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

3M Environmental Laboratory
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

Feces Analyses

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

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

This page has been reserved for specific country requirements.

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 H. Lieder — June 11, 2001
Paul Lieder, Ph.D., *Study Director* — Date

Kris J. Hansen — 05/24/01
Kris J. Hansen, Ph.D., *Principal Analytical Investigator* — Date

William Reagen — 06/04/01
William Reagen, Ph.D., *Laboratory Manager* — Date

John L. Butenhoff — June 6, 2001
John L. Butenhoff, Ph.D., *Sponsor Representative* — Date

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 10/2/00–10/6/00 10/9/00, 10/30/00	Data	10/9/00, 10/30/00	10/9/00, 10/30/00
12/14/00–12/15/00 12/18/00–12/20/00, 5/3/01, 5/4/01, 5/7/01, 5/8/01, 5/9/01	Draft Report	12/21/00, 5/10/01	12/21/00, 5/10/01

See Appendix H for individual contractor quality assurance statements.

Tanya K Rush 6/18/01

QAU Representative Date

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List of Tables

Study Personnel and Contributors

Study Director

Paul Lieder, Ph.D., DABT
3M Corporate Toxicology
3M Center, Building 220-2E-02
St. Paul, MN, 55144-1000
651-737-2678

Sponsor

3M Toxicology Services
Medical Department
3M Center, Building 220-2E-02
St. Paul, MN, 55133-3220
John L. Butenhoff, Ph.D., *Sponsor Representative*

Analytical Chemistry Laboratories

Liver, Serum and Urine Analyses
3M Environmental Laboratory
Kristen J. Hansen, Ph.D., *Analytical Investigator*

Feces Analyses
Centre Analytical Laboratories, Inc
Emily Stauffer, *Analytical Investigator*

3M Environmental Laboratory Contributing Personnel

David R. Barnidge, Ph.D.*
Lisa A. Clemen
Rhonda S. Dick*
Kelly J. Dorweiler*
Mark E. Ellefson
Sara E. Estes*
Barb A. Gramenz*
Sarah A. Heimdal*
Cari S. Hewitt*
Marlene M. Heying*
Megan C. Holloway*
Harold O. Johnson
Kelly J. Kuehlwein*
Glenn Langenburg*
Sally A. Linda*
Joseph C. Pilon*
Scott R. Post*
Ian A. Smith*
Bob W. Wynne*

*Contract lab professional service employees

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.

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.

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 subtreatment 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 Control	6	6	2
Group 2 Low-dose (3 mg/kg/day)	4	4	0
Group 3 Mid-dose (10 mg/kg/day)	6	6	2
Group 4 High-dose (30/20 mg/kg/day)[a]	6	6	0

[a]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.

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°C ±20°C and -20°C ±10°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°C ±20°C or -20°C ±10°C.

Chemical Characterization of Test Substance

Ammonium Perfluorooctanoate
CAS Number: 3825-26-1

Chemical Formula: $C_7F_{15}CO_2^-NH_4^+$ 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.

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 Perflurooctanesulfonate 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_{18} 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:

Time (minutes)	*%B*
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.

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.

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 ≥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 ±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.

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.

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.

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 TN-A-2052	Rabbit Serum TN-A-2307	Rabbit Liver TN-A-0808	Rabbit Serum TN-A-2391	Rabbit Serum 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 TN-A-0806	Rabbit Liver TN-A-0802	Rabbit Serum 99062-055	Monkey Urine 99062-067	Monkey Urine 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 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) TCR # MISSING	**O** (Ammonium luorooctanoate) TN-A-1479 SE-030	**APFO** (Ammonium Perfluorooctanoate) TN-A-0497 SD022	**APFO** (Ammonium Perfluorooctanoate) TCR-99030-30
Source	3M Specialty Chemical	ecialty 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 SE037	THPFOS 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%.

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

Test Material	Ammonium perfluorooctanoate (APFO/POAA)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor Representative	Paul Lieder, Ph.D., DABT 3M Toxicology Services Building 220-2E-02 651-737-2678
Study Director	Kristen Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Covance Laboratories, Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106
Proposed Study Timetable	
Analytical Start Date	November 12, 1998
Analytical Termination Date	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 °C ± 10 °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 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 °C ± 10 °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 N. Lieder — August 3, 1999

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

Kris J. Hansen — 8/4/99

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 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 W. Lieder — Feb 10, 2000

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

3M

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 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

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

John L. Butenhoff 10/18/00

John L. Butenhoff, Ph.D. Date
Sponsor Representative, Analytical Phase

Paul N. Lieder 10/23/00

Paul Lieder, Ph.D., DABT Date
3M Study Director

Emily R Stauffer 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 (Check one): ☐ SOP ☒ Method ☐ Equipment Procedure ☐ 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, 8/13/99, 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 affect.

kjh 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. 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: Kim A Clemen Date: 11/14/00

IV. Impact on Study / Project

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

kjb 11/17/00

Authorized By *(Study Director / Project Lead)*: Paul V. Lieder 11/20/00 / John D. Butenhoff 4 DEC 00 Date:

Sponsor: John Butenhoff Study Director: Paul Lieder

3M Environmental Laboratory
Form ETS-4-8.0

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 (*Check one*) ☐ SOP ☐ Method ☐ Equipment Procedure ☒ 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 Lisa 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*) Kristen Hu Date 4/29/99

Record of Deviation

I. Identification

Study / Project No. TOX026 Covance 6329-231

Deviation Type (Check one): ☐ SOP ☒ Method ☐ Equipment Procedure ☐ 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: Lisa 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): Kathy Date: 4/29/99

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	Date(s) of occurrence
FACT-M-1.0	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	Date
Lisa A Clemen	11/14/00

IV. Impact on Study / Project

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

kh 11/17/00

Authorized By *(Study Director / Project Lead)*	Date
Paul W. Lieder 11/20/00 / John T. Butenhoff 4 DEC 00	

Sponsor: John Butenhoff Study Director: Paul Lieder

Deviation No. 5

(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: 5/10/99, 5/11/99, 5/17/99, 5/19/99, 5/27/99, 6/16/99, 6/22/99, 6/23/99, 6/24/99, 7/13/99, 7/21/99

II. Description:

Required Procedure/process:

Method FACT-M-2.0 states the initial curve will be plotted using linear regression.

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: Lisa A Clemen

Date: 11/14/00

IV. Impact on Study / Project

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

kgh 11/17/00

Authorized By *(Study Director / Project Lead)*: Paul H. Lieder 11/20/00 / John T. Butenhoff 4 DEC 00

Date

Sponsor: John Butenhoff Study Director: Paul Lieder

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	Date(s) of occurrence
FACT-TDX-026	5/17/99, 5/18/99, 5/19/99, 5/20/99, 5/28/99

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	Date
Lisa A Clemen	11/14/00

IV. Impact on Study / Project

No adverse affects.

kh 11/17/00

Authorized By *(Study Director / Project Lead)*	Date
Paul H. Lieder 11/20/00 / John R. Butenhoff 4 DEC 00	

Sponsor: John Butenhoff Study Director: Paul Lieder

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, 7/21/99

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 Clemen

Date: 11/14/00

IV. Impact on Study / Project

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

kn 11/17/00

Authorized By *(Study Director / Project Lead)*: Paul N. Lieder 11/20/00 / John D. 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 (*Check one*) ☐ SOP ☐ Method ☐ Equipment Procedure ☒ Protocol ☐ Other:

Document Number	Date(s) of occurrence
FACT-TOX-026	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	Date
Lisa A Clemen	11/14/00

IV. Impact on Study / Project

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

kjn 11/17/00

Authorized By *(Study Director / Project Lead)* Date

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

Sponsor: John Butenhoff Study Director: Paul Lieder

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	Date(s) of occurrence
ETS-8-97.0	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	Date
Kisn A Clemen	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)	Date
Kitzen Hz	8/11/99

Deviation No. 10
(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	Date(s) of occurrence
ETS-8-97.0	8/24/99

II. Description:

Required Procedure/process:

The method states - analyze a mid range 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 +/- 30% of the spiked concentration.

Actual Procedure/process:

One continuing calibration verification per 15 samples had a percent recovery within +/- 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	Date
Lisa A. Clemen	11/16/00

IV. Impact on Study / Project

Data will be flagged to denote added uncertainty.

kjh 11/17/00

Authorized By *(Study Director / Project Lead)* — Date

Paul W. Leidy 11/20/00 / Glen T. Butterhoff 4 DEC 00

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Form ETS-4-8.0

Deviation No. 11
(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	Date(s) of occurrence
ETS-8-97.0	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	Date
Lisa A Clemen	(RE) LC 11/14/00 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. kh 11/17/00

Authorized By *(Study Director / Project Lead)*	Date
Paul W. Lieder 11/20/00 / John Z. Butenhoff 4 DEC 00	

Sponsor: John Butenhoff Study Director: Paul Lieder

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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	Date(s) of occurrence
ETS-8-97.0	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	Date
Lisa A. Clemen	11/14/00

IV. Impact on Study / Project

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

kjh 11/17/00

Authorized By *(Study Director / Project Lead)* Date

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

Sponsor: John Butenhoff Study Director: Paul Lieder

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. TOX036

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 LOQ.

* 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 MKU050499-H2O61K-1 is greater than half the LOQ. The area counts for the other nine H_2O blanks is less than half that of the LOQ.

III. Actions Taken:

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

Notified PI.

Recorded By

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 affect on the study. kjh (PAI) 10/02/00

Authorized By *(Study Director / Project Lead)*

John R. Butenhoff 11/7/00 Paul W. Lieder 11/6/00

Sponsor Rep. John Butenhoff Study Director. Paul Lieder

Deviation No. 14

(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 X Method ☐ Equipment Procedure ☐ Protocol ☐ Other:

Document Number(s): ETS-8-5.1, ETS-8-7.0, and ETS-8-97.0

Date(s) of occurrence: Entire study

II. Description:

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.

III. Actions Taken:

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

This deviation was written.

Recorded By: Lisa A Clemen Date: 02/12/01

IV. Impact on Study / Project

The updated calculations accommodate new information and are an improvement. No adverse affect on the study. KJh 02/12/01

Authorized By *(Study Director / Project Lead)*: Paul N. Lieder Date: 19 Feb 01

John T. Butenhoff 19 FEB 01

Sponsor Representative: John Butenhoff Study Director: Paul Lieder

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 16
(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 X Method ☐ Equipment Procedure ☐ Protocol ☐ 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 ± 30% of the spiked concentration.

Actual Procedure/process:

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

III. Actions Taken:

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

This deviation was written.

Recorded By	Date
Lisa A Clemen	01/11/01

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.

KH 01/11/01

Authorized By *(Study Director / Project Lead)*	Date
Paul N. Lieder 19 FEB 01	19 Feb 01

Sponsor Representative: John Butenhoff Study Director: Paul Lieder

Deviation No. 15
(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 X 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 ± 30% of the spiked concentration.

Actual Procedure/process:

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

III. Actions Taken:

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

This deviation was written.

Recorded By: Lisa 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.

KH 05/15/01

Authorized By *(Study Director / Project Lead)*: Paul N. Lieder 5/18/01 Date: John T. Butenhoff May 24, 2001

Study Director: Paul Lieder
Sponsor Representative: John Butenhoff

Deviation No. 17
(assigned by Study Director or Project Lead at the end of study or project)

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 FLUOROCHEMICAL 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:

D T Bn 6/3/99
Laboratory Manager Date

Kler Hz 6/1/99
Group Leader Date

Lisa A Clemen 6/01/99
Technical Reviewer Date

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

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 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 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O\text{-}PO_3H)$

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, (± 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 Sampule 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 µL to 50 µL

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 µm, 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™ 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™ 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_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 (µg/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, $C_8F_{13}SO_3H$ 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 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 MilliQ™ 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 MilliQ™ water, and rinse with another 2.5 ml of MilliQ™ water. Use Wheaton 6 ml plastic sampule 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

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.

Table 1
Approximate Spiking Amounts for Standards and Spikes Using 1.0 ml of Liver

Working Standard (Approx. Conc.)	µL	Approx. final conc. of 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 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).

12.18 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

13.1 Calculations:

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

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

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

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.

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 into the study notebook or include into study 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-1.1 and 2.1-V-1**.

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
1	*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.*	08/01/98

Study#_____ Matrix_____ Box#_____ Analyst/Date	Surrogate Std. approx. ppm actual ppm #W_____	FC Mix Std approx. 0.5 ppm actual ppm #W_____	FC Mix Std approx. 5 ppm actual ppm #W_____	FC Mix Std approx. 50 ppm actual ppm #W_____	Comments
H_2O Blank					
_____ Blank					

Blank	Std #	amount =
Extraction Method/Revision:		**Date & Initials**
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_2CO_3/0.25M $NaHCO_3$ 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		

MS/MSD/___ Cont. Checks: Spiked _____ uL of a _____ ppm std (_____) for a final concentration of _____ ppm. MS/MSD used sample _____. Cont. Checks used same matrix as for std curve.

MDL/LOQ values for Rabbit Liver:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate Concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate Concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate Concentrations to be used for preparing the Standard Calibration Curve
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					
Compound	Matrix	MDL	LOQ	Approximate Linear Range[1]	
				Low Standard	High Standard
PFOS	Rabbit	11.8 ppb	37.4 ppb	38 ppb	1000 ppb
	Bovine	n/d = not determined[2]		60 ppb	1200 ppb
	Rat	24.7 ppb	78.7 ppb	62 ppb	1200 ppb
	Monkey	n/v = not valid[3]		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:

[signature] 6/3/99
Laboratory Manager Date

[signature] 6/1/99
Group Leader Date

[signature] 6/01/99
Technical Reviewer Date

Initial RSD 6/5/01 Date

Exact Copy of Original

1.0 SCOPE AND APPLICATION

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

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

1.3 **Matrices:** Rabbit, rat, bovine, and monkey livers or other livers 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. 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

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 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

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 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 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

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 (± 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 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

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 µL 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 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 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 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

13.1 Calculations:

13.1.1 Calculate matrix spike percent recoveries using the following equation:

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

13.1.2 Calculate percent difference using the following equation:

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

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

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^2 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 ± 30% of the spiked concentration.

14.4 Continuing Calibration Verifications

14.4.1 Continuing calibration verification percent recoveries must be ± 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

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

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression. Section 12.2.2.4 Clarification of solvent ramp. 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 Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. 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 FLUOROCHEMICAL COMPOUNDS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

Method Number: ETS-8-6.0 **Adoption Date:** 07/22/99

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager	Date 7/22/99
Group Leader	Date 7/14/99
Technical Reviewer	Date 07/14/99

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

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.

2.0 SUMMARY OF METHOD

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 µm 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

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

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

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

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

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 sampule 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

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_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 (µg/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

9.1 All samples 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 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

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
Approximate Spiking Amounts for Calibration Standards

Working Standard (Approx. Conc.)	µl	Approx. final conc. of 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 sampule 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 sampule 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 sampule 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 sampule 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 matirx 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.

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

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

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

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

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

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

Study #_____ Matrix_____ Box #_____ Wk/Day_____ Date Spiked/Analyst	Surrogate Std approx. ppm actual ppm #_____	FC Mix Std approx. 0.5 ppm actual ppm #_____	FC Mix Std approx. 5 ppm actual ppm #_____	FC Mix Std approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank Liver Homogenate: Std #	Liver amount = g
Liver Extraction Method :	**Date & Initials**
Spike surrogate and Standard mix, Vortex 15 sec.	
Pipette 1 mL of Liver Solution	
Pipette 1 mL of †0.5 M TBA, pH 10. pH =	Std. #
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. #
Dispense 5ml of Methyl-t-Butyl Ether	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	Evaporator Temperature
Add 1.0 mL of Methanol	TN-A-
Vortex 30 sec.	
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial	

Cont. Cal. Verifications used the same matrix as for the standard curve.

MDL/LOQ values for rabbit liver

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) 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 Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	Std conc ug/mL	All Am't spiked mL	All Density 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 Final conc ng/g	PFOSA Final conc ng/g	PFOSAA Final conc ng/g	EtFOSE Final conc ng/g	PFOSEA Final conc ng/g	M556 Final conc ng/g	Std conc ng/g	Surrogate Std conc ng/mL	All Am't spiked 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			
29.9	29.9	29.9	29.9	29.9	29.9		**Surrogate**	
59.9	59.9	59.9	59.9	59.9	59.9		Final conc	
120	120	120	120	120	120		ng/mL	
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.					

