. **PROTOCOL READS:**

**ANALYTICAL TERMINATION DATE:** May 12, 1999 (under Proposed Study Timetable, page 2)

**AMEND TO READ:**

**ANALYTICAL TERMINATION DATE:** September 30, 2000

**REASON:**

To allow for the analyses of all samples.

6. **PROTOCOL READS:**

**LOCATION OF RAW DATA, RECORDS, AND FINAL REPORT** (Section 16., page 7)

**AMEND TO READ:**

Add: After issuing their final report, Centre will forward all original study-specific raw data to 3M Environmental Laboratories, together with copies of appropriate facility-specific raw data applicable to this study. Centre will maintain a copy of the applicable study-specific raw data, protocol and analytical report in the Centre archives.

**REASON:**

To specify the archival requirement for the portion of the data developed by Centre.

7. **PROTOCOL READS:**

**SAMPLE RETENTION** (Section 17., page 8)

**AMEND TO READ:**

After the analytical report on feces is signed by the PAI, all feces specimens of this study will be returned to 3M Environmental Laboratory. These specimens may then be discarded by written direction of the study director. Specimens of blood, plasma, red blood cells, serum, bile, and urine may also be discarded by written direction of the study director after

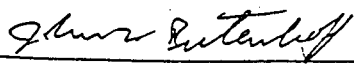
*Protocol TOX-026  
Amendment 4*

the analytical report for the 3M Environmental Laboratory analyses is signed by the study director.

**REASON:** To specify the handling of the above biological specimens, and to define when the quality assurance verification is considered complete.

Protocol TOX-026  
Amendment 4

Amendment Approval

 19 SEPT 2000  
\_\_\_\_\_  
John Butenhoff, Ph.D. Date  
Sponsor Representative, Analytical Phase

 19 SEPT 2000  
\_\_\_\_\_  
Paul Lieder, Ph.D., DABT Date  
3M Study Director

 9/26/00  
\_\_\_\_\_  
Enaksha Wickremesinhe, Ph.D. Date  
Centre Analytical Laboratories, Principal Analytical Investigator

**Study Title**  
26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO)  
in Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 5**

**Amendment Date:**  
October 12, 2000

**Performing Laboratory**  
3M Environmental Technology & Safety Services  
3M Environmental Laboratory  
935 Bush Avenue  
St. Paul, MN 55106

**Laboratory Project Identification**  
FACT-TOX-026  
ET&SS LRN U2782  
Covance 6329-231  
3M Medical Department Study: T-6889.3

Exact Copy of Original  
  iAC     11/09/00    
Initial      Date

3M Environmental Laboratory

Protocol FACT TOX-026  
Amendment No. 5

This amendment modifies the following portion(s) of the protocol:

1. **PROTOCOL READS:**

Principal Analytical Investigators: Kristen Hansen, Ph.D. and Enaksha Wickremesinhe, Ph.D.

2. **AMEND TO READ:**

Principal Analytical Investigators: Kristen Hansen, Ph.D. and Emily Stauffer.

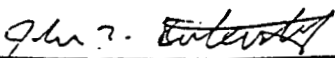
**REASON:**

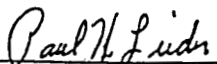
To change role of the Principal Analytical Investigator at Centre Analytical Laboratories.

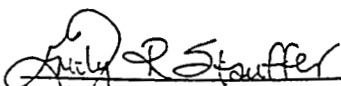
3M Environmental Laboratory

Protocol FACT TOX-026  
Amendment No. 5

### Amendment Approval

 10/12/00  
\_\_\_\_\_  
John L. Butenhoff, Ph.D. Date  
Sponsor Representative, Analytical Phase

 10/23/00  
\_\_\_\_\_  
Paul Lieder, Ph.D., DABT Date  
3M Study Director

 11/2/00  
\_\_\_\_\_  
Emily Stauffer Date  
Centre Analytical Laboratories, Principal Analytical Investigator

3M Environmental Laboratory



***Study Title***

26-Week Capsule Toxicity Study with Ammonium Perfluorooctanoate (APFO/POAA) in  
Cynomolgus Monkeys

**PROTOCOL AMENDMENT NO. 2**

***Amendment Date:***

January 11, 2001

***Performing Laboratories***

Covance Laboratories Inc.  
3301 Kinsman Boulevard  
Madison, WI 53704

3M Environmental Technology & Safety Services

3M Environmental Laboratory

935 Bush Avenue

St. Paul, MN 55106

***Laboratory Project Identification***

Covance Study: 6329-231

Proposal No. w6806a

3M Medical Department Study: T-6889.3

ET&SS LRN-U2782

FACT TOX-026

3M Environmental Laboratory

Exact Copy of Original  
IAC 02/12/01  
Initial Date

*Covance Study 6329-231  
Amendment 2*

This amendment modifies the following portion(s) of the protocol:

1. **PROTOCOL READS: 3M ANALYTICAL PHASE PROTOCOL, SECTION 9. SPECIMEN RECEIPT**  
It was expected that there would be 18 feces specimens and 18 urine specimens from the recovery animals collected and shipped for analysis.

**AMEND TO READ:**

There were 300 feces specimens collected and sent to Centre Analytical Laboratories for analysis. There were 300 urine specimens collected and sent to 3M Environmental Laboratory for analysis.

**REASON:** Feces and urine specimens were collected every two weeks during the main part of the study, in addition to the samples pulled from the recovery animals.

2. **PROTOCOL READS: COVANCE PROTOCOL 6329-231, PAGE 2**  
The study location is designated as Covance Laboratories, Inc.

**AMEND TO READ:**

The testing facility is 3M Toxicology Services—Medical Department, 3M Center, Building 220-2E-02, P.O. Box 33220, St. Paul, MN 55133-3220. The test sites are Covance Laboratories, Inc., 3301 Kinsman Boulevard, Madison, WI 53704 for the in-life phase and 3M Environmental Technology & Safety Services, 3M Environmental Laboratory, 935 Bush Avenue, St. Paul, MN 55106 for the analytical phase.

**REASON:** GLPs allow for only one study location per study. The study director and sponsor reside at 3M Toxicology Services.

3. **PROTOCOL READS: 3M ANALYTICAL PHASE PROTOCOL TOX-026 AND THE COVANCE 6329-231:** Originally there were two protocols for the same study.

**AMEND TO READ: AMENDMENT TO THE COVANCE PROTOCOL 6329-231:**

Covance Protocol 6329-231 is the one protocol for this study. Attached to this amendment are the protocol FACT TOX-026 and Amendments 1–5 for the analytical phase of the study. All of the following study, protocol and project identifications pertain to the same study.

Covance Study 6329-231 (In-Life Phase)  
Proposal No. w6806a  
FACT-TOX-026 (Analytical Phase)  
LRN # U2782  
3M Medical Department Study: T-6889.3

**REASON:** GLPs allow for only one protocol for a study. All study, protocol and project identifications are listed to provide a documented link between documents.

3M Environmental Laboratory

Covance Study 6329-231  
Amendment 2

**4. PROTOCOL READS: AMENDED 3M ANALYTICAL PHASE TOX-026 AMENDMENT #4:**

Peter Thomford was removed from position as study director, but his status was not redefined.

**AMEND TO READ:**

Dr. Peter Thomford will take on the position of Principal In-Life Investigator.

**REASON:** GLPs allow only one study director for a study. Dr. Thomford's role needs to be redefined once he was removed as study director.

**5. PROTOCOL READS: 3M ANALYTICAL PHASE TOX-026, SECTION 4.0 AND 3M AMENDMENT #3 TO TOX-026:**

Amendment #3 adds Centre to list of performing laboratories.

**AMEND TO READ: SECTION 4 OF TOX-026, ANALYTICAL PHASE:**

Centre Analytical Labs Quality Assurance Unit will audit the data they provide for the study, at their facility.

**REASON:**

To clarify that 3M QAU will not be responsible for auditing Centre's data, as the protocol currently states.

**6. PROTOCOL READS: 3M ANALYTICAL PHASE PROTOCOL FACT TOX-026, SECTION 7.**

**REFERENCE MATERIAL:**

Protocol states that the reference material used will be lot #377 or #245.

**AMEND TO READ:**

Ammonium perfluorooctanoate, lot #332, is the reference material used at Centre Analytical Labs.

**REASON:**

When protocol was written, it was anticipated that lot #377 or #245 would be used as reference material. Feces analyses performed at Centre Labs were not included in the original protocol but was added in Amendment 3. Centre actually used lot #332 for the reference.

3M Environmental Laboratory

Covance Study 6329-231  
Amendment 2

**Amendment Approval**

John L. Butenhoff  
John L. Butenhoff, Ph.D., Sponsor Representative

09/FEB/01  
Date

Paul H. Lieder  
Paul Lieder, Ph.D., Study Director

06 Feb 01  
Date

3M Environmental Laboratory



**Centre Analytical  
Laboratories, Inc.**

3048 Research Drive, State College, PA 16801.

Phone: (814) 231-8032, Facsimile: (814) 231-1253

|                                                                                                                                         |                                       |
|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| <b>PROTOCOL DEVIATION</b>                                                                                                               |                                       |
| Deviation Number: 1                                                                                                                     |                                       |
| Date of Occurrence: 10/3/00                                                                                                             |                                       |
| Centre Study Number: 023-011                                                                                                            | Sponsor Protocol Number: FACT-TOX-026 |
| <b>DESCRIPTION OF DEVIATION</b>                                                                                                         |                                       |
| 1. § 12.4 Page 6: Accepted recoveries of 66% for PFOSAA, 54% for M570, and 62% for M556 for Centre sample 0008435 Spk B in Set 100200A. |                                       |
| <b>ACTIONS TAKEN</b>                                                                                                                    |                                       |
| i.e., amendment issued, SOP revision, etc.,                                                                                             |                                       |
| 1. Protocol deviation issued                                                                                                            |                                       |
| Recorded By/Date: <u>Emily R. Stauffer 10/5/00</u>                                                                                      |                                       |
| <b>IMPACT ON THE STUDY</b>                                                                                                              |                                       |
| 1. No negative impact on the study.                                                                                                     |                                       |
| <br>                                                                                                                                    |                                       |
| <u>Emily R. Stauffer</u><br>Principal Investigator Signature                                                                            | <u>12/21/00</u><br>Date               |
| <u>John Z. Buterbaugh</u><br>Sponsor Representative Signature                                                                           | <u>10 JAN 2001</u><br>Date            |
| <u>Paul W. Lich</u><br>STUDY DIRECTOR                                                                                                   | <u>20 March 2001</u><br>DATE          |
| CAL QAU Review                                                                                                                          | <u>NCL 12/18/00</u>                   |

February 12, 1998/2



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### PROTOCOL DEVIATION

Deviation Number: 2

Date of Occurrence: (1) 10/07/00, (2) 10/10/00, (3) 10/10-11/00, (4) 10/10-11/00

Centre Study Number: 023-011

Sponsor Protocol Number: FACT-TOX-026

#### DESCRIPTION OF DEVIATION

1. § 12.4 Page 6: Accepted recovery of 137% for POAA for Centre sample 0008461 Spk B in Set 100600AD.
2. § 12.4 Page 6: Accepted recovery of 139% for POAA for Centre sample 0008482 Spk B in Set 100900A.
3. § 12.4 Page 6: Accepted recovery of 133% for POAA for Centre sample 0008500 Spk B in Set 101000A.
4. § 12.4 Page 6: Accepted  $r^2$  value of 0.970444 for EtFOSE-OH calibration curve in Set 101000A.

#### ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc.,

1-4. Protocol deviation issued.

Recorded By/Date:

Emily R. Stauffer 12/18/00

#### IMPACT ON THE STUDY

- 1-3. No negative impact on the study.
4. No negative impact on the study because still met method requirements for linearity.

Emily R. Stauffer  
Principal Investigator Signature

12/21/00  
Date

John J. Buterbaugh  
Sponsor Representative Signature

10 JAN 2001  
Date

Paul M. Luch  
STUDY DIRECTOR

CAL QAU Review

20 March 2001  
DATE  
NCL 12/18/00

February 12, 1998/2



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Laboratories, Inc.**

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### PROTOCOL DEVIATION

Deviation Number: 3

Date of Occurrence: (1) 10/18-19/00, (2) 10/19-20/00, (3) 10/20-21/00, (4) 10/22-23/00, (5) 10/23/00  
(6) 10/25/00

Centre Study Number: 023-011

Sponsor Protocol Number: FACT-TOX-026

### DESCRIPTION OF DEVIATION

1. § 12.4 Page 6: Accepted recovery of 136% for PFOSEA for Centre sample 0008585 Spk A in Set 101600AR.
2. § 12.4 Page 6: Accepted recovery of 141% for POAA for Centre sample 0008626 Spk B in Set 101800A.
3. § 12.4 Page 6: Accepted recovery of 138% for EtFOSE-OH for Centre sample 0008650 Spk A and 63% for PFOSAA and 57% for PFOSEA for Centre sample 0008651 Spk B in Set 101900A.
4. § 12.4 Page 6: Accepted recovery of 57% for PFOS for Centre sample 0008667 Spk B in Set 102000A.
5. § 12.4 Page 6: Accepted recovery of 170% for POAA for Centre sample 0008667 Spk B in Set 102000AD.
6. § 12.4 Page 6: Accepted recovery of 56% for POAA for Centre sample 0008689 Spk A in Set 102300AR.

### ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc.,

1-6. Protocol deviation issued.

Recorded By/Date:

Emily R Stauffer 10/27/00

### IMPACT ON THE STUDY

1-6. No negative impact on the study.

Principal Investigator Signature

John R. Butenloff

Sponsor Representative Signature

Paul H. Luch  
STUDY DIRECTOR

(CET 3/15/01)

Date

12/21/00  
10 JAN 2001

Date

20 March 2001

Date

12/18/00

CAL QAU Review

12

February 12, 1998/2



**Centre Analytical  
Laboratories, Inc.**

3048 Research Drive, State College, PA 16801.

Phone: (814) 231-8032, Facsimile: (814) 231-1253

### PROTOCOL DEVIATION

Deviation Number: 4

Date of Occurrence: (1 & 2) Entire Study

Centre Study Number: 023-011

Sponsor Protocol Number: FACT-TOX-026

#### DESCRIPTION OF DEVIATION

1. § 1 Amendment No. 3: Throughout the study, samples were analyzed for EtFOSE-OH, PFOSAA, M570, M556, PFOSEA, PFOS, PFOSA, and POAA, instead of only for POAA.
2. § 7 Reference Material Page 4: The lot of POAA used for this study was 332.

#### ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc.,

- 1-2. Protocol deviation issued.

Recorded By/Date:

Emily R Stauffer 12/19/00

#### IMPACT ON THE STUDY

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

Emily R Stauffer  
Principal Investigator Signature

12/21/00  
Date

John T. Benteleff  
Sponsor Representative Signature

10 JAN 2001  
Date

Paul M. Fied 20 Nov 2001  
STUDY DIRECTOR DATE

CAL QAU Review

NCL 12/20/00

February 12, 1998/2

# **APPENDIX B**

## **Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Method #00M-023-003, revision 2 and Deviations and Modifications**

TITLE

Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS  
(Revision 2)

AUTHORS

Enaksha Wickremesinha, Shaozhi Zheng, and John Flaherty

DATE ISSUED

June 02, 2000

SPONSOR

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

PERFORMING LABORATORY

Centre Analytical Laboratories, Inc. (Centre)  
3048 Research Drive  
State College, PA 16801

CENTRE STUDY NUMBER

023-003

CENTRE METHOD NUMBER

00M-023-003, revision 2

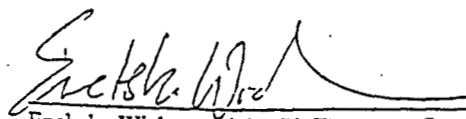
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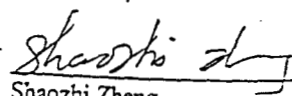
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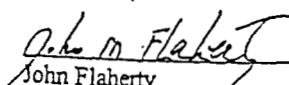
Centre Method No.: 00M-023-003, revision 2

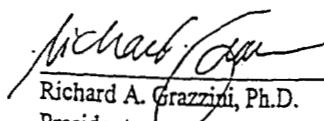
### MANAGEMENT APPROVAL

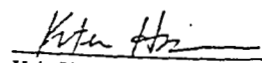
The work cited here was performed in conformance with applicable standard operating procedures and general GLP's.

 6-02-00  
Enaksha Wickremesinha, Ph.D. Date  
Principal Investigator  
Centre Analytical Laboratories, Inc

 6-02-00  
Shaozhi Zheng Date  
Principal Investigator  
Centre Analytical Laboratories, Inc.

 6/8/00  
John Flaherty Date  
Laboratory Manager  
Centre Analytical Laboratories, Inc.

 2-JUNE-00  
Richard A. Grazzini, Ph.D. Date  
President  
Centre Analytical Laboratories, Inc. (WD) 2-JUNE-00

 06/06/00  
Kris Hansen, Ph.D. Date  
Group Leader  
3M Environmental Laboratory

Centre Method No. : 00M-023-003, revision 2

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Centre Method No.: 00M-023-003, revision 2

## 1. SUMMARY

This document details a method of analysis for residues of perfluorooctane sulfonate (PFOS), perfluorooctane sulfonylamide (PFOSA), perfluorooctane sulfonylamidoethyl-acetate (PFOSAA), perfluorooctanesulfonylethylamide (PFOSEA), perfluorooctanoate (POAA), 2(N-ethylperfluorooctanesulfonamido)-ethyl alcohol (Et-FOSE-OH), M570, and M556 in monkey feces. The chemical formulas of the analytes are given in Section 2 of this method.

This method is being re-issued as method 00M-023-003 revision 2. It is being revised to add the rat feces as a matrix, to document the use of 20-mL vials instead of 15 mL tubes for sample extraction, to add the option of homogenizing feces samples before weighing, to clarify the preparation of QC recovery samples and to correct some typographical errors in method 00M-023-003 revision 1. Rat feces is being added to the method, however, it was validated for the analysis of PFOS only. Therefore, this method should be used for the analysis of PFOS only, in rat feces.

Residues of these fluorochemicals are extracted from feces with acetonitrile (ACN). The ACN extract is filtered and passed through a carbon solid-phase extraction (SPE) column. Approximately 3-4 drops of 1-octanol are added to the extract and evaporated to near dryness using rotary evaporation. Each extract is then reconstituted with methanol. Quantification of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM).

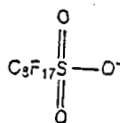
The proposed limit of quantitation (LOQ) for this method is 10 ppb each for PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA. This is based on studies conducted during the development of this method using control monkey feces.

## 2. FLUORO-CHEMICAL STANDARDS

Molecular structures of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA are given below.

### PFOS

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



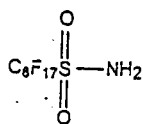
Chemical Name = Perfluorooctane sulfonate

Note: The neutral molecule and standard form that the PFOS (anion) is derived from is potassium perfluorooctane sulfonate [ $C_8F_{17}SO_3K$ ], molecular weight 538.

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**PFOSA**

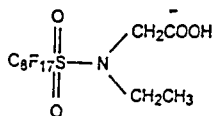
Molecular weight: 499



Chemical Name = Perfluorooctane sulfonylamide

**PFOSAA**

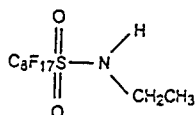
Molecular weight: 585



Chemical Name = perfluorooctane sulfonylamidoethylacetate

**PFOSEA**

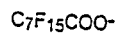
Molecular weight: 527



Chemical Name = Perfluorooctanesulfonylethylamide

**POAA**

Molecular weight: 413

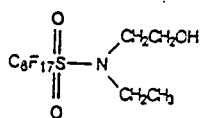


Chemical Name = Perfluorooctanoate

Note: The neutral molecule and standard form that the POAA (anion) is derived from is ammonium perfluorooctanoate [ $\text{C}_7\text{F}_{15}\text{COONH}_4$ ], molecular weight 431.

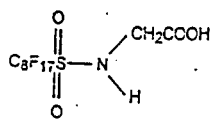
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Et-FOSE-OH  
Molecular weight: 571



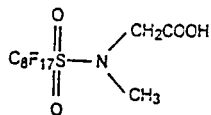
Chemical Name = 2-(N-ethylperfluorooctanesulfonamido)-ethyl alcohol

M 556  
Molecular weight: 557



Chemical Name = M556

M 570  
Molecular weight: 571



Chemical Name = M570

THPFOS  
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, C<sub>8</sub>F<sub>13</sub>SO<sub>3</sub>H

Chemical Name = 4-H, perfluorooctane sulfonic acid

Centre Method No. : 00M-023-003, revision 2

### 3. CHEMICALS AND SUPPLIES

#### 3.1. CHEMICALS

| Chemical              | Grade   | Source            | Catalog No. |
|-----------------------|---------|-------------------|-------------|
| Acetonitrile (ACN)    | HPLC    | (VWR) J. T. Baker | JT9017-3    |
| Carbon 120/400 mesh   | -       | Supelco           | 57210-U     |
| Methanol (MeOH)       | HPLC    | (VWR) J. T. Baker | JT9093-2    |
| 1-Octanol             | Reagent | Aldrich Chemical  | 111-87-5    |
| Ammonium Acetate      | Reagent | Sigma-Aldrich     | A-7330      |
| L-Ascorbic acid       | Reagent | Sigma-Aldrich     | L-5960      |
| Toluene               | Reagent | (VWR) J. T. Baker | JT9460-3    |
| Dimetyldichlorosilane | Reagent | Supelco           | 3-3009      |
| Water                 | Type I  | in house          | -           |

(Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25°C, from a Labconco waterpro workstation)

#### 3.2. FLUOROCHEMICAL STANDARDS

| Standard   | Source |
|------------|--------|
| PFOS       | 3M     |
| PFOSA      | 3M     |
| PFOSAA     | 3M     |
| PFOSEA     | 3M     |
| Et-FOSE-OH | 3M     |
| M556       | 3M     |
| M570       | 3M     |
| POAA       | 3M     |
| THPFOS     | 3M     |

#### 3.3. EQUIPMENT AND SUPPLIES

| Equipment                                       | Source  |
|-------------------------------------------------|---------|
| Balance, analytical, (display at least 0.0001g) | Mettler |
| Balance, top loading, (display at least 0.01 g) | Mettler |
| Rotary evaporator                               | Buchi   |
| Rotary evaporator trap                          | Buchi   |
| Pear-shaped flasks (125 mL)                     | Pyrex   |
| 20-mL SPE tubes (cat. no. N057177) with frits   | Supelco |
| Vacuum pump                                     | Buchi   |
| Visiprep vacuum manifold                        | Supelco |

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|                                                                                         |                               |
|-----------------------------------------------------------------------------------------|-------------------------------|
| Wrist-action shaker                                                                     | Burrell                       |
| 20-mL polyethylene scintillation vials with lids                                        | Wheaton (VWR)                 |
| 20-mL syringe                                                                           | VWR                           |
| 25-mm diameter glass fibre acrodisc (cat # 4523)                                        | Gelman                        |
| Disposable pipets, test tubes etc.                                                      | VWR                           |
| Disposable micropipets, 100 - 200 $\mu$ L.                                              | VWR                           |
| 125-mL LDPE narrow-mouth bottles                                                        | Nalgene                       |
| 2-mL clear HPLC vial kit (cat # 5181-3400)                                              | HP                            |
| Standard lab equipment (class A pipets and volumetric flask, graduated cylinders, etc.) | various                       |
| LC/MS/MS and HPLC systems                                                               | As described in Section 4.5.1 |

Notes:

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

### 3.4. SOLUTIONS

1. 50 mM Ammonium Acetate: Dissolve 3.85 g of ammonium acetate in 1 L of type I water.
2. 2 mM Ammonium Acetate: Dilute 40 mL of the 100 mM ammonium acetate solution in a liter of type I water, for mobile phase A.
3. Saturated Ascorbic Acid in Methanol: Dissolve ~ 10 g ascorbic acid in 100 mL methanol.
4. The 2% Ascorbic Acid in Methanol: Dissolve 2 g ascorbic acid in 100 mL methanol.

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

Centre Method No.: 00M-023-003, revision 2

### 3.5. PREPARATION OF STOCK, FORTIFICATION, AND CALIBRATION SOLUTIONS

Analytical standards are used for two purposes: (1) to fortify control matrix samples designated to be extracted and processed as calibration standards that will be then used to calibrate the response of the detector used in the analysis; and (2) to fortify other control matrix samples to determine analytical recovery.

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

#### 3.5.1. Stock solutions

To prepare stock solutions of 100 µg/mL each PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, POAA, and the surrogate standard THPFOS, weigh out 10 mg of analytical standard (corrected for purity and percent salt, if necessary) and dilute to 100 mL with methanol in a 100-mL volumetric flask. Prepare a separate solution for each analyte. These 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 6 months from the date of preparation.

#### 3.5.2. Fortification Solutions

- a. 10 µg/mL Mixed Fortification Solution – Pipet 10.0 mL each of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA stock solution to a 100 mL volumetric flask. Bring up to volume with methanol.
- b. 2.5 µg/mL Mixed Fortification Solution – Pipet 25.0 mL of the 10 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.
- c. 0.5 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 2.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.
- d. 0.1 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 0.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

Follow Steps a and b above to make a 2.5 µg/mL solution of the surrogate standard (THPFOS) from the stock solution.

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

### 3.5.3. Calibration Standards

Prepare LC/MS/MS calibration standards by fortifying weighed out aliquots of the same control feces sample (or a combined "bulk" control feces sample) before they are processed through the extraction procedure.

The following is a typical example; additional concentrations may be prepared as needed. It is recommended to prepare the fortification solutions so that the volume of fortification is between 50 and 200 µL and to use disposable micropipets for aliquoting fortification volumes.

| Conc. of<br>Mixed<br>Fortification<br>Solution<br>(µg/ml) | Fortification<br>Volume (µL) | Weight of<br>Control<br>Sample (g) | Conc. of<br>Extracted<br>Calibration<br>Standard<br>(ppb) | Vol. of<br>Surrogate<br>Standard*<br>added (µL) |
|-----------------------------------------------------------|------------------------------|------------------------------------|-----------------------------------------------------------|-------------------------------------------------|
| NA                                                        | NA                           | 1.0                                | NA                                                        | 200                                             |
| 0.1                                                       | 100                          | 1.0                                | 10                                                        | 200                                             |
| 0.1                                                       | 200                          | 1.0                                | 20                                                        | 200                                             |
| 0.5                                                       | 100                          | 1.0                                | 50                                                        | 200                                             |
| 0.5                                                       | 200                          | 1.0                                | 100                                                       | 200                                             |
| 2.5                                                       | 100                          | 1.0                                | 250                                                       | 200                                             |
| 2.5                                                       | 200                          | 1.0                                | 500                                                       | 200                                             |
| 10                                                        | 100                          | 1.0                                | 1000                                                      | 200                                             |

\* 2.5 µg/ml THPFOS fortification solution.

Calibration standard preparations may be stored in a refrigerator at 2°C to 6°C, if needed, and used over a period of time as long as its stability has been verified.

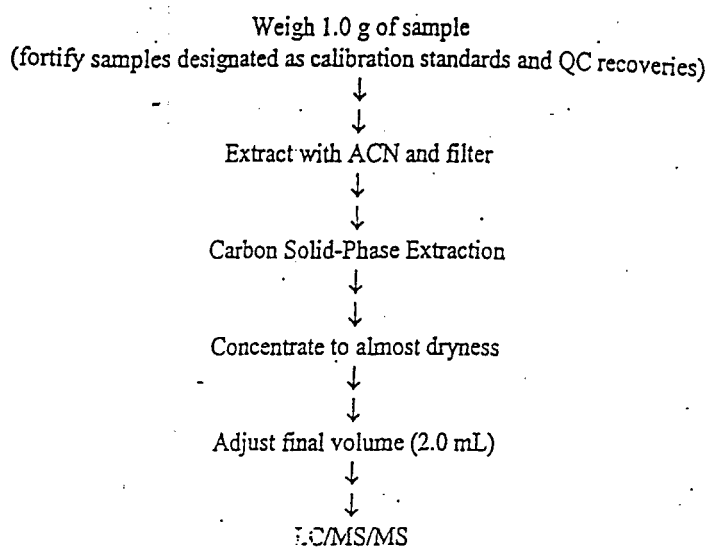
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#### 4. METHOD

##### 4.1. FLOW DIAGRAM

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

##### Method Flow Diagram



##### 4.2. SAMPLE PROCESSING

All samples are received frozen and will be kept frozen (below  $-10^{\circ}\text{C}$ ) until time of extraction. The rat feces do not need any processing, however, the monkey feces may need to be homogenized in order to be able to weigh out the specified amounts.