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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:

[signature]	7/22/99
Laboratory Manager	Date
[signature]	7/14/99
Group Leader	Date
[signature]	07/14/99
Technical Reviewer	Date

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

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

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 µL 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 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 uL/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:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

Final Volume (mL)

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 ± 30% of the spiked concentration.

14.5 Continuing Calibration Verification

14.5.1 Continuing calibration verification percent recoveries must be within ± 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

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 Dose	Sample#	Concentration ng/g	Initial Wt. g	Dilution Factor	Final Conc. 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:

[signature]	4/27/99
Laboratory Manager	Date
[signature]	4/26/99
Group Leader	Date
Lisa A Clemen	04/26/99
Technical Reviewer	Date

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

1.0 SCOPE AND APPLICATION

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

1.2 **Applicable compounds:** Fluorochemical surfactants or other fluorinated 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 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 µm 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

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

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

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

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 (± 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 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 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 (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 (µg/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

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

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.

Table 1 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of 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:

$$\frac{\text{mL of standard x concentration of standard (µg /mL)}}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

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

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

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

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

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
1	Section 12.21 Changed to include sample storage at room temperature. Section 12.13 Added the shaker speed. 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

Study #_____ Matrix_____ Box #_____ Wk/Day_____ DateSpiked/Analyst	Surrogate Std approx. ppm actual ppm #_____	FC-Mix approx. 0.5 pm actual ppm #_____	FC-Mix approx. 5 ppm actual ppm #_____	FC-Mix approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

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. pH = _____ Std. #_____	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer Std. #_____	
Dispense 5 mL of methyl-t-butyl ether 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 filter into a 1.5 mL autosample vial	

Cont. Cal. Verifications used same matrix as for std curve.

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 of standards (ppb) (ng/mL)	LCR from curve (ppb) (ng/mL)	% Recovery Range	RSD 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 Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE Std conc ug/mL	PFOSEA Std conc ug/mL	M556 Std conc ug/mL	All Am't spiked mL	All Final vol 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 Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	PFOSEA Final conc ng/mL	M556 Final conc ng/mL	Surrogate Std conc ng/mL	All Am't spiked 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		
24.8	25.1	26.3	24.8	25.2	25.8	**Surrogate**	
49.3	50.0	52.4	49.4	50.1	51.3	Final conc	
97.6	98.9	104	97.8	99.3	102	ng/mL	
248	251	263	248	252	258	500	
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 FLUOROCHEMICAL 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:

[signature] 10/1/98
Laboratory Manager Date

[signature] 9/28/98
Group Leader Date

Lisa A Clemen 9/28/98
Technical Reviewer Date

Excess RSD
Initial
Date 6/5/01

1.0 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

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 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 $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O\text{-}PO_3H)$

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, which 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 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 (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

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

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_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 (µg/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 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

9.1 All samples 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 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

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.

Table 1 Approximate spiking amounts for standards and spikes using 1.0 ml of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of 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

Table 2 Approximate spiking amounts for standards and spikes using 0.5 ml of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of 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 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.

12.18 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:

$$\frac{\text{mL of standard x concentration of standard (µg /mL)}}{\text{mL of standard + mL of surrogate standard + initial matrix volume (mL)}} =$$

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 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

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

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date
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 #	Sample Number set #	FC-Mix approx. 0.5 ppm actual ppm #W_____	FC-Mix approx. 5 ppm actual ppm #W_____	FC-Mix approx. 50 ppm actual ppm #W_____	Date and Initials for Std. or Comments
-	H_2O Blank	-	-	-	
-	_____ Blank	-	-	-	
-					
-					
-					
-					
-					
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-					
[1]		-	-	-	
		-	-	-	
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		-	-	-	
		-	-	-	

[1] 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_2CO_3/0.25M $NaHCO_3$ 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			

MS/MSD/___ Cont. Checks: Spiked _____ uL of a _____ ppm std (_____) for a final concentration of _____ ppm. MS/MSD used sample _____. Cont. Checks used same matrix as for std curve.

Surrogate Standard: Spiked _____ uL of a _____ ppm std (_____) to all samples, standards, and blanks

MDL/LOQ values for Rabbit Serum:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
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:

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate Concentrations to be used for preparing the Standard Calibration Curve
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[2] 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[1]	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 Std conc ug/mL	PFOSA Std conc ug/mL	PFOSAA Std conc ug/mL	EtFOSE-OH Std conc ug/mL	POAA Std conc ug/mL	PFOSEA Std conc ug/mL	Monoester Std conc ug/mL	All Am't spiked mL	All 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 Final conc ng/mL	PFOSA Final conc ng/mL	PFOSAA Final conc ng/mL	EtFOSE Final conc ng/mL	POAA Final conc ng/mL	PFOSEA Final conc ng/mL	Monoester Std conc ng/mL	Surrogate Std conc ng/mL	All 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		
24.8	25.1	26.3	24.8	25.2	25.8	24.8	**Surrogate**	
49.3	50.0	52.4	49.4	50.1	51.3	49.4	Final conc ng/mL	
97.6	98.9	104	97.8	99.3	102	97.8	81.0	
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:

[signature]	4/26/99
Laboratory Manager	Date
[signature]	4/26/99
Group Leader	Date
Lisa A Clemen	04/26/99
Technical Reviewer	Date

Initial Date

Exact Copy of Original

1.0 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

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 µL 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 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:

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

13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\text{(Initial Volume of matrix (mL) + mL of Surrogate Standard)}} \times \frac{1\ \mu g}{1000\ ng}$$
$$\text{Final Volume (mL)}$$

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 ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

14.5.1 Continuing calibration verification percent recoveries must be ± 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

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, 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: ETS-8-5.1 Data summary spreadsheet.

18.0 REFERENCES

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

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

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. 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. Section 12.2.2.4 Clarification of solvent ramp. 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 Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. 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 — Date: 10/1/98

Group Leader — Date: 9/29/98

Technical Reviewer — Date: 9/29/98

Initial Date

Exact Mass

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 (± 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 µL 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 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 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.

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 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:

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

13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/ml):

$$\frac{\text{(ng of PFOS calc. from std. Curve x Dilution Factor)}}{\text{(Initial Volume of matrix (ml) + ml of Surrogate Standard)}} \times \frac{1\ \mu g}{1000\ ng}$$
$$\text{Final Volume (mL)}$$

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 ± 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 ± 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

18.1 FACT-EP-3.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Platform Systems"

19.0 AFFECTED DOCUMENTS

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

Revision Number.	Reason For Revision	Revision Date
1	*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.*	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 Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. 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 FLUOROCHEMICAL 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 RSD Date 8/5/01

Exact Copy of Original

1.0 SCOPE AND APPLICATION

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

1.2 **Applicable compounds:** Fluorochemicals or other fluorinated compounds.

1.3 **Matrices:** Human, rat, and monkey urine or other fluids 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 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 µm 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

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

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

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

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 (± 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 2.5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 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

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_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), 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 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 $C_8F_{13}SO_3H$) 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_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 in the Standard Logbook.

8.11.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (μg/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 100-120 ppm. Record the actual volume transferred in the Standard Logbook.

9.0 SAMPLE HANDLING

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 2.0 ml methanol is used as a solvent blank.

10.1.2 Extract two 2.0 ml aliquots of Milli-Q™ 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

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.

Table 1 Approximate spiking amounts for standards and spikes Using 2.0 ml of matrix			
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix	Approx. final conc. 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

13.1 Calculations

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

$$\frac{\text{ml of standard x concentration of standard (µg /ml)}}{\text{ml of standard + ml of surrogate standard + initial matrix volume (ml)}} =$$

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 **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

Revision Number	Reason For Revision	Revision Date

Extraction Worksheet ETS-8-96.0

Study #_____ Matrix_____ Box #_____ Wk/Day_____ DateSpiked/Analyst	Surrogate Std Approx. ppm Actual ppm #_____	FC-Mix approx. 0.5 ppm actual ppm #_____	FC-Mix approx. 5 ppm actual ppm #_____	FC-Mix approx. 50 ppm actual ppm #_____	Comments
CCV					
MS					
MSD					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
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		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

Blank	Std #	amount =	ml
Urine Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix , spike with appropriate surrogate or FC-Mix	Volume	ml	
Pipette 1 ml of 0.5 M TBA, pH 10. pH = _____		Std. #_____	
Pipette 2 ml of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer		Std. #_____	
Dispense 5 ml of methyl-t-butyl ether		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 filter into an autosample vial			

Cont. Cal. Verifications used same matrix as for std curve.

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 Std conc ug/ml	PFOSA Std conc ug/ml	PFOSAA Std conc ug/ml	EtFOSE Std conc ug/ml	POAA Std conc ug/ml	M556 Std conc ug/ml	M570 Std conc ug/ml	All Am't spiked ml	All Final vol 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 Final conc ng/ml	PFOSA Final conc ng/ml	PFOSAA Final conc ng/ml	EtFOSE Final conc ng/ml	POAA Final conc ng/ml	M556 Final conc ng/ml	M570 Final conc ng/ml	Surrogate Std conc ng/ml	All Am't 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		
6.17	6.18	6.17	6.18	6.15	6.17	6.18	**Surrogate**	
12.5	12.5	12.5	12.5	12.4	12.5	12.5	Final conc	
24.8	24.9	24.8	24.9	24.8	24.8	24.9	ng/ml	
61.7	61.8	61.7	61.8	61.5	61.7	61.8	125	
125	125	125	125	124	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 Final conc ng/ml	PFOSA Final conc ng/ml	PFOSAA Final conc ng/ml	EtFOSE Final conc ng/ml	POAA Final conc ng/ml	M556 Final conc ng/ml	M570 Final conc ng/ml	Surrogate Std conc ng/ml	All Am't 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		
25.0	25.1	25.0	25.1	25.0	25.0	25.1	**Surrogate**	
50.0	50.1	50.0	50.1	49.9	50.0	50.1	Final conc	
100	100	100	100	100	100	100	ng/ml	
250	251	250	251	250	250	251	500	
500	501	500	501	499	500	501		
750	752	750	752	749	750	752		
1000	1002	1000	1002	998	1000	1002		
1500	1503	1500	1503	1497	1500	1503		

Prep Date(s):
Analyst(s):
Sample Matrix:
Method/Revision:
Target Analyte(s):

Study Number:
Equipment Number:
Final Solvent & TN Number:
Matrix Blank/Identifier:
Box:

Sample ID	Initial Wt./Vol. g/mL/L			Comments

Summary of method:

Notes:

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:

D J Bo 7/28/99
Laboratory Manager Date

Kiter H 7/28/99
Group Leader Date

Lisa A. Clemen 7/27/99
Technical Reviewer 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

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

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

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

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 µL 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 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 uL/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:

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

13.1.5 Calculate percent difference using the following equation:

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

13.1.6 Calculate actual concentration of PFOS, or other fluorochemical, in matrix (µg/mL):

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

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^2 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 ± 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

17.1 Attachment A: ETS-8-97.0 Data summary spreadsheet.

18.0 References

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

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

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 Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. 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:	
Inter-assay	<5.4%
Intra-assay	<21%
System Precision	<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
Sera		**µg/mL**	
FACT-M-4.1	10/11/98	0.0137	11/16/98 to 1/27/99 (Day 9, Wk 4, Wk 12, Wk 14)
ETS-8-5.1	4/26/99	0.0137	4/30/99 to 6/23/99 (Wk 6–Wk 10, Wk 16–Wk 34)
ETS-8-5.1	4/26/99	0.0137	7/14/99 to 7/21/99 (Wk 36–40)
Urine		**µg/mL**	
ETS-8-97.0	7/28/99	0.0182	8/19/99 to 9/28/99 (Wk 2–Wk 40)
Liver		**µg/g**	
FACT-M-2.1	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

Serum	
Sample Name	**% Recovery**
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
Average	94
Standard Deviation	11

Table 8. Recovery Summaries of Fortified Samples in FACT TOX-026

Urine	
Sample Name	**% Recovery**
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
Average	88
Standard Deviation	17

Table 9. Recovery Summaries of Fortified Samples in FACT TOX-026

Liver	
Sample Name	**% Recovery**
I05709M-MS-3	74
I05709-MSD-3	62
I05718M-MS-11	113
I05718M-MSD-12	111
Average	90
Standard Deviation	26

Table 10. TOX 026 Data Summary of POAA Concentration—Serum (µg/mL)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Timepoint				
Day 9	0.0613 ± 0.0472 (n=6)	126 ± 36.1 (n=5)	189 ± 48.9 (n=6)	1597 ± 2392 (n=6)
Week 4	0.206 ± 0.105 (n=6)	98.4 ± 42.7[a] (n=3)	172 ± 71.9 (n=5)	1084 ± 1839 (n=6)
Week 6	0.103 ± 0.0113 (n=6)	102 ± 33.6 (n=4)	95.8 ± 20.6 (n=6)	145 ± 21.6 (n=5)
Week 8	<LOQ (n=6)	94.7 ± 27.3 (n=4)	97.6 ± 23.5 (n=6)	166 ± 98.9 (n=5)
Week 10	0.126 ± 0.0348 (n=6)	105 ± 37.7 (n=4)	93.6 ± 13.7 (n=6)	237 ± 158 (n=5)
Week 12	0.123 ± 0.0507 (n=6)	79.6 ± 25.9 (n=4)	90.6 ± 24.5 (n=6)	140 ± 77.5 (n=5)
Week 14	0.162 ± 0.0643 (n=6)	90.0 ± 28.9 (n=4)	92.2 ± 27.6 (n=6)	79.4 ± 28.3 (n=5)
Week 16	0.128 ± 0.0721 (n=6)	68.6 ± 26.0 (n=4)	98.5 ± 42.3 (n=6)	83.9 ± 58.5 (n=5)
Week 18	0.183 ± 0.0637 (n=6)	29.4 ± 25.4 (n=4)	17.4 ± 14.3 (n=6)	36.2 ± 21.2 (n=5)
Week 20	0.224 ± 0.0730 (n=6)	33.0 ± 26.0 (n=4)	96.6 ± 26.6 (n=6)	97.7 ± 129 (n=5)
Week 22	0.232 ± 0.131 (n=6)	77.6 ± 24.9 (n=3)	105 ± 36.3 (n=6)	58.6 ± 45.6 (n=5)
Week 24	<LOQ (n=4)	72.2 ± 68.8 (n=3)	90.9 ± 21.2 (n=6)	62.7 ± 74.3 (n=5)
Week 26	0.209 ± 0.156 (n=6)	118 ± 27.5 (n=3)	77.4 ± 16.9 (n=4)	77.8 ± 126 (n=5)
Week 26/27	0.223 ± 0.105 (n=6)	52.5 ± 9.14 (n=5)	74.1 ± 33.1 (n=6)	51.5 ± 77.6 (n=6)
Week 28	0.181 ± 0.0391 (n=2)	NS	25.9 ± 7.07 (n=2)	NS
Week 30	0.144 ± 0.0238 (n=2)	NS	11.3 ± 5.11 (n=2)	NS
Week 32	0.110 ± 0.0216 (n=2)	NS	7.60 ± 3.90 (n=2)	NS
Week 34	0.0861 ± 0.0256 (n=2)	NS	3.97 ± 2.09 (n=2)	NS
Week 36	0.111 ± 0.0445 (n=2)	NS	2.75 ± 1.88 (n=2)	NS

Table 10. TOX 026 Data Summary of POAA Concentration—Serum (µg/mL)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Week 38	0.0941 ± 0.0324 (n=2)	NS	1.84 ± 1.40 (n=2)	NS
Week 40	0.0738 ± 0.00256 (n=2)	NS	1.18 ± 0.827 (n=2)	NS