##### 4.3. SAMPLE PREPARATION

Prepare the following blank and calibration samples (Sections 4.3.1 to 4.3.4) from same control feces sample (or a combined "bulk" control feces sample).

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#### *4.3.1. Matrix Blanks*

Prepare two blank samples without any fortifications, per set of samples analyzed. These will be used as the matrix blank.

#### *4.3.2. Matrix Zero Blanks*

Prepare two matrix blank samples fortified with the surrogate, per set of samples analyzed. These will be used as the matrix zero blanks.

#### *4.3.3. Calibration Standards*

Prepare calibration standards as described in Section 3.5.3.

#### *4.3.4. Continuing Calibration Verification Standards*

Two of the extracted calibration standards, a low-mid and a mid-high level standard, will be used as continuing calibration verification (CCV) standards.

#### *4.3.5. QC Recovery Samples*

Prepare QC recovery samples to represent every control matrix included in the study. Prepare at least one QC recovery sample in the low-range, and one in the high-range, to approximately bracket the expected range of analyte in the samples. For sets containing more than 20 samples, prepare an additional QC recovery sample for every group of 10 additional samples (for example, if one set has 24 samples, then 3 levels of QC recovery samples will be prepared). Prepare these additional QC recovery samples to bracket the range of analyte found in the samples, as appropriate.

Note: An example of a sample extraction worksheet is attached, as Attachment 1.

#### 4.4. EXTRACTION

Note: A. Preparation/conditioning of SPE columns is described in Section 4.4.1.

B. Silanization of pear-shaped flasks is described in Section 4.4.2.

C. Evaluation/standardization of SPE columns (when a different supplier is used etc.) is described in Section 4.4.3.

- a. Weigh approximately 1.0 g ( $\pm 0.05$  g) of sample in to 20 mL polyethylene scintillation vial (Note: designated samples need to be fortified with appropriate aliquots of fortification standards).
- b. Add 10 mL of ACN (note: break-up any clumps using a spatula or glass rod), shake on a wrist-action shaker for 30 minutes.

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- c. Let the vials sit for ~ 5 minutes, carefully decant, directly on to a 20 mL disposable syringe barrel connected to a glass acrodisc (Gelman) filter.
- d. Transfer the filtrate to a conditioned SPE column (see Section 4.4.1). Collect the elute in a 125-mL "silanized" (see Section 4.4.2) pear-shaped flask.

Note: The 20 mL SPE column fits well inside the mouth (sleeve) of the pear-shaped flask. Make sure that the pear-shaped flask is well supported and will not topple. No vacuum is needed for this step.

- e. Add 10 mL of ACN and then 20 mL of 90:10 ACN : 2% ascorbic acid in MeOH to the SPE column and collect the eluate in to the same pear shaped flask, combining the eluates.
- f. Add 3-4 drop of 1-octanol to the pear shaped flask and rotary evaporate to near dryness (a minute amount of 1-octanol will remain) at a reduced pressure of ~ 40°C.
- g. Reconstitute by adding 2.0 mL methanol (final volume = 2 mL) and swirl/mix to dissolve. Transfer the extract to HPLC vials using disposable pipets.

#### 4.4.1 SPE Column Preparation

Pack 20-mL SPE tubes with 2 g carbon. Condition columns with 10 mL of saturated ascorbic acid in MeOH followed by 10 mL ACN. Discard all washes. Do not allow the column to dry.

Note: The SPE columns may be packed and conditioned at any point. Use the vacuum manifold to condition the columns. Do not let the SPE columns to run dry at any time.

#### 4.4.2 Silanization of Pear-Shaped Flasks

Silanize the pear-shaped flasks before use, by rinsing with a 30% dimethyl-dichlorosilane in toluene solution followed by a toluene rinse and finally a methanol rinse.

#### 4.4.3 Standardization of SPE Columns

When using carbon from a different supplier, SPE columns should be standardized in the following manner, prior to analyzing samples:

- a. Using a mixed standard with a concentration between 100 and 500 ng/mL (each of all eight analytes) follow the elution schemes as outlined in steps 2 and 3. Collect all eluting fractions.

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- b. Collect a post-elution fraction, after step 3, eluting with an additional 20 mL of 90:10 (ACN : 2% ascorbic acid in MeOH).
- c. Adjust the final volume of all the fractions to 10 mL with methanol.
- d. Analyze all the fractions by LC/MS/MS.
- e. If the target fraction contains a minimum of 85% of the respective analytes, it may be considered acceptable.
- f. If the "post-elution" fraction contains significant amount of analytes, the target elution volume may be increased.

#### 4.5. ANALYSIS BY LC/MS/MS

##### 4.5.1. LC/MS/MS System and Operating Conditions (Electrospray)

Mass Spec: Micromass Quattro Ultima (Micromass)  
Interface: Electrospray (Micromass)  
Harvard infusion pump  
Computer: COMPAQ Professional Workstation AP200  
Software: Windows NT, MassLynx 3.3  
HPLC: Hewlett Packard (HP) Series 1100  
HP Quat Pump  
HP Vacuum Degasser  
HP Autosampler  
HP Column Oven

Note: A 4 x 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) 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.

HPLC Column: Genesis C<sub>8</sub> (Jones Chromatography), 2.1 mm x 50 mm, 4 $\mu$   
Column Temp.: 35° C  
Injection Vol.: 10  $\mu$ L  
Mobile Phase (A): 2 mM Ammonium Acetate in Type I water  
Mobile Phase (B): Methanol

| Time | % A | % B | Flow Rate (mL/min) |
|------|-----|-----|--------------------|
| 0    | 60  | 40  | 0.300              |
| 0.4  | 60  | 40  | 0.300              |
| 1.0  | 10  | 90  | 0.300              |
| 7.0  | 10  | 90  | 0.300              |
| 7.5  | 0   | 100 | 0.300              |
| 9.0  | 0   | 100 | 0.400              |
| 9.5  | 60  | 40  | 0.400              |
| 13.5 | 60  | 40  | 0.400              |
| 14.0 | 60  | 40  | 0.300              |

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

Ions monitored:

| <u>Analyte</u> | <u>Mode</u> | <u>Parent Ion</u> | <u>Product Ion</u> | <u>Approximate Retention Time (min)</u> |
|----------------|-------------|-------------------|--------------------|-----------------------------------------|
| PFOS           | negative    | 499               | 99                 | 5.3                                     |
| PFOSA          | negative    | 498               | 78                 | 6.0                                     |
| PFOSAA         | negative    | 584               | 526                | 5.7                                     |
| PFOSEA         | negative    | 526               | 169                | 6.7                                     |
| Et-FOSE-OH     | negative    | 630               | 59                 | 6.7                                     |
| POAA           | negative    | 413               | 369                | 5.1                                     |
| M556           | negative    | 556               | 498                | 5.4                                     |
| M570           | negative    | 570               | 419                | 5.6                                     |
| THPFOS         | negative    | 427               | 80                 | 5.1                                     |