NS—No sample
<LOQ—Below limit of quantitation
[a]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)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Timepoint				
Week 2	<LOQ (n=6)	73.5 ± 38.1 (n=4)	221 ± 124 (n=6)	909 ± 269 (n=6)
Week 4	0.152 ± 0.337 (n=6)	54.9 ± 4.62 (n=4)	190 ± 91.6 (n=6)	240 ± 161 (n=6)
Week 6	0.0587 ± 0.0716 (n=6)	65.7 ± 46.9 (n=4)	128 ± 50.0 (n=6)	272 ± 140 (n=5)
Week 8	0.0161 ± 0.00940 (n=6)	47.6 ± 20.6 (n=4)	206 ± 73.1 (n=6)	180 ± 109 (n=5)
Week 10	0.0177 ± 0.0114 (n=6)	39.9 ± 18.7 (n=4)	175 ± 92.3 (n=6)	359 ± 449 (n=5)
Week 12	0.0141 ± 0.00648 (n=6)	48.4 ± 18.8 (n=4)	201 ± 92.7 (n=6)	118 ± 111 (n=5)
Week 14	7.96 ± 19.5 (n=6)	63.1 ± 47.2 (n=4)	139 ± 52.2 (n=6)	72.9 ± 84.0 (n=5)
Week 16	0.0299 ± 0.0339 (n=6)	50.2 ± 21.0 (n=4)	139 ± 57.0 (n=6)	50.2 ± 67.9 (n=5)
Week 18	0.0256 ± 0.0248 (n=6)	37.7 ± 19.3 (n=4)	186 ± 63.9 (n=6)	43.1 ± 84.6 (n=5)
Week 20	0.0211 ± 0.0130 (n=6)	52.1 ± 9.63 (n=4)	144 ± 135 (n=6)	44.0 ± 59.9 (n=5)
Week 22	0.0231 ± 0.00688 (n=6)	95.8 ± 80.8 (n=3)	158 ± 78.4 (n=6)	98.5 ± 134 (n=5)
Week 24	0.0125 ± 0.00749 (n=6)	46.3 ± 8.52 (n=3)	157 ± 63.3 (n=6)	56.0 ± 77.2 (n=5)
Week 26	0.0268 ± 0.0265 (n=6)	51.6 ± 13.7 (n=3)	109 ± 75.2 (n=6)	19.2 ± 27.0 (n=5)
Week 28	0.118 ± 0.142 (n=2)	NS	0.327 ± 0.0182 (n=2)	NS
Week 30	<LOQ (n=2)	NS	0.361 ± 0.118 (n=2)	NS
Week 32	<LOQ (n=2)	NS	0.114 ± 0.0608 (n=2)	NS
Week 34	<LOQ (n=2)	NS	0.121 ± 0.0305 (n=2)	NS
Week 36	0.0117 ± 0.00841 (n=2)	NS	0.0502 ± 0.0166 (n=2)	NS
Week 38	<LOQ (n=2)	NS	0.0284 ± 0.0102 (n=2)	NS

Table 11. TOX 026 Data Summary of POAA Concentration—Urine (µg/mL)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Week 40	<LOQ (n=2)	NS	0.0254 ± 0.0101 (n=2)	NS

<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)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Timepoint				
Week 20	NS	18.3 (n=1)	NS	NS
Week 27	0.117 ± 0.0730 (n=4)	15.3 ± 3.02 (n=4)	14.0 ± 7.55 (n=4)	42.8 ± 63.3 (n=6)
Week 40	<LOQ (n=2)	NS	0.114 ± 0.0441 (n=2)	NS

NS—No sample
<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)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Timepoint				
Week 2	<LOQ (n=6)	7.43 ± 6.54 (n=4)	15.4 ± 10.2 (n=6)	56.6 ± 73.7 (n=6)
Week 4	0.0214 ± 0.0178 (n=6)	10.4 ± 12.0 (n=4)	23.4 ± 10.6 (n=6)	22.0 ± 6.23 (n=6)
Week 6	0.0108 ± 0.00192 (n=6)	12.1 ± 14.1 (n=4)	23.3 ± 8.46 (n=6)	101 ± 86.7 (n=5)
Week 8	0.0782 ± 0.103 (n=6)	9.46 ± 9.21 (n=4)	41.0 ± 25.0 (n=6)	36.7 ± 34.2 (n=5)
Week 10	<LOQ (n=6)	3.96 ± 3.68 (n=4)	26.0 ± 17.4 (n=6)	48.0 ± 34.0 (n=5)
Week 12	0.0498 ± 0.0894 (n=6)	7.15 ± 5.65 (n=4)	10.3 ± 6.07 (n=6)	32.0 ± 42.9 (n=5)
Week 14	0.139 ± 0.308 (n=6)	7.50 ± 2.43 (n=4)	27.2 ± 29.4 (n=6)	19.9 ± 25.2 (n=5)
Week 16	0.0572 ± 0.0762 (n=6)	6.88 ± 2.62 (n=4)	31.4 ± 23.3 (n=6)	18.2 ± 28.8 (n=5)
Week 18	0.258 ± 0.604 (n=6)	5.72 ± 7.15 (n=4)	17.3 ± 13.3 (n=6)	22.1 ± 31.7 (n=5)
Week 20	0.450 ± 1.08 (n=6)	6.81 ± 4.89 (n=4)	52.4 ± 39.5 (n=6)	37.8 ± 58.1 (n=5)

Table 13. TOX 026 Data Summary of POAA Concentration from Centre Analytical Laboratory—Feces (µg/g)

	Group 1 0.0 mg/kg/day Average ± SD	Group 2 3 mg/kg/day Average ± SD	Group 3 10 mg/kg/day Average ± SD	Group 4 30 mg/kg/day Average ± SD
Week 22	15.5 ± 36.9 (n=6)	13.8 ± 5.22 (n=3)	39.5 ± 21.0 (n=6)	25.2 ± 36.0 (n=5)
Week 24	0.517 ± 1.13 (n=6)	6.22 ± 5.45 (n=3)	40.5 ± 21.8 (n=6)	34.6 ± 47.7 (n=5)
Week 26	0.0172 ± 0.00892 (n=6)	2.92 ± 1.35 (n=3)	43.0 ± 36.9 (n=6)	10.3 ± 20.8 (n=5)
Week 28–34	0.279 ± 0.732 (n=8)	NS	0.387 ± 0.372 (n=8)	NS
Week 36–40	0.0103 ± 0.00684 (n=6)	NS	0.0336 ± 0.0313 (n=6)	NS

NS—No sample
<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
Group 1	I05709M	0.0645	0.299	<LOQ	<LOQ	0.191	0.207	0.275	0.275	0.263	0.342	0.393	E
0.0 mg/kg/day	I05714M	0.137	0.230	0.126	<LOQ	0.105	0.0994	0.156	<LOQ	0.244	0.212	0.157	E
Control	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
Group 2	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
3.0 mg/kg/day	I05706M	177	3611[a]	115	101	114	85.3	112	72.6	16.0	11.4	105	146
Low Dose	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		
I05721M replacement	I05723M	119											
Group 3	I05707M	230	197	84.4	97.2	97.4	94.0	68.0	99.5	13.4	107	122	89.1
10 mg/kg/day	I05708M	103	84.6	78.8	67.8	72.8	56.9	103	60.2	9.67	61.9	63.7	61.4
Mid-Dose	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
Group 4	I05703M	6338	286	162	175	313	235	84.4	68.2	68.8	38.9	16.4	20.4
30 mg/kg/day	I05704M	313	251	111	117	128	103	109	113	20.0	92.5	92.7	90.4
High Dose	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
[a]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
Group 1	I05709M	0.471	0.377							
0.0 mg/kg/day	I05714M	0.108	0.219							
Control	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							
Group 2	I05702M	125	47.9							
3.0 mg/kg/day	I05706M	141	64.5							
Low Dose	I05717M	87.6	44.1							
	I05721M		46.0							
I05721M replacement	I05723M		60.0							
Group 3	I05707M	77.9	121							
10 mg/kg/day	I05708M	55.4	32.1							
Mid-Dose	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							
Group 4	I05703M	11.1	7.48							
30 mg/kg/day	I05704M	61.7	58.6							
High Dose	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
Group 1	I05709M	<LOQ	<LOQ	0.172	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0344	<LOQ	0.0208
0.0 mg/kg/day	I05714M	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0287	<LOQ	<LOQ
Control	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
Group 2	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
3.0 mg/kg/day	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
Mid Dose	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			
Group 3	I05707M	412	195	157	230	272	170	127	165	157	1648*	58.0	184	256
10 mg/kg/day	I05708M	199	271	195	221	252	144	171	187	228	362	168	216	66.6
Mid-high Dose	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
Group 4	I05703M	525	383	354	167	1131	175	0.181	0.179	<LOQ	0.363	0.352	0.306	0.223
30 mg/kg/day	I05704M	811	47.6	193	282	328	169	156	136	170	124	255	155	57.7
High Dose	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											

<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 (µg/mL)

	Animal ID	Week 28	Week 30	Week 32	Week 34	Week 36	Week 38	Week 40
Group 1	I05709M							
0.0 mg/kg/day	I05714M							
Control	I05715M							
	I05718M	0.218	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	I05720M	<LOQ	<LOQ	<LOQ	<LOQ	0.0176	<LOQ	<LOQ
	I05725M							
Group 2	I05702M							
3.0 mg/kg/day	I05706M							
Mid Dose	I05717M							
	I05721M							
Group 3	I05707M							
10 mg/kg/day	I05708M							
Mid-high Dose	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							
Group 4	I05703M							
30 mg/kg/day	I05704M							
High Dose	I05711M							
	I05713M							
	I05722M							
	I05724M							

<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**
Group 1 0.0 mg/kg/day Control	I05709M		0.0913	
	I05714M		<LOQ	
	I05715M		0.226	
	I05725M		<LOQ	
	I05718M			<LOQ
	I05720M			<LOQ
Group 2 3.0 mg/kg/day Mid Dose	I05702M		15.2	
	I05706M		18.5	
	I05717M		11.3	
	I05723M		16.3	
	I05721M	18.3		
Group 3 10 mg/kg/day Mid-high Dose	I05707M		21.9	
	I05708M		6.29	
	I05710M		8.86	
	I05719M		18.8	
	I05712M			0.146
	I05716M			0.0833
Group 4 30 mg/kg/day 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%.

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

	POAA	Dilutions
Blks	A111698003, 4 & 79, 80	1/1
Grp 1	111698015-20	1/1
Grp 2	112498018, 12039845-47	1/75, 1/750
Grp 3	12039851-56	1/1000
Grp 4	111698041, 12039859-63	1/1, 1/5000, 1/2000
MS, MSD	111698075-76	1/1

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			
	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%
Group 1	I05709M	0.50	0.9582	1	33.7	0.0645			
Control	I05714M	0.50	0.9582	1	71.4	0.137			
0.0 mg/kg/day	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			77.1
	I05725M	0.50	0.9582	1	26.0	0.0499		0.0613	0.0472
Group 2	I05702M	0.70	0.9582	750	89.2	91.6			
Low Dose	I05706M	0.50	0.9582	750	123	177			
3.0 mg/kg/day	I05717M	0.60	0.9582	750	96.8	116			
	I05721M	-	0.9582	NR	NR	NR			28.7
	I05723M	0.30	0.9582	75	497	119		126	36.1
Group 3	I05707M	0.50	0.9582	1000	120	230			
Mid-High Dose	I05708M	0.60	0.9582	1000	64.5	103			
10 mg/kg/day	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			25.9
	I05719M	0.50	0.9582	1000	115	220		189	48.9
Group 4	I05703M	0.40	0.9582	5000	529	6338			
High Dose	I05704M	0.50	0.9582	2000	81.8	313			
30 mg/kg/day	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	*		150
	I05724M	0.40	0.9582	2000	183	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 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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.		Mean POAA ug/mL	RSD Std. Dev. 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	I05709M	0.0645			
Control	I05714M	0.137			
0.0 mg/kg/day	I05715M	<LOQ			
	I05718M	0.0889			
	I05720M	<LOQ			77.1
	I05725M	0.0499		0.0613	0.0472
Group 2	I05702M	91.6			
Low Dose	I05706M	177			
	I05717M	116			
	I05721M	NR			28.7
	I05723M	119		126	36.1
Group 3	I05707M	230			
Mid-High Dose	I05708M	102.9			
10 mg/kg/day	I05710M	173			
	I05712M	178			
	I05716M	228			25.9
	I05719M	220		189	48.9
Group 4	I05703M	6338			
High Dose	I05704M	313			
30 mg/kg/day	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

	POAA	Dilutions
Blks	A111698003, 4 & 79, 80	1/1
Grp 1	111698045-50	1/1
Grp 2	12039866-68, 010499021	1/250, 750, 1875
Grp 3	112498041-46	1/150
Grp 4	112498049-50,52	1/200
Grp 4	12039881,83 010499020	1/60, 2000, 5000
MS, MSD	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%	
Group 1	I05709M	0.50	0.9582	1	156	0.299				
Control	I05714M	0.50	0.9582	1	120	0.230				
0.0 mg/kg/day	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	
Group 2	I05702M	0.60	0.9582	750	80.8	96.8				
Low Dose	I05706M	0.40	0.9582	1875	804	3611	*	98.4	43.4*	43.4
3.0 mg/kg/day	I05717M	0.50	0.9582	750	98.7	142		98.4*	42.7*	42.7
	I05721M	0.40	0.9582	250	23576	56.5			180	
	I05723M	-	0.9582	NR	NR	NR		977	1757	
Group 3	I05707M	0.50	0.9582	150	684	197				
Mid-High Dose	I05708M	0.60	0.9582	150	353	84.6				
10 mg/kg/day	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	
Group 4	I05703M	0.40	0.9582	200	598	286				
High Dose	I05704M	0.40	0.9582	200	524	251				
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.		Mean POAA ug/mL	RSD Std. Dev. 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	I05709M	0.299			
Control	I05714M	0.230			
0.0 mg/kg/day	I05715M	0.0785			
	I05718M	0.347			
	I05720M	0.167			51.1
	I05725M	0.114		0.206	0.105
Group 2	I05702M	96.8			
Low Dose	I05706M	3611	*		43.4*
	I05717M	142		98.4*	42.7*
	I05721M	56.5			180
	I05723M	NR		977	1757
Group 3	I05707M	197			
Mid-High Dose	I05708M	84.6			
10 mg/kg/day	I05710M	268			
	I05712M	E			
	I05716M	119			41.7
	I05719M	194		172	71.9
Group 4	I05703M	286			
High Dose	I05704M	251			
30 mg/kg/day	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

	Filenames POAA	Dilutions
Blks	A061099031, 32 & 99, 100	1/1
Grp 1	061099043-50	1/1
Grp 2	060799015-18,64	1/100&1000
Grp 3	060799022-23,27-28,67-68	1/100&1000
Grp 4	060799031-32,71-73, 0616990115	1/100,200,&1000
MS, MSD	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)		
	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%
Group 1	I05709M	0.54	0.9582	1	56.3	<LOQ (0.0983 ug/mL)		
Control	I05714M	0.74	0.9582	1	100	0.126		
0.0 mg/kg/day	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)		11.0
	I05725M	0.67	0.9582	1	21.0	<LOQ (0.0983 ug/mL)	0.103	0.0113
Group 2	I05702M	0.70	0.9582	100	493	67.5		
Low Dose	I05706M	0.55	0.9582	100	662	115		
3.0 mg/kg/day	I05717M	0.56	0.9582	100	489	83.7		
	I05721M	0.65	0.9582	1000	97.1	143		32.8
	I05723M	NR	0.9582	1	NR	NR	102	33.6
Group 3	I05707M	0.72	0.9582	1000	63.4	84.4		
Mid-High Dose	I05708M	0.55	0.9582	100	452	78.8		
10 mg/kg/day	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		21.5
	I05719M	0.40	0.9582	100	562	135	95.8	20.6
Group 4	I05703M	0.49	0.9582	200	414	162		
High Dose	I05704M	0.39	0.9582	100	450	111		
30 mg/kg/day	I05711M	0.61	0.9582	1000	102	161		
	I05713M	0.59	0.9582	1000	95.1	154		14.9
	I05722M	0.49	0.9582	1000	70.0	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, 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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	<LOQ (0.0983 ug/mL)		
Control	I05714M	0.126		
0.0 mg/kg/day	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
Group 2	I05702M	67.5		
Low Dose	I05706M	115		
	I05717M	83.7		
	I05721M	143		32.8
	I05723M	NR	102	33.6
Group 3	I05707M	84.4		
Mid-High Dose	I05708M	78.8		
10 mg/kg/day	I05710M	103		
	I05712M	87.9		
	I05716M	86.2		21.5
	I05719M	135	95.8	20.6
Group 4	I05703M	162		
High Dose	I05704M	111		
30 mg/kg/day	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 POAA	Dilutions
Blks	A061099031, 32 & 99, 100	1/1
Grp 1	061099057-62	1/1
Grp 2	060799038-41	1/100
Grp 3	060799044-46,50-51,80	1/100&1000
Grp 4	060799054-56,84-85	1/100&1000
MS, MSD	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%
Group 1	I05709M	0.30	0.9582	1	32.5	<LOQ (0.0983 ug/mL)		
Control	I05714M	0.67	0.9582	1	86.2	<LOQ (0.0983 ug/mL)		
0.0 mg/kg/day	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
Group 2	I05702M	0.54	0.9582	100	431	76.4		
Low Dose	I05706M	0.50	0.9582	100	529	101		
3.0 mg/kg/day	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
Group 3	I05707M	0.58	0.9582	100	588	97.2		
Mid-High Dose	I05708M	0.63	0.9582	100	446	67.8		
10 mg/kg/day	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
Group 4	I05703M	0.36	0.9582	100	659	175		
High Dose	I05704M	0.42	0.9582	100	513	117		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	<LOQ (0.0983 ug/mL)		
Control	I05714M	<LOQ (0.0983 ug/mL)		
0.0 mg/kg/day	I05715M	<LOQ (0.0983 ug/mL)		
	I05718M	<LOQ (0.0983 ug/mL)		
	I05720M	<LOQ (0.0983 ug/mL)		NA
	I05725M	<LOQ (0.0983 ug/mL)	<LOQ	NA
Group 2	I05702M	76.4		
Low Dose	I05706M	101		
	I05717M	70.5		
	I05721M	130		28.8
	I05723M	NR	94.7	27.3
Group 3	I05707M	97.2		
Mid-High Dose	I05708M	67.8		
10 mg/kg/day	I05710M	88.5		
	I05712M	82.3		
	I05716M	102		24.1
	I05719M	133	97.6	23.5
Group 4	I05703M	175		
High Dose	I05704M	117		
30 mg/kg/day	I05711M	102		
	I05713M	100		59.6
	I05722M	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:	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

	Filenames POAA	Dilutions
Blks	M051799028, 29, 98-99, & 109, 110	1/1
Grp 1	051799040-44, 47	1/1
Grp 2	051999016-19	1/100
Grp 3	051999022-27	1/100
Grp 4	051999030-34, 052499058	1/250&500
MS, MSD	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			
	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	I05709M	0.43	0.9582	1	85.5	0.191			
Control	I05714M	0.83	0.9582	1	90.7	0.105			
0.0 mg/kg/day	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			27.7
	I05725M	0.45	0.9582	1	51.5	0.110		0.126	0.0348
Group 2	I05702M	0.52	0.9582	100	412	75.9			
Low Dose	I05706M	0.54	0.9582	100	641	114			
3.0 mg/kg/day	I05717M	0.42	0.9582	100	328	74.8			
	I05721M	0.33	0.9582	100	532	154			36.0
	I05723M	NR	0.9582	NR	NR	NR		105	37.7
Group 3	I05707M	0.55	0.9582	100	559	97.4			
Mid-High Dose	I05708M	0.77	0.9582	100	585	72.8			
10 mg/kg/day	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			14.6
	I05719M	0.63	0.9582	100	708	108		93.6	13.7
Group 4	I05703M	0.50	0.9582	250	654	313			
High Dose	I05704M	0.73	0.9582	250	392	128			
30 mg/kg/day	I05711M	0.68	0.9582	250	191	67.4			
	I05713M	0.55	0.9582	250	477	208			66.8
	I05722M	0.53	0.9582	500	517	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:	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%
Group 1	I05709M	0.191			
Control	I05714M	0.105			
0.0 mg/kg/day	I05715M	0.0919			
	I05718M	0.129			
	I05720M	0.128			27.7
	I05725M	0.110		0.126	0.0348
Group 2	I05702M	75.9			
Low Dose	I05706M	114			
	I05717M	74.8			
	I05721M	154			36.0
	I05723M	NR		105	37.7
Group 3	I05707M	97.4			
Mid-High Dose	I05708M	72.8			
10 mg/kg/day	I05710M	87.9			
	I05712M	103			
	I05716M	102			14.6
	I05719M	108		93.6	13.7
Group 4	I05703M	313			
High Dose	I05704M	128			
30 mg/kg/day	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

	POAA	Dilutions
Blks	M012199003, 4 & 79, 80	1/1
Grp 1	012199016-21	1/1
Grp 2	012199024-27	1/150
Grp 3	012199030-35, 012799026	1/150, 1/400
Grp 4	012199039-40, 012799027-29	1/50, 1/150, 1/250, 1/400, 1/500
MS, MSD	012199074-75	1/1

Sample Data

Week 12 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	<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%
Group 1	I05709M	0.50	0.9582	1	108	0.207		
Control	I05714M	0.70	0.9582	1	73.0	0.0994		
0.0 mg/kg/day	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
Group 2	I05702M	0.60	0.9582	150	268	64.0		
Low Dose	I05706M	0.60	0.9582	150	358	85.3		
3.0 mg/kg/day	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
Group 3	I05707M	0.50	0.9582	150	329	94.0		
Mid-High Dose	I05708M	0.60	0.9582	150	239	56.9		
10 mg/kg/day	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
Group 4	I05703M	0.70	0.9582	500	345	235		
High Dose	I05704M	0.60	0.9582	150	433	103		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	0.207		
Control	I05714M	0.0994		
0.0 mg/kg/day	I05715M	0.0639		
	I05718M	0.139		
	I05720M	0.140		41.4
	I05725M	0.0869	0.123	0.0507
Group 2	I05702M	64.0		
Low Dose	I05706M	85.3		
	I05717M	55.5		
	I05721M	114		32.6
	I05723M	NR	79.6	25.9
Group 3	I05707M	94.0		
Mid-High Dose	I05708M	56.9		
10 mg/kg/day	I05710M	79.0		
	I05712M	131		
	I05716M	84.4		27.1
	I05719M	98	90.6	24.5
Group 4	I05703M	235		
High Dose	I05704M	103		
30 mg/kg/day	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
Blks	M012199003, 4 & 79, 80	1/1
Grp 1	012199045-50	1/1
Grp 2	012199053-56, 012799033	1/100, 1/200
Grp 3	012199061-62, 64 012799034-36	1/100, 1/200
Grp 4	012199067-71	1/150
MS, MSD	012199074-75	1/1

Sample Data

Week 14 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	<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%
Group 1	I05709M	0.50	0.9582	1	144	0.275		
Control	I05714M	0.50	0.9582	1	81.6	0.156		
0.0 mg/kg/day	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
Group 2	I05702M	0.50	0.9582	100	381	72.7		
Low Dose	I05706M	0.50	0.9582	200	294	112		
3.0 mg/kg/day	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
Group 3	I05707M	0.50	0.9582	200	178	68.0		
Mid-High Dose	I05708M	0.50	0.9582	200	270	103		
10 mg/kg/day	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
Group 4	I05703M	0.50	0.9582	150	295	84.4		
High Dose	I05704M	0.50	0.9582	150	380	109		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	0.275		
Control	I05714M	0.156		
0.0 mg/kg/day	I05715M	0.0981		
	I05718M	0.153		
	I05720M	0.182		39.8
	I05725M	0.105	0.162	0.0643
Group 2	I05702M	72.7		
Low Dose	I05706M	112		
	I05717M	58.4		
	I05721M	117		32.1
	I05723M	NR	90.0	28.9
Group 3	I05707M	68.0		
Mid-High Dose	I05708M	103		
10 mg/kg/day	I05710M	61.3		
	I05712M	79.2		
	I05716M	134		30.0
	I05719M	108	92.2	27.6
Group 4	I05703M	84.4		
High Dose	I05704M	109		
30 mg/kg/day	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

	Filenames POAA	Dilutions
Blks	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	I05709M	0.54	0.9582	1	155	0.275		
Control	I05714M	0.66	0.9582	1	70.7	<LOQ (0.0983 ug/mL)		
0.0 mg/kg/day	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	I05702M	0.60	0.9582	100	346	55.3		
Low Dose	I05706M	0.55	0.9582	100	417	72.6		
3.0 mg/kg/day	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	I05707M	0.57	0.9582	100	592	99.5		
Mid-High Dose	I05708M	0.68	0.9582	100	427	60.2		
10 mg/kg/day	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	I05703M	0.57	0.9582	250	162	68.2		
High Dose	I05704M	0.75	0.9582	250	355	113		
30 mg/kg/day	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
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 3.1
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 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	0.02578
QC - 250 ppb	RBS05129-MS-7	103%	*		
	RBS05129-MSD-7	106%	*	104%	3%
	RBS05129-MS-8	103%	*		
	RBS05129-MSD-8	127%	*	115%	20%
Group 1	I05709M	0.275			
Control	I05714M	<LOQ (0.0983 ug/mL)			
0.0 mg/kg/day	I05715M	<LOQ (0.0983 ug/mL)			
	I05718M	<LOQ (0.0983 ug/mL)			
	I05720M	<LOQ (0.0983 ug/mL)			56.4
	I05725M	<LOQ (0.0983 ug/mL)		0.128	0.0721
Group 2	I05702M	55.3			
Low Dose	I05706M	72.6			
	I05717M	43.4			
	I05721M	103			37.9
	I05723M	NR		68.6	26.0
Group 3	I05707M	100			
Mid-High Dose	I05708M	60.2			
10 mg/kg/day	I05710M	73.2			
	I05712M	180			
	I05716M	91.3			43.0
	I05719M	86.5		98.5	42.3
Group 4	I05703M	68.2			
High Dose	I05704M	113			
30 mg/kg/day	I05711M	13.3			
	I05713M	167			
	I05722M	57.7			69.7
	I05724M	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

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
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

	POAA	POAA	Dilutions
Blks	M051899013, 14 & 89, 90		1/1
Grp 1	051899026-33		1/1
Grp 2	052599086-87, 89	60799061	1/50&250
Grp 3	052599092-97		1/50
Grp 4	052499030, 32, 34, 052599101, 103		1/50
MS, MSD	051899084-87		1/1

Sample Data

Week 18 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		
	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%
Group 1	I05709M	0.63	0.9582	1	173	0.263		
Control	I05714M	0.57	0.9582	1	145	0.244		
0.0 mg/kg/day	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
Group 2	I05702M	0.69	0.9582	50	160	11.1		
Low Dose	I05706M	0.63	0.9582	50	210	16.0		
3.0 mg/kg/day	I05717M	0.45	0.9582	250	125	66.8		86.5
	I05721M	0.47	0.9582	50	234	23.8	29.4	25.4
Group 3	I05707M	0.52	0.9582	50	145	13.4		
Mid-High Dose	I05708M	0.68	0.9582	50	137	9.67		
10 mg/kg/day	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
Group 4	I05703M	0.46	0.9582	50	660	68.8		
High Dose	I05704M	0.66	0.9582	50	276	20.0		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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	I05709M	0.263		
Control	I05714M	0.244		
0.0 mg/kg/day	I05715M	0.0950		
	I05718M	0.165		
	I05720M	0.193		34.8
	I05725M	0.138	0.183	0.0637
Group 2	I05702M	11.1		
Low Dose	I05706M	16.0		
	I05717M	66.8		86.5
	I05721M	23.8	29.4	25.4
Group 3	I05707M	13.4		
Mid-High Dose	I05708M	9.67		
10 mg/kg/day	I05710M	46.4		
	I05712M	13.7		
	I05716M	10.1		82.1
	I05719M	11.3	17.4	14.3
Group 4	I05703M	68.8		
High Dose	I05704M	20.0		
30 mg/kg/day	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

	POAA	Dilutions
Blks	M051899013, 14 & 89, 90	1/1
Grp 1	051899055-62	1/1
Grp 2	052599107-110	1/50
Grp 3	052499045, 061099075-76, 061699018-20	1/50&500
Grp 4	052499051, 53, 55, 061699021-22	1/50&500
MS, MSD	051899084-87	1/1

Sample Data

Week 20 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		
	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%
Group 1	I05709M	0.59	0.9582	1	211	0.342		
Control	I05714M	0.66	0.9582	1	146	0.212		
0.0 mg/kg/day	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
Group 2	I05702M	0.73	0.9582	50	271	17.8		
Low Dose	I05706M	0.65	0.9582	50	155	11.4		
3.0 mg/kg/day	I05717M	0.63	0.9582	50	438	33.3		78.8
	I05721M	0.24	0.9582	50	348	69.6	33.0	26.0
Group 3	I05707M	0.43	0.9582	50	964	107		
Mid-High Dose	I05708M	0.70	0.9582	50	905	61.9		
10 mg/kg/day	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
Group 4	I05703M	0.49	0.9582	50	398	38.9		
High Dose	I05704M	0.53	0.9582	500	102	92.5		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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	I05709M	0.342		
Control	I05714M	0.212		
0.0 mg/kg/day	I05715M	0.115		
	I05718M	0.224		
	I05720M	0.242		32.6
	I05725M	0.207	0.224	0.0730
Group 2	I05702M	17.8		
Low Dose	I05706M	11.4		
	I05717M	33.3		78.8
	I05721M	69.6	33.0	26.0
Group 3	I05707M	107		
Mid-High Dose	I05708M	61.9		
10 mg/kg/day	I05710M	75.2		
	I05712M	137		
	I05716M	91.4		27.5
	I05719M	107	96.6	26.6
Group 4	I05703M	38.9		
High Dose	I05704M	92.5		
30 mg/kg/day	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

	POAA	Dilutions
Blks	A052099035, 36 & 98, 99	1/1
Grp 1	052099047-51, 061099086	1/1
Grp 2	052699025-27	1/100
Grp 3	052699028-32, 34-35, 052899104	1/100&200
Grp 4	052699036-42	1/10&100
MS, MSD	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%
Group 1	I05709M	0.62	0.9582	1	254	0.393		
Control	I05714M	0.68	0.9582	1	111	0.157		
0.0 mg/kg/day	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
Group 2	I05702M	0.75	0.9582	100	547	69.8		
Low Dose	I05706M	0.32	0.9582	100	352	105		32.1
3.0 mg/kg/day	I05717M	0.68	0.9582	100	408	57.5	77.6	24.9
Group 3	I05707M	0.42	0.9582	100	533	122		
Mid-High Dose	I05708M	0.55	0.9582	100	366	63.7		
10 mg/kg/day	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
Group 4	I05703M	0.35	0.9582	10	601	16.4		
High Dose	I05704M	0.28	0.9582	100	271	92.7		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	0.393		
Control	I05714M	0.157		
0.0 mg/kg/day	I05715M	0.106		
	I05718M	0.269		
	I05720M	0.372		56.5
	I05725M	<LOQ (0.0983 ug/mL)	0.232	0.131
Group 2	I05702M	69.8		
Low Dose	I05706M	105		32.1
	I05717M	57.5	77.6	24.9
Group 3	I05707M	122		
Mid-High Dose	I05708M	63.7		
10 mg/kg/day	I05710M	87.7		
	I05712M	168		
	I05716M	106		34.5
	I05719M	85.2	105	36.3
Group 4	I05703M	16.4		
High Dose	I05704M	92.7		
30 mg/kg/day	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

	POAA	Dilutions
Blks	A052099035, 36 & 98, 99	1/1
Grp 1	061099087-90	1/1
Grp 2	052699045, 47, 052899105	1/100&200
Grp 3	052699052-59	1/100
Grp 4	052699051, 63-64, 052899106-107	1/1,10,20,&200
MS, MSD	061099095-96	1/1