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

#### 4.5.2. Example Tune File Parameters

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

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

| <u>Analyte</u> | <u>Dwell (s)</u> | <u>Collision Energy (eV)</u> | <u>Cone (V)</u> |
|----------------|------------------|------------------------------|-----------------|
| PFOS           | 0.2              | 43                           | 76              |
| PFOSA          | 0.2              | 28                           | 34              |
| PFOSAA         | 0.2              | 20                           | 37              |
| PFOSEA         | 0.2              | 28                           | 49              |
| Et-FOSE-OH     | 0.2              | 31                           | 30              |
| POAA           | 0.2              | 11                           | 25              |
| M556           | 0.2              | 28                           | 50              |
| M570           | 0.2              | 20                           | 60              |
| THPFOS         | 0.2              | 35                           | 34              |

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| <u>Source</u>      | <u>Set</u> |
|--------------------|------------|
| Capillary          | 2.56 kV    |
| Hexapole 1         | 0.5 V      |
| Aperture 1         | 0.2 V      |
| Hexapole 2         | 0.8 V      |
| Source Block Temp. | 100°C      |
| Desolvation Temp.  | 400°C      |

| <u>Analyzer</u> | <u>Set</u> |
|-----------------|------------|
| LM Res 1        | 13.0 V     |
| HM Res 1        | 13.0 V     |
| IEnergy 1       | 0.7 V      |
| Entrance        | -2 V       |
| Exit            | 1 V        |
| LM Res 2        | 11.0 V     |
| HM Res 2        | 11.0 V     |
| IEnergy 2       | 1.0 V      |
| Multiplier      | 650 V      |

| <u>Gas Flows</u> | <u>Set</u> |
|------------------|------------|
| Cone Gas         | ~ 150 L/hr |
| Desolvation      | ~ 700 L/hr |

| <u>Pressures</u> | <u>Read back</u> |
|------------------|------------------|
| Gas Cell         | ~ 3.0e-3 mbar    |

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

#### 4.5.3. Calibration Procedures

- Inject an aliquot (10 µL) of each calibration standard into the LC/MS/MS system. Include standards corresponding to six or more concentration levels (ranging from the LOQ to the highest concentration level to be included in the analysis) in an analytical set.
- Use a linear  $y = mx + b$  function 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 the Windows NT, MassLynx 3.3 (or equivalent) software system. Any extracted standards that fall outside the 70 to 130%, based on the average response factor of the surrogate standard, must be excluded from the calibration curve.

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- c. The correlation coefficient (R) for calibration curves generated must be  $\geq 0.98$  ( $R^2 \geq 0.96$ ). If calibration results fall outside these limits, take appropriate steps to adjust instrument operation, and reanalyze the relevant sets of samples.

#### 4.5.4. Sample Analysis

- a. Each set of samples analyzed must include matrix blanks (without surrogate standard), matrix zero blanks (with the surrogate standard), a set of extracted standards, and QC recovery samples.
- b. Inject an aliquot (10  $\mu$ L) of each standard/sample/QC recovery/control into the LC/MS/MS system. Begin a set of samples with an entire set of calibrations. Include two continuing calibration verification (CCV) standards (one from the low-mid and one from the mid-high range) after every 5 to 10 samples, at the most.
- c. Determine the concentration of each sample/QC recovery/control from the standard curve, based on the peak area of each analyte. The standard responses must bracket responses of the residue found in each sample set. Samples that fall outside the range of the standard curve must be diluted appropriately and re-analyzed.
- d. Evaluate the ongoing acceptability of instrumental analysis based on the CCV results. Samples analyzed will be acceptable as long as both CCV's fall within  $\pm 30\%$  of their true value. Calculate the CCV concentration found using the linear calibration curve. If the CCV recovery is outside 70% to 130%, re-analyze samples analyzed after the last acceptable check.
- e. Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The response is calculated using the "average response factor" function using MassLynx 3.3 (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The average response factor for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% to 140% ( $\pm 40\%$ ). Those deviating by greater than 30% (outside 70% to 130% range) must be checked for possible errors and may need to be re-extracted. Any responses deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.
- f. If analysis is delayed, samples must be stored refrigerated at approximately 2°C to 6°C until analysis, and analyzed preferably within a week.

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#### 4.6. PERFORMANCE CRITERIA

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

First Criterion - Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (the 10 ppb extracted standard) and obtain a signal to noise ratio of at least 9:1 for all analytes. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion - Run a set of standards of six or more concentration levels, ranging from the proposed LOQ up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.98 for each analyte.

#### 4.7. TIME REQUIRED FOR ANALYSIS

A set of 14 samples can be taken through the extraction procedure in approximately six hours by one person. The LC/MS/MS analysis (12-14 standards and 14 samples) will take approximately seven hours.

### 5. CALCULATIONS

#### 5.1. ANALYTE FOUND

Calculate the amount of analyte found (in ppb) using the standard curve generated by the Mass Lynx software program using Equation 1. Correction for sample weight (1 g) and final volume (2 mL) is not required since the samples and standards are extracted the same way.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{(\text{peak area} - \text{intercept}) \times \text{DF}}{\text{slope}}$$

Where DF = dilution factor, if samples were diluted.

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### 5.2. QC RECOVERY

For samples fortified with known amounts of analytes prior to extraction, calculate the percent recovery from Equation 2.

Equation 2:

% Recovery =

$$\frac{\text{analyte found (ppb)} - \text{analyte found in matrix blank (ppb)}}{\text{amount analyte added (ppb)}} \times 100$$

### 5.3. MEAN RESPONSE FACTOR

The mean response factor (MRF) is calculated by the Mass Lynx software program by averaging the mean response for all calibration standards (response/amount added) and dividing by the amount added. The actual concentration from each injection is then calculated by dividing the individual response by the MRF. The recovery for each injection is the percent of the actual concentration to the amount added. This calculation is performed by the MassLynx 3.3 (or equivalent) software.

## 6. SAFETY

All samples must be treated as potential biohazards and appropriate universal precautions must be taken (follow standard operating procedures for the handling of biofluids). These include, but are not limited to, the use of double layers of latex or vinyl gloves, goggles, dust mask, and lab coat. All disposable materials must be treated as biohazards and handled accordingly.

Acetonitrile, methanol, dimethyldichlorosilane and toluene are highly flammable. Open and use under fume hood only.

All organic waste and solvent waste must be segregated and disposed of accordingly.

ATTACHMENT 1.



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#### PREPARATION OF SAMPLES FOR EXTRACTION AND ANALYSIS

Matrix: Feces

Analytes: PFOS, PFOSA, POAA, Et-FOSE-OH, PFOSEA, PFOSAA, M 570, M 556, THPFOS

If a sample is not justified, draw a line through the justification section. Vertical arrows in a column indicate identical values.