Sample Data

Week 24 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%
Group 1	I05709M	0.40	0.9582	1	Ext	Ext		
Control	I05714M	0.58	0.9582	1	Ext	Ext		
0.0 mg/kg/day	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
Group 2	I05702M	0.61	0.9582	100	64.9	10.2		
Low Dose	I05706M	0.69	0.9582	200	527	146		95.3
3.0 mg/kg/day	I05717M	0.43	0.9582	100	270	60.2	72.2	68.8
Group 3	I05707M	0.71	0.9582	100	660	89.1		
Mid-High Dose	I05708M	0.64	0.9582	100	410	61.4		
10 mg/kg/day	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
Group 4	I05703M	0.57	0.9582	20	606	20.4		
High Dose	I05704M	0.72	0.9582	100	679	90.4		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	Ext		
Control	I05714M	Ext		
0.0 mg/kg/day	I05715M	<LOQ (0.0983 ug/mL)		
	I05718M	<LOQ (0.0983 ug/mL)		
	I05720M	<LOQ (0.0983 ug/mL)		NA
	I05725M	<LOQ (0.0983 ug/mL)	<LOQ	NA
Group 2	I05702M	10.2		
Low Dose	I05706M	146		95.3
	I05717M	60.2	72.2	68.8
Group 3	I05707M	89.1		
Mid-High Dose	I05708M	61.4		
10 mg/kg/day	I05710M	81.8		
	I05712M	116		
	I05716M	82.3		23.3
	I05719M	115	90.9	21.2
Group 4	I05703M	20.4		
High Dose	I05704M	90.4		
30 mg/kg/day	I05711M	4.29		
	I05713M	181		119
	I05722M	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 & 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

	POAA	Dilutions
Blks	A051799055, 56 & 95, 96	1/1
Grp 1	051799067-70	1/1
Grp 2	052699015-17	1/200
Grp 3	052099120-121, 123 & 052699020	1/100&200
Grp 4	051799086, 052099111, 126 & 052699021-22	1/1,10,20,100,&500
MS, MSD	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%
Group 1	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
Group 2	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
Group 3	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
Group 4	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05709M	0.471		
Control	I05714M	0.108		
0.0 mg/kg/day	I05715M	0.101		
	I05718M	0.206		
	I05720M	0.308		74.5
	I05725M	0.0623	0.209	0.156
Group 2	I05702M	125		
Mid Dose	I05706M	141		23.3
	I05717M	87.6	118	27.5
Group 3	I05707M	77.9		
Mid-High Dose	I05708M	55.4		
10 mg/kg/day	I05710M	96.5		21.8
	I05719M	79.6	77.4	16.9
Group 4	I05703M	11.1		
High Dose	I05704M	61.7		
30 mg/kg/day	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	Filenames		
Analytical Equipment System Number:	Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Blks**	A050399003, 4 & 42, 43	1/1
Filename:	See Attachments	**Grp 1**	043099065-70	1/1
R-Squared Value:	See Attachments	**Grp 2**	050399016-18,36-37	1/100
Slope:	See Attachments	**Grp 3**	050399021-28, 050599116	1/100&1000
Y-Intercept:	See Attachments	**Grp 4**	050399029-32, 050499016, 050599117	1/100&1000
Date of Extraction/Analyst:	04/29/99 SAH	**MS, MSD**	043099073-74	1/1
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 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%
Group 1	I05709M Wk27	1.0	0.9582	1	393	0.377		
Control	I05714M Wk27	1.0	0.9582	1	229	0.219		
0.0 mg/kg/day	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
Group 2	I05702M Wk27	1.0	0.9582	100	500	47.9		
Mid Dose	I05706M Wk27	1.0	0.9582	100	673	64.5		
3.0 mg/kg/day	I05717M Wk27	1.0	0.9582	100	460	44.1		
	I05721M Moribund	1.0	0.9582	100	480	46.0		17.4
5721M replacement	I05723M	1.0	0.9582	100	626	60.0	52.5	9.14
Group 3	I05707M Wk27	1.0	0.9582	1000	126	121		
Mid-High Dose	I05708M Wk27	1.0	0.9582	100	335	32.1		
10 mg/kg/day	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
Group 4	I05703M Wk27	1.0	0.9582	100	78.1	7.48		
High Dose	I05704M Wk27	1.0	0.9582	100	612	58.6		
30 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	<LOQ (0.00983 ug/mL)		
	H2O Blk-2	<LOQ (0.00983 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	<LOQ (0.00983 ug/mL)		
	Rabbit Sera Blk-2	<LOQ (0.00983 ug/mL)	<LOQ	NA
QC - 250 ppb	I05709M-MS	79%		
	I05714M-MS	108%	94%	17%
Group 1	I05709M Wk27	0.377		
Control	I05714M Wk27	0.219		
0.0 mg/kg/day	I05715M Wk27	0.0945		
	I05718M Wk26	0.206		
	I05720M Wk26	0.308		47.1
	I05725M Wk27	0.136	0.223	0.105
Group 2	I05702M Wk27	47.9		
Mid Dose	I05706M Wk27	64.5		
	I05717M Wk27	44.1		
	I05721M Moribund	46.0		17.4
	I05723M	60.0	52.5	9.14
Group 3	I05707M Wk27	121		
Mid-High Dose	I05708M Wk27	32.1		
10 mg/kg/day	I05710M Wk27	47.6		
	I05712M Wk26	101		
	I05716M Wk26	78.1		44.7
	I05719M Wk27	65.2	74.1	33.1
Group 4	I05703M Wk27	7.48		
High Dose	I05704M Wk27	58.6		
30 mg/kg/day	I05711M Wk27	0.877		
	I05713M Wk27	184		
	I05722M Wk27	6.65		151
	I05724M Moribund	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	Filenames		
Analytical Equipment System Number:	Soup 020199, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Blks**	S062299003-5 & 44-46	1/1
Filename:	See Attachments	**Grp 1**	062299016-17	1/1
R-Squared Value:	See Attachments	**Grp 3**	062399016-17	1/50
Slope:	See Attachments	**MS, MSD**	062299040-41	1/1
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 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		
	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%
Group 1	I05718M	0.53	0.9582	1	84.8	0.153		21.6
Control, 0.0 mg/kg/day	I05720M	0.56	0.9582	1	122	0.209	0.181	0.0391
Group 3	I05712M	0.61	0.9582	50	394	30.9		27.2
10 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05718M	0.153		21.6
Control, 0.0 mg/kg/day	I05720M	0.209	0.181	0.0391
Group 3	I05712M	30.9		27.2
10 mg/kg/day	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 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		
	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%
Group 1	I05718M	0.65	0.9582	1	86.1	0.127		16.6
Control, 0.0 mg/kg/day	I05720M	0.62	0.9582	1	104	0.161	0.144	0.0238
Group 3	I05712M	0.75	0.9582	50	234	14.9		45.2
10 mg/kg/day	I05716M	0.55	0.9582	50	88.4	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

Sample Data

Week 30 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-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%
Group 1	I05718M	0.127		16.6
Control, 0.0 mg/kg/day	I05720M	0.161	0.144	0.0238
Group 3	I05712M	14.9		45.2
10 mg/kg/day	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
Blks	S062299003-5 & 44-46	1/1
Grp 1	062299022-23	1/1
Grp 3	062399024-25	1/50
MS, MSD	062299040-41	1/1

Sample Data

Week 32 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		
	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%
Group 1	I05718M	0.38	0.9582	1	37.4	0.0943		19.8
Control, 0.0 mg/kg/day	I05720M	0.48	0.9582	1	62.6	0.125	0.110	0.0216
Group 3	I05712M	0.72	0.9582	50	156	10.4		51.2
10 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05718M	0.0943		19.8
Control, 0.0 mg/kg/day	I05720M	0.125	0.110	0.0216
Group 3	I05712M	10.4		51.2
10 mg/kg/day	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

	POAA	Dilutions
Blks	S062299003-5 & 44-46	1/1
Grp 1	062299024-25	1/1
Grp 3	062399028-29	1/50
MS, MSD	062299040-41	1/1

Sample Data

Week 34 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		
	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%
Group 1	I05718M	0.43	0.9582	1	30.5	0.0680		29.7
Control, 0.0 mg/kg/day	I05720M	0.45	0.9582	1	49.0	0.104	0.0861	0.0256
Group 3	I05712M	0.76	0.9582	50	86.4	5.45		52.7
10 mg/kg/day	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. 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%
Group 1	I05718M	0.0680		29.7
Control, 0.0 mg/kg/day	I05720M	0.104	0.0861	0.0256
Group 3	I05712M	5.45		52.7
10 mg/kg/day	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

	POAA	Dilutions
Blks	S071499003, 4, 16-17, & 39, 40	1/1
Grp 1	071499021-22	1/1
Grp 3	072199096-97	1/10
MS, MSD	071499018-19	1/1
	Box 99-130	

Sample Data

Week 36 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)		
	H2O Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	1.0	NA	1	228	92%		
	MKS07139-MSD-1	1.0	NA	1	208	84%	88%	9%
Group 1	I05718M	0.45	0.9582	1	37.3	0.0795		40.1
Control, 0.0 mg/kg/day	I05720M	0.48	0.9582	1	71.4	0.142	0.111	0.0445
Group 3	I05712M	0.64	0.9582	10	273	4.08		68.4
10 mg/kg/day	I05716M	0.65	0.9582	10	96.3	1.42	2.75	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	<LOQ (0.0248 ug/mL)		
	H2O Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	92%		
	MKS07139-MSD-1	84%	88%	9%
Group 1	I05718M	0.0795		40.1
Control, 0.0 mg/kg/day	I05720M	0.142	0.111	0.0445
Group 3	I05712M	4.08		68.4
10 mg/kg/day	I05716M	1.42	2.75	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	Dilutions
Blks	S071499003, 4, 16-17, & 39, 40	1/1
Grp 1	071499027-28	1/1
Grp 3	071499030, 072199098	1/1&10
MS, MSD	071499018-19	1/1
	Box 99-130	

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)		
	H2O Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	1.0	NA	1	228	92%		
	MKS07139-MSD-1	1.0	NA	1	208	84%	88%	9%
Group 1	I05718M	0.63	0.9582	1	46.8	0.0711		34.5
Control, 0.0 mg/kg/day	I05720M	0.49	0.9582	1	59.8	0.117	0.0941	0.0324
Group 3	I05712M	0.75	0.9582	10	221	2.83		76.5
10 mg/kg/day	I05716M	0.75	0.9582	1	659	0.842	1.84	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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	<LOQ (0.0248 ug/mL)		
	H2O Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	92%		
	MKS07139-MSD-1	84%	88%	9%
Group 1	I05718M	0.0711		34.5
Control, 0.0 mg/kg/day	I05720M	0.117	0.0941	0.0324
Group 3	I05712M	2.83		76.5
10 mg/kg/day	I05716M	0.842	1.84	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

	POAA	Dilutions
Blks	S071499003, 4, 16-17, & 39, 40	1/1
Grp 1	071499033-34	1/1
Grp 3	071499036, 072199099	1/1&2
MS, MSD	071499018-19	1/1
	Box 99-130	

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)		
	H2O Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	1.0	0.9582	1	0.00	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	1.0	NA	1	228	92%		
	MKS07139-MSD-1	1.0	NA	1	208	84%	88%	9%
Group 1	I05718M	0.37	0.9582	1	27.8	0.0719		3.47
Control, 0.0 mg/kg/day	I05720M	0.50	0.9582	1	39.4	0.0756	0.0738	0.00256
Group 3	I05712M	0.49	0.9582	2	452	1.77		69.8
10 mg/kg/day	I05716M	0.36	0.9582	1	225	0.600	1.18	0.827

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 Dose	Sample #	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	<LOQ (0.0248 ug/mL)		
	H2O Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Rabbit Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
Matrix Blk	Monkey Sera Blk-1	<LOQ (0.0248 ug/mL)		
	Monkey Sera Blk-2	<LOQ (0.0248 ug/mL)	<LOQ	NA
QC - 250 ppb	MKS07139-MS-1	92%		
	MKS07139-MSD-1	84%	88%	9%
Group 1	I05718M	0.0719		3.47
Control, 0.0 mg/kg/day	I05720M	0.076	0.0738	0.00256
Group 3	I05712M	1.77		69.8
10 mg/kg/day	I05716M	0.600	1.18	0.827

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

				Filenames		
					POAA	**Dilutions**
Study:	6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys			**Blks**	S062399047-48 & 89-90	1/1
Product Number(Test Substance):	T-6889.2 (POAA)			**Grp 1**	062399059-62	1/1
Matrix:	Monkey Liver	Filename:	See List to Right	**Grp 2**	062399065-68	1/100
Method/Revision:	FACT-M-1.1 & FACT-M-2.1	R-Squared Value:	See Attachments	**Grp 3**	062399071-74	1/50,100
Analytical Equipment System Number:	Soup 020199	Slope:	See Attachments	**Grp 4**	062399077-80,82 100599077	1/1,2,10,100,200,500
Instrument Software/Version:	MassLynx 3.2	Y-Intercept:	See Attachments	**MS, MSD**	062399085-86	1/1
Date of Extraction/Analyst:	06/21/99 SAH				reanalyzed on 06/24/99 with confirmation of low recoveries	
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 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%
Group 1	I05709M Wk27	1.0153	NA	0.9582	96.7	1	91.3	0.0913		
Control	I05714M Wk27	0.9931	NA	0.9582	56.0	1	54.0	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M Wk27	1.0104	NA	0.9582	160	100	15206	15.2		
Mid Dose	I05706M Wk27	1.0002	NA	0.9582	193	100	18531	18.5		
3.0 mg/kg/day	I05717M Wk27	0.9931	NA	0.9582	117	100	11307	11.3		19.7
5721M replacement	I05723M	1.0163	NA	0.9582	173	100	16301	16.3	15.3	3.02
Group 3	I05707M Wk27	1.0143	NA	0.9582	232	100	21917	21.9		
Mid-High Dose	I05708M Wk27	1.0061	NA	0.9582	132	50	6294	6.29		
10 mg/kg/day	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
Group 4	I05703M Wk27	1.0036	NA	0.9582	137	10	1309	1.31		
High Dose	I05704M Wk27	1.0036	NA	0.9582	1679	10	16026	16.0	*	
30 mg/kg/day	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 Valu	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 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-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%
Group 1	I05709M Wk27	91.3	0.0913		
Control	I05714M Wk27	54.0	<LOQ		
0.0 mg/kg/day	I05715M Wk27	226	0.226		62.4
	I05725M Wk27	42.0	<LOQ	0.117	0.0730
Group 2	I05702M Wk27	15206	15.2		
Mid Dose	I05706M Wk27	18531	18.5		
3.0 mg/kg/day	I05717M Wk27	11307	11.3		19.7
5721M replacement	I05723M	16301	16.3	15.3	3.02
Group 3	I05707M Wk27	21917	21.9		
Mid-High Dose	I05708M Wk27	6294	6.29		
10 mg/kg/day	I05710M Wk27	8864	8.86		54.1
	I05719M Wk27	18753	18.8	14.0	7.55
Group 4	I05703M Wk27	1309	1.31		
High Dose	I05704M Wk27	16026	16.0		
30 mg/kg/day	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

				Filenames		
Study:	6 Month Capsule Toxicity Study with APFO in Cynomolgus Monkeys				**POAA**	**Dilutions**
Product Number(Test Substance):	T-6889.2 (POAA)			**Blks**	A071399003-4, 30-31	1/1
Matrix:	Monkey Liver	Filename:	See List to Right	**Grp 1**	071399019-20	1/1
Method/Revision:	FACT-M-1.1 & FACT-M-2.1	R-Squared Value:	See Attachments	**Grp 2**	072899016	1/100
Analytical Equipment System Number:	Soup020199	Slope:	See Attachments	**Grp 3**	071399023-24	1/1
Instrument Software/Version:	MassLynx 3.2	Y-Intercept:	See Attachments	**MS, MSD**	071399015-16	1/1
Date of Extraction/Analyst:	7/13/99 SEE				Box 99-131	
Date of Analysis/Analyst:	07/13/99 DRB					
Date of Data Reduction/Analyst:	07/14/99, 11/02/00, 11/06/00 DRB/KJH					

Sample Data

MONKEY LIVER Week 20 and 40

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-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%
Group 1, Wk40	I05718M	0.9933	NA	0.9582	14.0	1	13.5	<LOQ		
0.0 mg/kg/day	I05720M	0.9997	NA	0.9582	22.5	1	21.6	<LOQ	<LOQ	NA
Group 2, Wk20	I05721M	1.0083	NA	0.9582	193	100	18321	18.3	NA	NA
Group 3, Wk40	I05712M	1.0157	NA	0.9582	154	1	146	0.146		38.6
10 mg/kg/day	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	Filename:	
Product Number(Test Substance):	T-6889.2 (POAA)	R-Squared Valu	
Matrix:	Monkey Liver	Slope:	
Method/Revision:	FACT-M-1.1 & FACT-M-2.1	Y-Intercept:	
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		

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	I05718M	13.5	<LOQ		0.00
0.0 mg/kg/day	I05720M	21.6	<LOQ	<LOQ	NA
Group 2, Wk20	I05721M	18321	18.3	NA	NA
Group 3, Wk40	I05712M	146	0.146		38.6
10 mg/kg/day	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

		PFOA
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	Filenames					
Product Number(Test Substance):	T-6889.2 (POAA)		**POAA**	**Dilutions**		**POAA**	**Dilutions**
Matrix:	Monkey Urine	**Blks 08/04/99**	081399004-5, A000927018,32	1/1	**MS/MSDs 08/04/99**	A000927045-48, A001017020-21	1/1
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	1/1
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	1/1
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	1/1
Filename:	See listing to the right	**Blks 09/21/99**	092299003-4, 092899059-60	1/1	**MS/MSDs 08/20/99**	082399016-17	1/1
R-Squared Value:	See Attachments	**Box**	99-160-1, 99-162-3, 99-166-7		**MS/MSDs 08/20/99**	A001017025-26	1/1
Slope:	See Attachments				**MS/MSDs 09/21/99**	092299016-17	1/1
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 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 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
	MKU09219 H2O Blk-1	1	0.9582	1	6.90	<LOQ		
	MKU09219 H2O Blk-2	1	0.9582	1	0.270	<LOQ	NA	NA
Matrix Blk	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
	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
QC-MS	MKU08049 MS-1	1	NA	1	100	133% *		
61.6 ppb	MKU08049 MSD-1	1	NA	1	75.1	92% *	112%	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

* Confirmed results. LAC 10/26/00

POAA = Perfluorooctanoate $C_8F_{17}COO^-$

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
	MKU09219 H2O Blk-1	6.90	<LOQ			
	MKU09219 H2O Blk-2	0.270	<LOQ		NA	NA
Matrix Blk	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
	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
QC-MS	MKU08049 MS-1	100	133%	*		
61.6 ppb	MKU08049 MSD-1	75.1	92%	*	112%	36%
	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

* Confirmed results. LAC 10/26/00

POAA = Perfluorooctanoate 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
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:	08/19/99, 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

Filenames	POAA	Dilutions
Grp 1	081999022-23, 082399020-21, 092299026, 092799050	1/1
Grp 2	090999018-21	1/1000
Grp 3	090899049,78, 091099019-21, 091599150	1/500, 1/250, 1/1000, 1/2000
Grp 4	091599035, 122-126	1/2000, 1/8000
Box	99-160-1, 99-162-3, 99-166-7	

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
Group 1	I05709M	1	0.9582	1	8.27	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	57.1	54.7		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	2000	215	412		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	2000	274	525		
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	8.27	<LOQ		
0.0 mg/kg/day	I05714M	0.00	<LOQ		
	I05715M	12.0	<LOQ		
	I05718M	10.6	<LOQ		
	I05720M	10.3	<LOQ		NA
	I05725M	4.36	<LOQ	<LOQ	NA
Group 2	I05702M	57.1	54.7		
3.0 mg/kg/day	I05706M	62.7	60.1		
	I05717M	51.1	48.9		51.9
	I05723M	136	130	73.5	38.1
Group 3	I05707M	215	412		
10 mg/kg/day	I05708M	208	199		
	I05710M	257	123		
	I05712M	254	60.9		
	I05716M	252	242		56.2
	I05719M	300	288	221	124
Group 4	I05703M	274	525		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999024-25, 082399022-3,092799045,51	1/1, 1/5
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999022-28	1/1000
Filename:	See listing to the right	**Grp 3**	090899057,79, 091099022-27	1/500, 1/250, 1/1000
R-Squared Value:	See Attachments	**Grp 4**	091599043-45, 129-130, 092799047	1/1000, 1/4000, 1/2000
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:	08/19/99, 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 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
Group 1	I05709M	1	0.9582	1	10.6	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	57.7	55.3		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	204	195		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	4000	99.9	383		
30 mg/kg/day	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 A = Below LOQ **POAA** = Perfluorooctanoate $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	10.6	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	57.7	55.3		
3.0 mg/kg/day	I05706M	57.9	55.5		
	I05717M	62.6	60.0		8.43
	I05721M	50.9	48.7	54.9	4.62
Group 3	I05707M	204	195		
10 mg/kg/day	I05708M	283	271		
	I05710M	276	264		
	I05712M	149	36		
	I05716M	277	133		48.1
	I05719M	253	242	190	91.6
Group 4	I05703M	99.9	383		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999028-29, 082399026-27, 092799052, 65	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999028-34	1/1000
Filename:	See listing to the right	**Grp 3**	091599151-157, 160	1/1000, 1/250
R-Squared Value:	See Attachments	**Grp 4**	091599051-52, 131-133	1/1000, 1/4000
Slope:	See Attachments	**Box**	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 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 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
Group 1	I05709M	1	0.9582	1	179	0.172		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	138	132		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	164	157		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	4000	92.3	354		
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	179	0.172		
0.0 mg/kg/day	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
Group 2	I05702M	138	132		
3.0 mg/kg/day	I05706M	58.0	55.6		
	I05717M	55.5	53.2		71.4
	I05721M	22.8	21.8	65.7	46.9
Group 3	I05707M	164	157		
10 mg/kg/day	I05708M	203	195		
	I05710M	156	150		
	I05712M	227	54.4		
	I05716M	122	117		39.1
	I05719M	98.4	94.3	128	50.0
Group 4	I05703M	92.3	354		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999030-31, 082399028-29, 092299029, 092799053	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999035-40	1/1000
Filename:	See listing to the right	**Grp 3**	090899074, 091099028-34	1/500, 1/250, 1/1000
R-Squared Value:	See Attachments	**Grp 4**	082499061, 0915099056, 136-138	1/50, 1/10, 1/1000, 1/2000
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:	08/19/99, 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
Group 1	I05709M	1	0.9582	1	13.5	<LOQ			
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	49.2	49.2			
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	240	230			
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	2000	87.0	167			
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	13.5	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	49.2	49.2		
3.0 mg/kg/day	I05706M	78.7	75.4		
	I05717M	40.0	38.3		43.2
	I05721M	28.7	27.5	47.6	20.6
Group 3	I05707M	240	230		
10 mg/kg/day	I05708M	230	221		
	I05710M	311	298		
	I05712M	196	75.2		
	I05716M	200	192		35.5
	I05719M	230	220	206	73.1
Group 4	I05703M	87.0	167		
30 mg/kg/day	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

A = Below LOQ

POAA = Perfluorooctanoate 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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999034-35, 082399032-33, 092299030, 092799054	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999041-44	1/1000
Filename:	See listing to the right	**Grp 3**	090899072-73,80, 091099035-39	1/500, 1/250, 1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399017, 091599059,63,140, 092399022	1/20, 1/1000, 1/2000, 1/4000
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, 09/10/99, 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
Group 1	I05709M	1	0.9582	1	10.9	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	34.7	33.3		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	284	272		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	4000	295	1131		
30 mg/kg/day	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

A = Below LOQ

POAA = Perfluorooctanoate $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	10.9	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	34.7	33.3		
3.0 mg/kg/day	I05706M	66.5	63.7		
	I05717M	45.5	43.6		46.9
	I05721M	20.0	19.2	39.9	18.7
Group 3	I05707M	284	272		
10 mg/kg/day	I05708M	263	252		
	I05710M	243	233		
	I05712M	188	45.1		
	I05716M	197	94.3		52.7
	I05719M	322	154	175	92.3
Group 4	I05703M	295	1131		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999036-37, 082399034-35, 092299033, 092799057	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999047-50	1/1000
Filename:	See listing to the right	**Grp 3**	082499016-21	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399018-19, 091599143-145	1/20, 1/2000
Slope:	See Attachments	**Box**	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			

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 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
Group 1	I05709M	1	0.9582	1	14.7	<LOQ			
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	68.2	65.3			
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	177	170			
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	2000	91.3	175			
30 mg/kg/day	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

A = Below LOQ

POAA = Perfluorooctanoate

$C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL		RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	14.7	<LOQ			
0.0 mg/kg/day	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
Group 2	I05702M	68.2	65.3			
3.0 mg/kg/day	I05706M	65.6	62.8			
	I05717M	40.5	38.8			38.9
	I05721M	27.8	26.6	48.4		18.8
Group 3	I05707M	177	170			
10 mg/kg/day	I05708M	150	144			
	I05710M	381	365			
	I05712M	270	259			
	I05716M	148	142			46.0
	I05719M	135	130	201		92.7
Group 4	I05703M	91.3	175			
30 mg/kg/day	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**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999040, 082399052-3, 090899020, 092299034-35	1/1, 1/250
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999051-56	1/1000
Filename:	See listing to the right	**Grp 3**	090899021-028	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399023-25,71, 092399019	1/10,1/500, 1/1000
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			

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 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
Group 1	I05709M	0.15	0.9582	1	1.19	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	61.2	58.7		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	133	127		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	10	18.9	0.181		
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	1.19	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	61.2	58.7		
3.0 mg/kg/day	I05706M	136	131		
	I05717M	42.2	40.4		74.8
	I05721M	23.9	22.9	63.1	47.2
Group 3	I05707M	133	127		
10 mg/kg/day	I05708M	178	171		
	I05710M	193	185		
	I05712M	52.5	50.3		
	I05716M	120	115		37.7
	I05719M	191	183	139	52.2
Group 4	I05703M	18.9	0.181		
30 mg/kg/day	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	Filenames		
Method/Revision:	ETS-8-96.0 & ETS-8-97.0		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999042-43, 082399054-55, 092299036, 092799058	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999057-62	1/1000
Filename:	See listing to the right	**Grp 3**	090899029-036	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399029,31,72, 091599080, 092399018	1/1,1/10, 1/500, 1/5
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, 09/15/99, 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 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
Group 1	I05709M	1	0.9582	1	17.8	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	39.1	37.4		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	172	165		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	10	18.7	0.179		
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	17.8	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	39.1	37.4		
3.0 mg/kg/day	I05706M	65.1	62.4		
	I05717M	76.2	73.1		41.8
	I05721M	29.3	28.1	50.2	21.0
Group 3	I05707M	172	165		
10 mg/kg/day	I05708M	196	187		
	I05710M	169	162		
	I05712M	44.6	42.7		
	I05716M	102.4	98.1		40.9
	I05719M	188	181	139	57.0
Group 4	I05703M	18.7	0.179		
30 mg/kg/day	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 ApplicablePOAA = 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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999060-61, 082399058-59, 092299037, 092799059	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999063-65, 091599118	1/500, 1/1000
Filename:	See listing to the right	**Grp 3**	090899037-044	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399035,38, 091599081,87, 092399017	1/1,1/5, 1/10, 1/500
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, 09/15/99, 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
Group 1	I05709M	1	0.9582	1	11.0	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	38.3	36.7		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	1000	164	157		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	10	0.00	<LOQ		
30 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	11.0	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	38.3	36.7		
3.0 mg/kg/day	I05706M	60.8	58.3		
	I05717M	45.6	43.7		51.3
	I05721M	25.0	12.0	37.7	19.3
Group 3	I05707M	164	157		
10 mg/kg/day	I05708M	238	228		
	I05710M	203	194		
	I05712M	78.9	75.6		
	I05716M	213	204		34.3
	I05719M	269	258	186	63.9
Group 4	I05703M	0.0	<LOQ		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999062-63, 082399060-61, 092299040, 092799060	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999069-72	1/1000
Filename:	See listing to the right	**Grp 3**	082499049-53, 092899073	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399044,73, 09159982,89-90	1/1,1/10,1/20,1/500
Slope:	See Attachments	**Box**	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
Group 1	I05709M	1	0.9582	1	17.7	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	51.0	48.9		
3.0 mg/kg/day	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
Group 3	I05707M	1	0.9582	5000	344	1648 *		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	1	379	0.363		
30 mg/kg/day	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

** Conc. was below the LOQ

POAA = Perfluorooctanoate

$C_8F_{17}COO^-$

+ 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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.		Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	17.7	<LOQ			
0.0 mg/kg/day	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
Group 2	I05702M	51.0	48.9			
3.0 mg/kg/day	I05706M	69.0	66.1			
	I05717M	46.2	44.3			18.5
	I05721M	51.1	48.9		52.1	9.63
Group 3	I05707M	344	1648	*		
10 mg/kg/day	I05708M+	378	362			
	I05710M+	185	177			
	I05712M+	61.9	59.3			
	I05716M+	109	104			93.9
	I05719M+	18.8	18.0	**	144	135
Group 4	I05703M	379	0.363			
30 mg/kg/day	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

** Conc. was below the LOQ

POAA = Perfluorooctanoate 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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999066-67, 082399064-5, 092299041, 092799061	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999075-77, 091599119	1/500, 1/1000
Filename:	See listing to the right	**Grp 3**	082499056-58,60-61, 091099040	1/1000
R-Squared Value:	See Attachments	**Grp 4**	091599093-94, 092399016,24-25	1/1,1/10,1/20, 1/1000
Slope:	See Attachments	**Box**	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			

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 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
Group 1	I05709M	1	0.9582	1	35.9	0.0344		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	46.7	44.7		
3.0 mg/kg/day	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
Group 3	I05707M+	1	0.9582	1000	60.6	58.0		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	1	367	0.352		
30 mg/kg/day	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 $C_8F_{17}COO^-$

+ 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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	35.9	0.0344		
0.0 mg/kg/day	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
Group 2	I05702M	46.7	44.7		
3.0 mg/kg/day	I05706M	112	53.7		
	I05717M	197	189		84.4
	I05721M	NA	NA	95.8	80.8
Group 3	I05707M+	60.6	58.0		
10 mg/kg/day	I05708M+	175	168		
	I05710M+	246	235		
	I05712M	90.7	86.9		
	I05716M+	150	144		49.7
	I05719M+	266	255	158	78.4
Group 4	I05703M	367	0.352		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999068-69, 082399066-67, 092299042-43	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999078-82	1/1000
Filename:	See listing to the right	**Grp 3**	082499064-65,67-69, 091099041	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399055,65, 091599083,95-96	1/1,1/10,1/500
Slope:	See Attachments	**Box**	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			

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 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
Group 1	I05709M	0.4	0.9582	1	7.42	<LOQ			
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	38.4	36.8			
3.0 mg/kg/day	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
Group 3	I05707M+	1	0.9582	1000	192	184			
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	1	319	0.306			
30 mg/kg/day	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 $C_8F_{17}COO^-$

+ 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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	7.42	<LOQ		
0.0 mg/kg/day	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
Group 2	I05702M	38.4	36.8		
3.0 mg/kg/day	I05706M	55.6	53.3		
	I05717M	50.9	48.7		18.4
	I05721M	NA	NA	46.3	8.52
Group 3	I05707M+	192	184		
10 mg/kg/day	I05708M+	226	216		
	I05710M	222	212		
	I05712M+	56.3	54.0		
	I05716M+	117	112		40.3
	I05719M+	170	163	157	63.3
Group 4	I05703M	319	0.306		
30 mg/kg/day	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		**POAA**	**Dilutions**
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498	**Grp 1**	081999072-73, 082399070-71, 092299044, 092799064	1/1
Instrument Software/Version:	MassLynx 3.2	**Grp 2**	090999083-85	1/1000
Filename:	See listing to the right	**Grp 3**	082499072-75, 091099042, 092799046	1/1000
R-Squared Value:	See Attachments	**Grp 4**	081399066, 091599099-100, 092399015,23	1/1,1/10,1/250
Slope:	See Attachments	**Box**	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
Group 1	I05709M	1	0.9582	1	21.7	0.0208		
0.0 mg/kg/day	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
Group 2	I05702M	1	0.9582	1000	38.6	37.0		
3.0 mg/kg/day	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
Group 3	I05707M+	1	0.9582	1000	268	256		
10 mg/kg/day	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
Group 4	I05703M	1	0.9582	1	233	0.223		
30 mg/kg/day	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 $C_8F_{17}COO^-$