Initials: Date: / /

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|-------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| Page 1                                                                                                                                                            |                                                                                        |
| <b>METHOD DEVIATION</b>                                                                                                                                           |                                                                                        |
| Deviation Number: 1                                                                                                                                               |                                                                                        |
| Date of Occurrence: 10/04-05/00                                                                                                                                   |                                                                                        |
| Centre Study Number: 023-011                                                                                                                                      | Sponsor Protocol Number: FACT-TOX-026                                                  |
| <b>DESCRIPTION OF DEVIATION</b>                                                                                                                                   |                                                                                        |
| Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LCMS/MS (Revision 2)", Centre method number OOM-023-003, revision 2: |                                                                                        |
| 1. Section 4.5.4.d: CCV recoveries of 60%, 54%, 65%, 66%, 57%, and 60% were accepted for EtFOSE-OH in Set 100200AR.                                               |                                                                                        |
| <b>ACTIONS TAKEN</b><br><u>i.e., amendment issued, SOP revision, etc...</u>                                                                                       |                                                                                        |
| 1. Method deviation issued.                                                                                                                                       |                                                                                        |
| Recorded By/Date: <u>Emily R. Stauffer 10/5/00</u>                                                                                                                |                                                                                        |
| <b>IMPACT ON THE STUDY</b>                                                                                                                                        |                                                                                        |
| 1. No negative impact                                                                                                                                             |                                                                                        |
| <u>Emily R. Stauffer</u><br>Principal Investigator Signature                                                                                                      | <u>12/21/00</u><br>Date                                                                |
| <u>Paul H. Kish 2</u><br>Study Director                                                                                                                           | CAL QAU Review <u>NCL 12/19/00</u><br><u>21 March 2001</u><br>DATE February 12, 1998/2 |



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|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------|
| Page 1                                                                                                                                                             |                                       |
| <b>METHOD DEVIATION</b>                                                                                                                                            |                                       |
| Deviation Number: 2                                                                                                                                                |                                       |
| Date of Occurrence: (1) 10/10/00, (2) 10/10/00, (3) 10/11-12/00                                                                                                    |                                       |
| Centre Study Number: 023-011                                                                                                                                       | Sponsor Protocol Number: FACT-TOX-026 |
| <b>DESCRIPTION OF DEVIATION</b>                                                                                                                                    |                                       |
| Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)", Centre method number 00M-023-003, revision 2: |                                       |
| 1. Section 4.5.4.d: CCV recoveries of 50%, 51%, 59%, 60%, 61%, and 61% were accepted for EtFOSE-OH in Set 100900A.                                                 |                                       |
| 2. Section 4.5.4.e: Surrogate recovery of 46% was accepted for Centre sample 0008495 in Set 100900A.                                                               |                                       |
| 3. Section 4.5.4.e: Surrogate recoveries of 57% and 56% were accepted for Centre samples 0008514 and 0008515, respectively in Set 101000AD.                        |                                       |
| <b>ACTIONS TAKEN</b><br><i>i.e., amendment issued, SOP revision, etc...</i>                                                                                        |                                       |
| 1. Method deviation issued.                                                                                                                                        |                                       |
| Recorded By/Date: <u>Emily P. Stauffer 12/19/00</u>                                                                                                                |                                       |
| <b>IMPACT ON THE STUDY</b>                                                                                                                                         |                                       |
| 1. No negative impact                                                                                                                                              |                                       |
| <u>Emily P. Stauffer</u><br>Principal Investigator Signature                                                                                                       | <u>12/21/00</u><br>Date               |
| <u>Paul H. Leach</u><br>STUDY DIRECTOR                                                                                                                             | <u>21 March 2001</u><br>DATE          |
| CAL QAU Review <u>NEK 12/22/00</u>                                                                                                                                 |                                       |
| February 12, 1998/2                                                                                                                                                |                                       |



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|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|
| Page 1                                                                                                                                                                                                                                |                                               |
| <b>METHOD DEVIATION</b>                                                                                                                                                                                                               |                                               |
| Deviation Number: 3                                                                                                                                                                                                                   |                                               |
| Date of Occurrence: (1) 10/18-19/00, (2) 10/17-18/00, (3) 10/22-23/00, (4) 10/25/00,<br>(5) 10/24-25/00, (6) 10/26/00                                                                                                                 |                                               |
| Centre Study Number: 023-011                                                                                                                                                                                                          | Sponsor Protocol Number: FACT-TOX-026         |
| <b>DESCRIPTION OF DEVIATION</b>                                                                                                                                                                                                       |                                               |
| Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)", Centre method number 00M-023-003, revision 2:                                                                    |                                               |
| 1. Section 4.5.4.e: Surrogate recovery of 53% was accepted for Centre sample 0008595 in Set 101600AR.                                                                                                                                 |                                               |
| 2. Section 4.5.4.d: CCV recovery of 153% was accepted for EtFOSE-OH in Set 101700A.                                                                                                                                                   |                                               |
| 3. Section 4.5.4.d & e: In Set 102000A, a surrogate recovery of 149% was accepted for Centre sample 0008681 and CCV recoveries of 54%, 59%, 54%, 58%, and 55% for EtFOSE-OH were accepted.                                            |                                               |
| 4. Section 4.5.4.d & e: In Set 102300AR, a surrogate recovery of 54% was accepted for Centre sample 0008690 Spk B and one of 51% was accepted for 0008703. CCV recoveries of 54%, 51%, 51%, 53%, and 57% for EtFOSE-OH were accepted. |                                               |
| 5. Section 4.5.4.d: CCV recoveries of 62%, 63%, and 55% were accepted for EtFOSE-OH in Set 102400A.                                                                                                                                   |                                               |
| 6. Section 4.5.4.d: CCV recoveries of 66%, 64%, 43%, and 43% were accepted for EtFOSE-OH in Set 102500A.                                                                                                                              |                                               |
| <b>ACTIONS TAKEN</b><br><u>i.e., amendment issued, SOP revision, etc...</u>                                                                                                                                                           |                                               |
| 1-6. Method deviation issued.                                                                                                                                                                                                         |                                               |
| Recorded By/Date: <u>Emily R. Stauffer 12/19/00</u>                                                                                                                                                                                   |                                               |
| <b>IMPACT ON THE STUDY</b>                                                                                                                                                                                                            |                                               |
| 1-6. No negative impact                                                                                                                                                                                                               |                                               |
| <u>Emily R. Stauffer</u><br>Principal Investigator Signature                                                                                                                                                                          | <u>12/21/00</u><br>Date                       |
| <u>STUDY DIRECTOR</u>                                                                                                                                                                                                                 | <u>DATE</u><br>CAL QAU Review <u>12/22/00</u> |
| February 12, 1998/2                                                                                                                                                                                                                   |                                               |



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|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|-------------------------------------------|
| <b>METHOD DEVIATION</b>                                                                                                                                                |  | Page 1                                    |
| Deviation Number: 4                                                                                                                                                    |  |                                           |
| Date of Occurrence: (1) 8/15/00 and 9/26/00, (2) Entire study                                                                                                          |  |                                           |
| Centre Study Number: 023-011                                                                                                                                           |  | Sponsor Protocol Number: FACT-TOX-026     |
| <b>DESCRIPTION OF DEVIATION</b>                                                                                                                                        |  |                                           |
| Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)", Centre method number 00M-023-003, revision 2:     |  |                                           |
| 1. Section 3.5.1: Did not correct stock standard solutions for % purity.<br>2. Section 4.3.5: Only prepared 2 QC samples for sets that contained more than 20 samples. |  |                                           |
| <b>ACTIONS TAKEN</b><br><u>i.e., amendment issued, SOP revision, etc...</u>                                                                                            |  |                                           |
| 1-2. Method deviation issued                                                                                                                                           |  |                                           |
| Recorded By/Date: <u>Emily R. Stauffer</u> <u>12/8/00</u>                                                                                                              |  |                                           |
| <b>IMPACT ON THE STUDY</b>                                                                                                                                             |  |                                           |
| 1-2. No negative impact                                                                                                                                                |  |                                           |
| Principal Investigator Signature <u>Emily R. Stauffer</u>                                                                                                              |  | Date <u>12/21/00</u>                      |
| STUDY DIRECTOR <u>Paul H. Leach</u> <u>5/14/01</u>                                                                                                                     |  | CAL QAU Review <u>NCL</u> <u>12/15/00</u> |
| DATE                                                                                                                                                                   |  | February 12, 1998/2                       |



## Centre Analytical Laboratories, Inc.

3048 Research Drive, State College, PA 16801.

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Page 1 of 2

### METHOD MODIFICATION FORM

Modification No.: 1  
Study Director: Paul Lieder

Centre Study Number: 023-011

Sponsor Protocol Number: FACT-TOX-026

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS  
(Revision 2) Centre Method Number: 00M-023-003, revision 2

#### MODIFICATION:

1. Section 3.4 Solutions The 100 mM ammonium acetate solution should be 50 mM ammonium acetate.
2. Section 4.5.4 Sample Analysis (Item c.)

Should read as follows:

Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The concentration is calculated using the "average response factor" function from MassLynx 3.3 software (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The percent recovery for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% and 140%. Those deviating by greater than 30% must be checked for possible errors and may need re-extracted. Any recoveries deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.

3. Section 5.1 Analyte Found: Remove second sentence.

4. Section 5.1 Equations

Modify Equation 1 to:

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

Change Equation 2 to:

$$\text{Analyte found (ppb)} = \frac{(\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF})}{\text{sample wt. (g)}}$$

Add Equation 3 (where DF = dilution factor of spk, FV = final volume):

$$\text{Recovery (\%)} = \frac{((\text{anal. found (ng/mL)} - (\text{anal. found in corresponding sample (ng/mL)/DF}) \times \text{FV (mL)} \times \text{DF}) \times 100}{\text{amount added (ng)}}$$

5. Section 5.3 Mean Response Factor: Modify first sentence to read:

The mean response factor (MRF) is calculated by the MassLynx software program by averaging the response of the surrogate standard for all calibration standards and dividing by the amount added.



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Page 2 of 2

METHOD MODIFICATION FORM

Modification No.: 1

Study Director: Paul Lieder

Centre Study Number: 023-011

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Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS  
(Revision 2) Centre Method Number: 00M-023-003, revision 2

MODIFICATION (continued):

6. Section 4.1 Flow Diagram: The statement under Weigh 1.0 g of sample should read:  
(fortify designated samples with appropriate fortification solutions and surrogate standard)
7. Section 4.4.a Extraction: The Note should read as follows:  
(Note: designated samples need to be fortified with appropriate aliquots of fortification standards and all samples except matrix blanks need to be fortified with the surrogate standard).

JUSTIFICATION:

1. To correct for a typographical error.
2. To clarify SS acceptable criteria.
3. Remove incorrect statement because calculations did include sample weight and final volume.
4. Modify calculations to correspond with those used in study.
5. Clarify calculation of mean response factor
- 6.-7. Clarify that all samples except the matrix blanks need to be fortified with the surrogate standard.

EFFECT ON STUDY:

No negative impact.

Originator: Emily R Stauffer 12/19/00  
Date

Principal Investigator: Emily R Stauffer 12/21/00  
Date

Sponsor Management: John C. Fichtenhelf 10 JAN 2001  
Date

Paul M Lieder  
STUDY DIRECTOR

21 March 2001 February 12, 1998/3  
DATE

3M Medical Department Study: T-6889.3

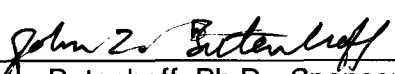
Analytical Report: FACT TOX-026  
LRN-U2782

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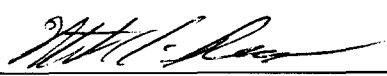
## Appendix I: Report Signature Page

  
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Paul Lieder, Ph.D., DABT, *Study Director*

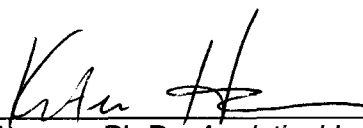
*June 11, 2001*  
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Date

  
\_\_\_\_\_  
John L. Butenhoff, Ph.D., *Sponsor Representative*

*June 6, 2001*  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
William Reagen, Ph.D., *Laboratory Manager*

*06/04/01*  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Kris J. Hansen, Ph.D., *Analytical Investigator*

*05/24/01*  
\_\_\_\_\_  
Date

3M Medical Department Study: T-6889.3

Analytical Report: FACT TOX-026  
LRN-U2782

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## Appendix J: Amendment 1 to FACT TOX-026 Final Report

### TOX-026 Final Study Report Amendment 1

Study number: TOX-026

Study title: 26-Week Capsule Study with Ammonium Perfluorooctanoate (APFO/POAA) in Cynomolgus Monkeys

Study Director: Paul Lieder, Ph.D.

Amendment date: June 15, 2001

Amendment number: 1

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This amendment modifies the following portion of the final report:

- GLP Study—Quality Assurance Statement, page 4: The dates 5/3/01, 5/8/01 and 5/9/01 need to be added to the draft report inspection dates.
- Statement of Data Quality, page 16: The sentence, “The average fortified sample recovery for sera was 93% with a standard deviation of 11%” should read, “The average fortified sample recovery for sera was 94% with a standard deviation of 11%.” A draft version of page 16 with the 93% value was inadvertently inserted into the final report and still included the draft watermark. The only revision without the draft watermark is the final version, which lists the recovery value correctly as 94%. The correct page 16 was inserted into the report.
- Location of Archives, page 8: The statement, “Specimens analyzed at Centre Analytical Laboratories will be returned to 3M Environmental Laboratory upon completion of analysis and submission of the subcontract final report” should be deleted. In its place should be the following sentence, “Feces specimens will be returned from Centre Analytical Laboratories for archiving at the 3M Environmental Laboratory and will be maintained at 3M Environmental Laboratory according to GLP requirements.”

Other changes to the TOX-013 report include:

- The cover page was updated to reflect the total number of pages and the title was changed to say “Amended Analytical Report Title.”
- The Table of Contents was updated to reflect the added amendment.

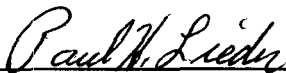
3M Medical Department Study: T-6889.3

Analytical Report: FACT TOX-026

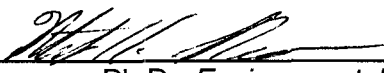
LRN-U2782

Approved by:

  
\_\_\_\_\_  
Kristen H. Hansen, Ph.D., *Principal Analytical Investigator* 06/19/01  
Date

  
\_\_\_\_\_  
Paul Lieder, Ph.D., *Study Director* 6/21/01  
Date

  
\_\_\_\_\_  
John Butenhoff, Ph.D., *Sponsor's Representative* 6/19/01  
Date

  
\_\_\_\_\_  
Bill Reagan, Ph.D., *Environmental Laboratory Manager* 06/19/01  
Date