+ 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 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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05709M	21.7	0.0208		
0.0 mg/kg/day	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
Group 2	I05702M	38.6	37.0		
3.0 mg/kg/day	I05706M	55.9	53.6		
	I05717M	67.0	64.2		26.6
	I05721M	NA	NA	51.6	13.7
Group 3	I05707M+	268	256		
10 mg/kg/day	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
Group 4	I05703M	233	0.223		
30 mg/kg/day	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	Filenames		
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999074-75	1/1
Filename:	See listing to the right	**Grp 3**	082499052-53	1/10
R-Squared Value:	See Attachments	**Box**	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, 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 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 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
Group 1	I05718M	1	0.9582	1	228	0.218		
0.0 mg/kg/day	I05720M	1	0.9582	1	11.9	<LOQ	0.118	0.142
Group 3	I05712M	1	0.9582	10	32.8	0.314		
10 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	228	0.218		
0.0 mg/kg/day	I05720M	11.9	<LOQ	0.118	0.142
Group 3	I05712M	32.8	0.314		
10 mg/kg/day	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
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
Grp 1	081999078-79	1/1
Grp 3	091599018, 159	1/1&10
Box	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 30

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
Group 1	I05718M	1	0.9582	1	2.73	<LOQ		
0.0 mg/kg/day	I05720M	1	0.9582	1	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	1	0.9582	1	290	0.278		
10 mg/kg/day	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 $C_8F_{17}COO^-$

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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	2.73	<LOQ		
0.0 mg/kg/day	I05720M	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	290	0.278		
10 mg/kg/day	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		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999080-81	1/1
Filename:	See listing to the right	**Grp 3**	091599019-20	1/1
R-Squared Value:	See Attachments	**Box**	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 32

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
Group 1	I05718M	1	0.9582	1	4.68	<LOQ		
0.0 mg/kg/day	I05720M	1	0.9582	1	5.14	<LOQ	<LOQ	NA
Group 3	I05712M	1	0.9582	1	164	0.157		
10 mg/kg/day	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 $C_8F_{17}COO^-$

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 32

Group Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	4.68	<LOQ		
0.0 mg/kg/day	I05720M	5.14	<LOQ	<LOQ	NA
Group 3	I05712M	164	0.157		
10 mg/kg/day	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	Filenames		
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999084-85	1/1
Filename:	See listing to the right	**Grp 3**	091599021-22	1/1
R-Squared Value:	See Attachments	**Box**	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 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
Group 1	I05718M	1	0.9582	1	0.00	<LOQ		
0.0 mg/kg/day	I05720M	1	0.9582	1	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	1	0.9582	1	104	0.0993		
10 mg/kg/day	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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	0.00	<LOQ		
0.0 mg/kg/day	I05720M	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	104	0.0993		
10 mg/kg/day	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	Filenames		
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999086-87	1/1
Filename:	See listing to the right	**Grp 3**	091599025-26	1/1
R-Squared Value:	See Attachments	**Box**	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 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
Group 1	I05718M	1	0.9582	1	0.00	<LOQ			
0.0 mg/kg/day	I05720M	1	0.9582	1	18.4	0.0176	0.0117	A	0.00841
Group 3	I05712M	1	0.9582	1	40.1	0.0384			
10 mg/kg/day	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 $C_8F_{17}COO^-$

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 36

Group Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	0.00	<LOQ		
0.0 mg/kg/day	I05720M	18.4	0.0176	0.0117 A	0.00841
Group 3	I05712M	40.1	0.0384		
10 mg/kg/day	I05716M	64.6	0.0619	0.0502	0.0166

Limit of Quantitation (LOQ): 0.0182 ug/mL

NA = Not Analyzed/Not Applicable

A = Below LOQ

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	Filenames		
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999090-91	1/1
Filename:	See listing to the right	**Grp 3**	091599027-28	1/1
R-Squared Value:	See Attachments	**Box**	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 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
Group 1	I05718M	1	0.9582	1	0.00	<LOQ		
0.0 mg/kg/day	I05720M	1	0.9582	1	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	1	0.9582	1	37.2	0.0357		
10 mg/kg/day	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 $C_8F_{17}COO^-$

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 38

Group Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	0.00	<LOQ		
0.0 mg/kg/day	I05720M	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	37.2	0.0357		
10 mg/kg/day	I05716M	22.1	0.0212	0.0284	0.0102

Limit of Quantitation (LOQ): 0.0182 ug/mL

POAA = Perfluorooctanoic Acid Anion

$C_8F_{17}COO^-$

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	Filenames		
Analytical Equipment System Number:	Soup 020199, Madeline 040198, Amelia 062498		**POAA**	**Dilutions**
Instrument Software/Version:	MassLynx 3.2	**Grp 1**	081999092-93	1/1
Filename:	See listing to the right	**Grp 3**	091599031-32	1/1
R-Squared Value:	See Attachments	**Box**	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 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.
Group 1	I05718M	1	0.9582	1	0.00	<LOQ		
0.0 mg/kg/day	I05720M	1	0.9582	1	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	1	0.9582	1	34.0	0.0325		
10 mg/kg/day	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 Dose	Sample #	POAA Conc. ng/mL	Concentration of POAA ug/mL or % Rec.	Mean POAA ug/mL	RSD Std. Dev. MS/MSD RPD
Group 1	I05718M	0.00	<LOQ		
0.0 mg/kg/day	I05720M	0.00	<LOQ	<LOQ	NA
Group 3	I05712M	34.0	0.0325		
10 mg/kg/day	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

$$\text{AR (ng/mL)} \times \text{DF} \times \text{SC} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{Reported Concentration µg/mL)}$$

Calculation Used for Group 2, Week 27, Animal ID 105702M

$$500\ \text{ng/mL} \times 100 \times 0.9582 \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{47.9 µg/mL}$$

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

$$\text{AR (ng/g)} \times \frac{\partial\ \text{curve}^{(1)}}{\partial\ \text{sample}} \times \text{SC} \times \text{DF} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{[POAA] sample (µg/g)}$$

(1) ∂ curve is assumed to be: $\frac{1\ \text{g liver}}{5\ \text{mL}\ H_2O}$

Calculation Used for Group 2, Week 27, Animal ID 105702M

$$160\ \text{ng/g} \times \frac{1\ \text{g/}\ 5\ \text{mL}}{1.0104\ \text{g/}\ 5\ \text{mL}} \times 0.9582 \times 100 \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{15.2 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (g sample/ 5 mL H_2O)
SC—POAA salt correction constant (0.9582)
DF— Dilution factor

Formula Used for Urine Analyses in Study FACT TOX-026

$$\text{AR (ng/mL)} \times \text{DF} \times \text{SC} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{Reported Concentration µg/mL)}$$

Calculation Used for Group 2, Week 26, Animal ID 105702M

$$38.6\ \text{ng/mL} \times 1000 \times 0.9582 \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{37.0 µg/mL}$$

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

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 — 4/2/01

Emily R. Stauffer
Principal Investigator
Centre Analytical Laboratories, Inc.

Date

Paul H. Lieder — 5/4/01

Paul Lieder, Ph.D., DABT
Study Director
3M Toxicology Services

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.

Phase	Date Inspected	Date Reported to Centre Management	Date Reported to Study Director and Sponsor Management
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
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.
3048 Research Drive
State College, PA 16801
(814) 231-8032

Principal Investigator, Centre:

Emily R Stauffer — 4/2/01

Emily R. Stauffer
Scientist
Centre Analytical Laboratories, Inc.

Date

Centre Analytical Laboratories, Inc. Facility Management:

John M Flaherty — 4/2/01

John Flaherty
Laboratory Manager
Centre Analytical Laboratories, Inc.

Date

Study Director, 3M:

Paul H. Lieder — 5/17/01

Paul Lieder, Ph.D., DABT
3M Toxicology Services

Date

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 3M Center, Building 220-2E-02 St. Paul, MN 55133-3320
STUDY DIRECTOR:	From 11/12/98 to 2/10/00: Kris Hansen, Ph.D. 3M Environmental Technology and Safety Services Phone: (651) 778-6018 From 2/10/00 to Present: Paul H. Lieder, Ph.D., DABT 3M Toxicology Services Phone: (651) 737-2678
TESTING FACILITY:	Centre Analytical Laboratories, Inc. 3048 Research Drive State College, PA 16801
PRINCIPAL INVESTIGATOR	From 9/19/00 to 10/23/00: Enaksha Wickremesinhe Ph.D., Centre Analytical Laboratories, Inc. Phone: (814) 231-8032 From 10/23/00 to Present: Emily R. Stauffer Centre Analytical Laboratories, Inc. Phone: (814) 231-8032

ANALYTICAL PHASE TIMETABLE:	Study Initiation Date:	11/12/98
	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:

Name	Title
Enaksha Wickremesinhe	Group/Team Leader
Emily Stauffer	Scientist
Karen Smith	Scientist
David Bell	Scientist
Tiffany Proctor	Technician
Angela Morgan	Technician
Ed Carnes	Sample Custodian
Rickey Keller	Sample Custodian
Lawrence Ord	Sample Custodian
David Shirk	Sample Custodian

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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°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.

Compound	Centre Control No.	Batch No.	Purity (%)	Expiration Date
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

$C_7F_{15}COO^-$

Note: The neutral molecule and standard form which POAA (anion) is derived from is ammonium perfluoroctanoate [$C_7F_{15}COONH_4$], molecular weight 431.

THPFOS
Chemical Name: 4-H, perfluorooctane sulfonic acid
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, $C_8F_{13}SO_3H$

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 ± 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 µg/mL by dissolving 10 mg of the standard (corrected for purity and salt content where appropriate) in methanol. From this solution, a mixed 10 µg/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 µg/mL by dissolving 10 mg of the standard in methanol. An individual 10 µg/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 Hewlet 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_2 Flow Rate: ~650 L/hr
Nebuliser N_2 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 :

Analyte	Transition Monitored	Dwell (secs)	Coll Energy (eV)	Cone (V)
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:

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

Equation 2:

$$\text{Analyte found } (\mu g/g) = \frac{\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times DF \times 1(\mu g)}{\text{sample wt. (g)} \times 1000 \text{ (ng)}}$$

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:

$$\text{Recovery (\%)} = \frac{\text{((anal. found (ng/mL) - (anal. found in corresponding sample (ng/mL)/DF) x FV (mL) x DF) x 100}}{\text{amount added (ng)}}$$

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

final vol. = 2 mL
sample wt. = 0.97 g

From equation 1:

Analyte found (ng/mL) = $\frac{[53080 - 3141.11]}{1928.40}$

= 25.9 ng/mL

From equation 2:

Analyte found (µg/g) = $\frac{[25.9\ ng/mL \times 2\ mL \times 1 \times 1\ \mu g]}{0.97\ g \times 1000\ ng}$

= 0.0534 µg/g

From equation 3:

% Recovery = $\frac{(25.9\ ng/mL \times 2\ mL \times 1)}{50\ ng} \times 100$

= 104%

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:

Analyte found (ng/mL): $\frac{\text{peak area}}{\text{mean response factor}}$

Other statistical methods used in analyzing this data were:

$$\textit{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad \textit{Mean} = \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Relative Standard Deviation (RSD or Coefficient of Variation (CV)) =

$$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100\%$$

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 ID	Centre ID	Set Number	Extraction Date	Analysis Date	Amount Added (ng)	% 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
				Average:	**NQ**
				Standard Deviation:	**NQ**
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
				Average:	**7.43**
				Standard Deviation:	**6.54**
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
				Average:	**15.4**
				Standard Deviation:	**10.2**
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
				Average:	**56.6**
				Standard Deviation:	**73.7**

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 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
				Average:	**0.0214**
				Standard Deviation:	**0.0178**
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
				Average:	**10.4**
				Standard Deviation:	**12.0**
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
				Average:	**23.4**
				Standard Deviation:	**10.6**
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
				Average:	**22.0**
				Standard Deviation:	**6.23**

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
				Average:	**0.0782**
				Standard Deviation:	**0.103**
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
				Average:	**9.46**
				Standard Deviation:	**9.21**
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
				Average:	**41.0**
				Standard Deviation:	**25.0**
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
				Average:	**36.7**
				Standard Deviation:	**34.2**

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
				Average:	**0.0498**
				Standard Deviation:	**0.0894**
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
				Average:	**7.15**
				Standard Deviation:	**5.65**
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
				Average:	**10.3**
				Standard Deviation:	**6.07**
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
				Average:	**32.0**
				Standard Deviation:	**42.9**

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
				Average:	**0.139**
				Standard Deviation:	**0.308**
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
				Average:	**7.50**
				Standard Deviation:	**2.43**
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
				Average:	**27.2**
				Standard Deviation:	**29.4**
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
				Average:	**19.9**
				Standard Deviation:	**25.2**

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
				Average:	**0.0572**
				Standard Deviation:	**0.0762**
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
				Average:	**6.88**
				Standard Deviation:	**2.62**
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
				Average:	**31.4**
				Standard Deviation:	**23.3**
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
				Average:	**18.2**
				Standard Deviation:	**28.8**

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 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
				Average:	**0.258**
				Standard Deviation:	**0.604**
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
				Average:	**5.72**
				Standard Deviation:	**7.15**
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
				Average:	**17.3**
				Standard Deviation:	**13.3**
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
				Average:	**22.1**
				Standard Deviation:	**31.7**

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 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
				Average:	**0.450**
				Standard Deviation:	**1.08**
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
				Average:	**6.81**
				Standard Deviation:	**4.89**
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
				Average:	**52.4**
				Standard Deviation:	**39.5**
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
				Average:	**37.8**
				Standard Deviation:	**58.1**

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
				Average:	**15.5**
				Standard Deviation:	**36.9**
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
				Average:	**13.8**
				Standard Deviation:	**5.22**
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
				Average:	**39.5**
				Standard Deviation:	**21.0**
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
				Average:	**25.2**
				Standard Deviation:	**36.0**

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 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
				Average:	**0.517**
				Standard Deviation:	**1.13**
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
				Average:	**6.22**
				Standard Deviation:	**5.45**
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
				Average:	**40.5**
				Standard Deviation:	**21.8**
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
				Average:	**34.6**
				Standard Deviation:	**47.7**

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 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
				Average:	**0.279**
				Standard Deviation:	**0.732**
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
				Average:	**0.387**
				Standard Deviation:	**0.372**

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
				Average:	0.0103
				Standard Deviation:	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
				Average:	0.0336
				Standard Deviation:	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

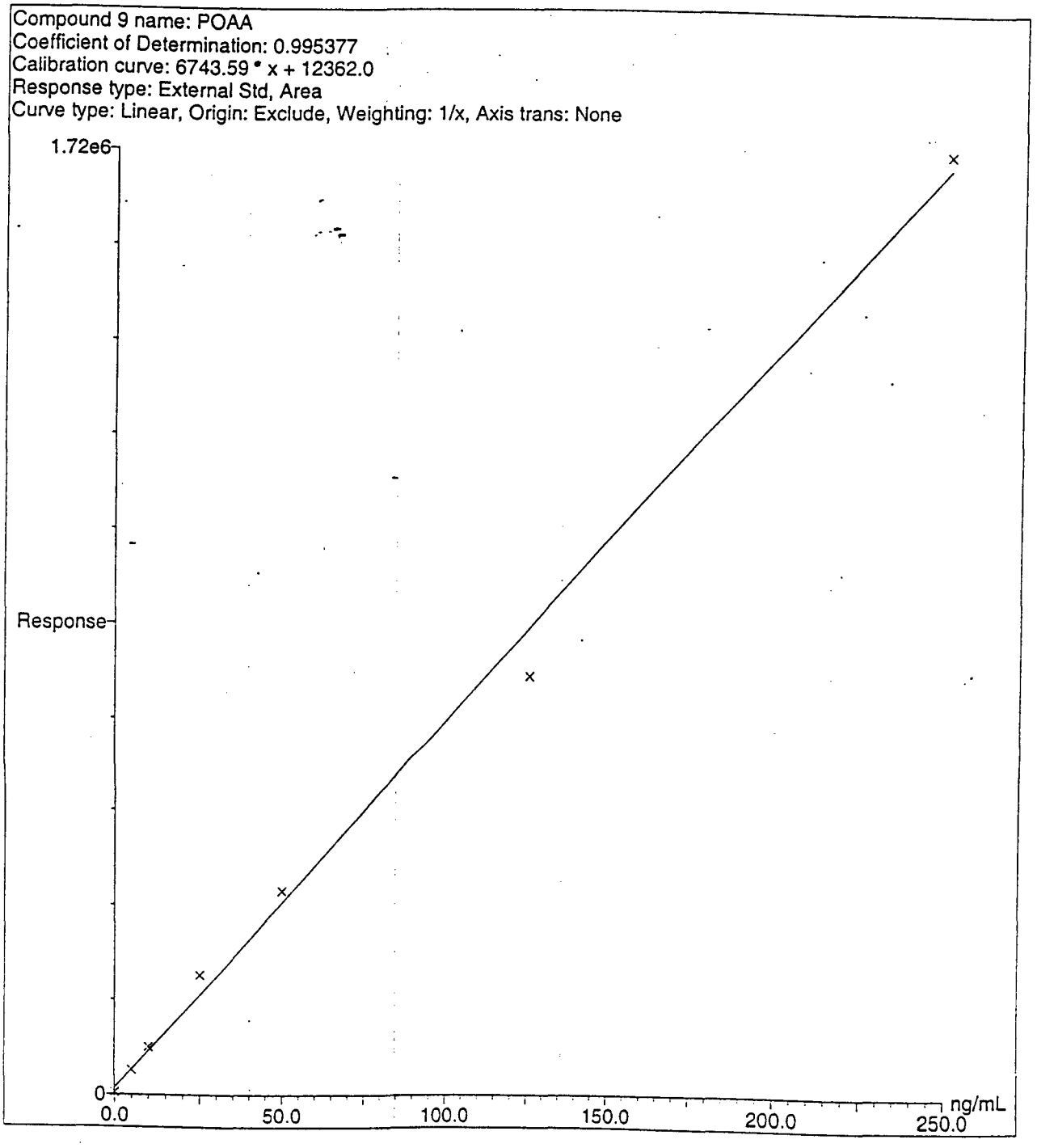


Figure 2. Typical Mean Response Factor for THPFOS

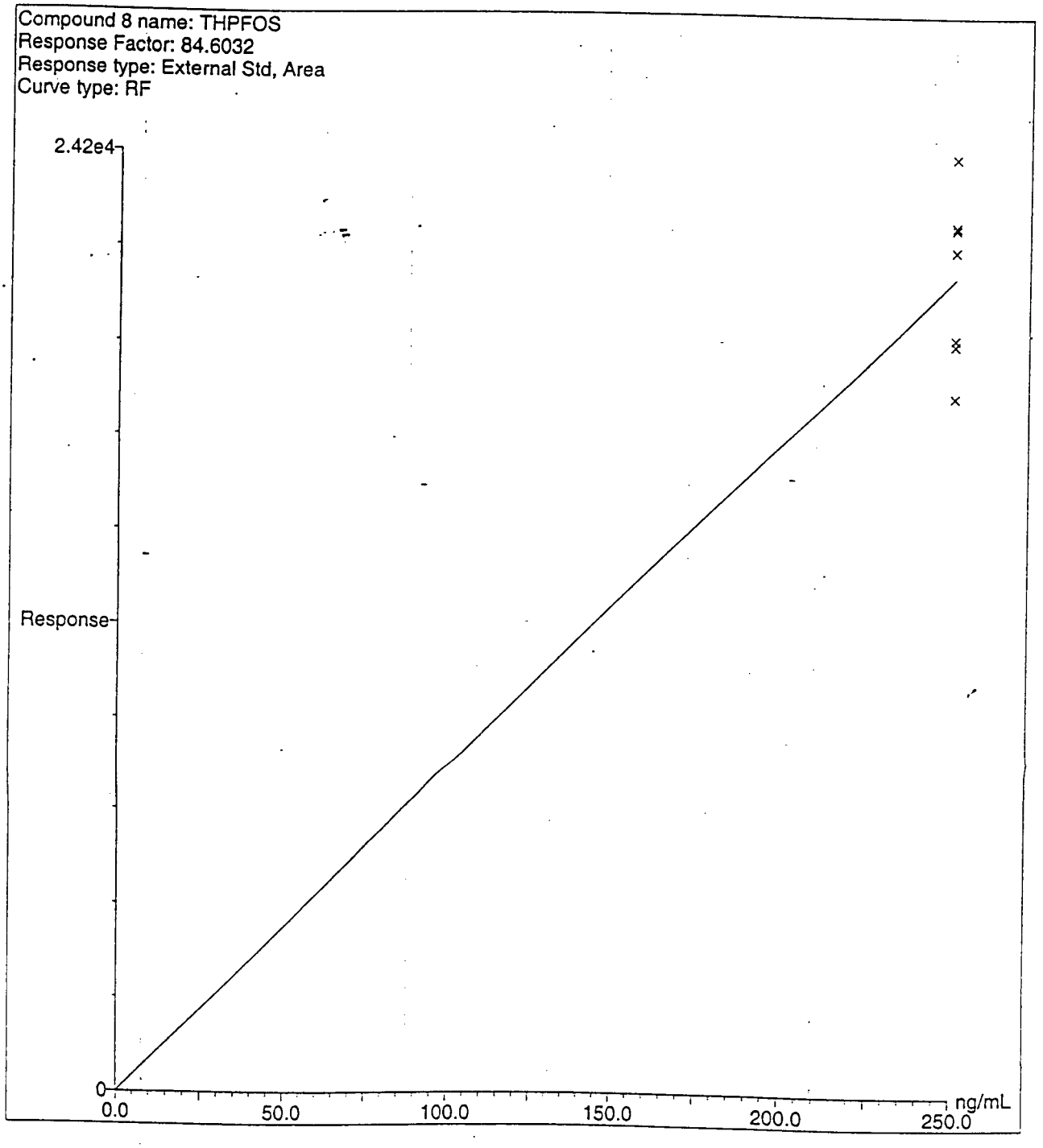


Figure 3. Chromatogram Representing a 5 ng/mL extracted standard for POAA and 250 ng/mL extracted standard for THPFOS

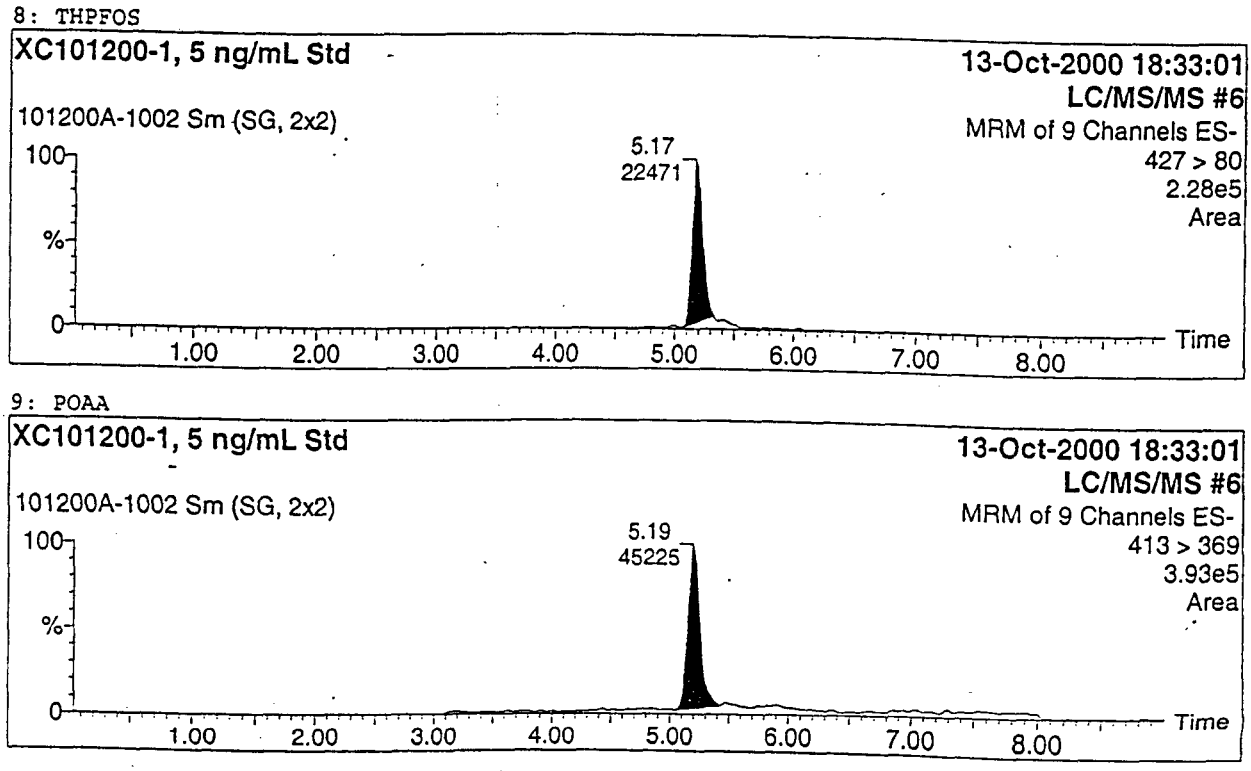


Figure 4. Chromatogram Representing a 125 ng/mL extracted standard for POAA and 250 ng/mL extracted standard for THPFOS

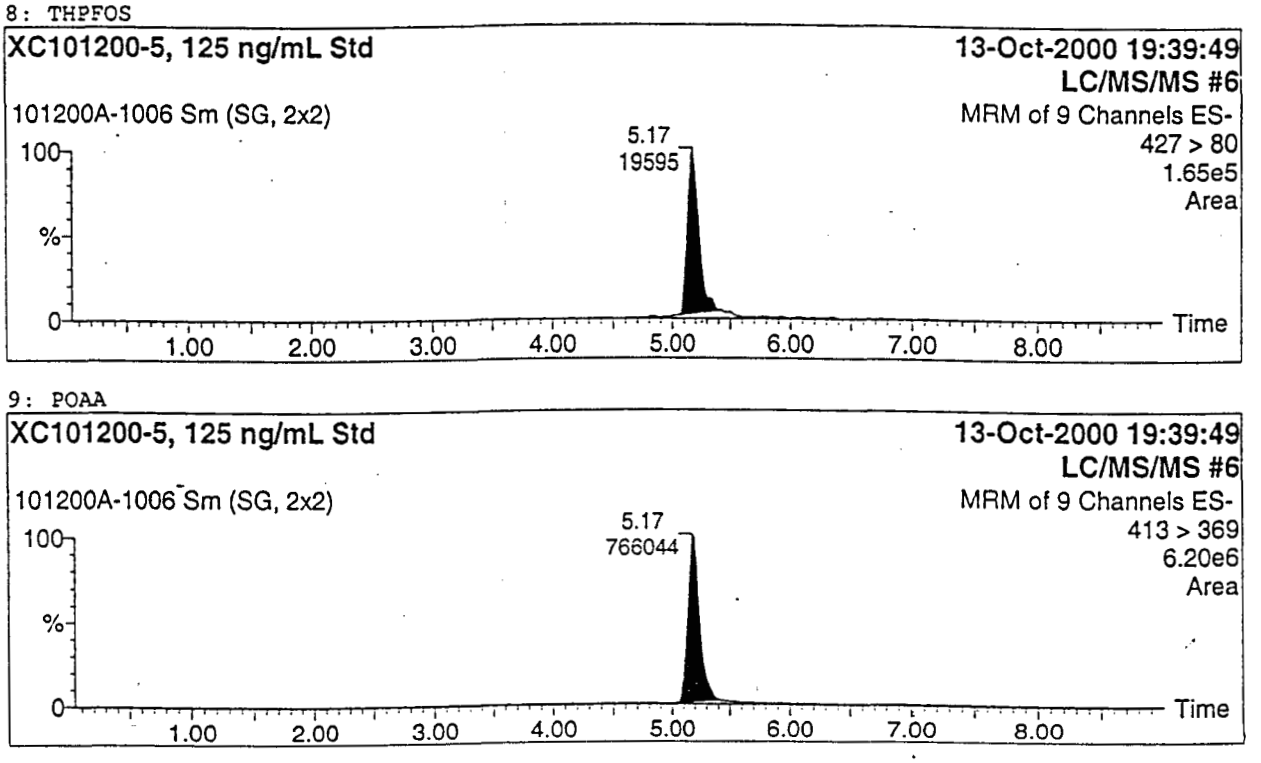


Figure 5. Chromatogram Representing Control Monkey Feces for POAA and THPFOS (Centre ID: 0009684 Blank A, Set: 101200A)

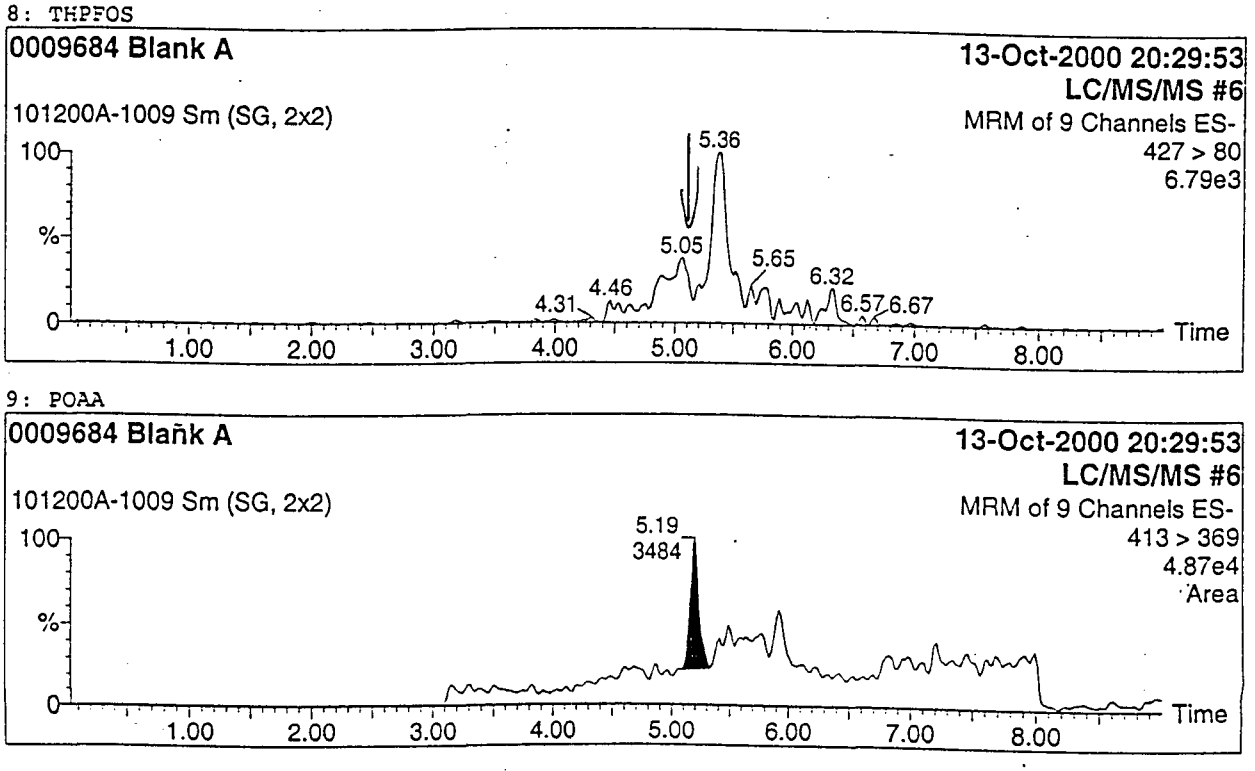


Figure 6. Chromatogram Representing Control Monkey Feces Fortified with 50 ng/g of POAA and 500 ng/g of THPFOS (Centre ID: 0008545 Spk A, Set: 101200A)

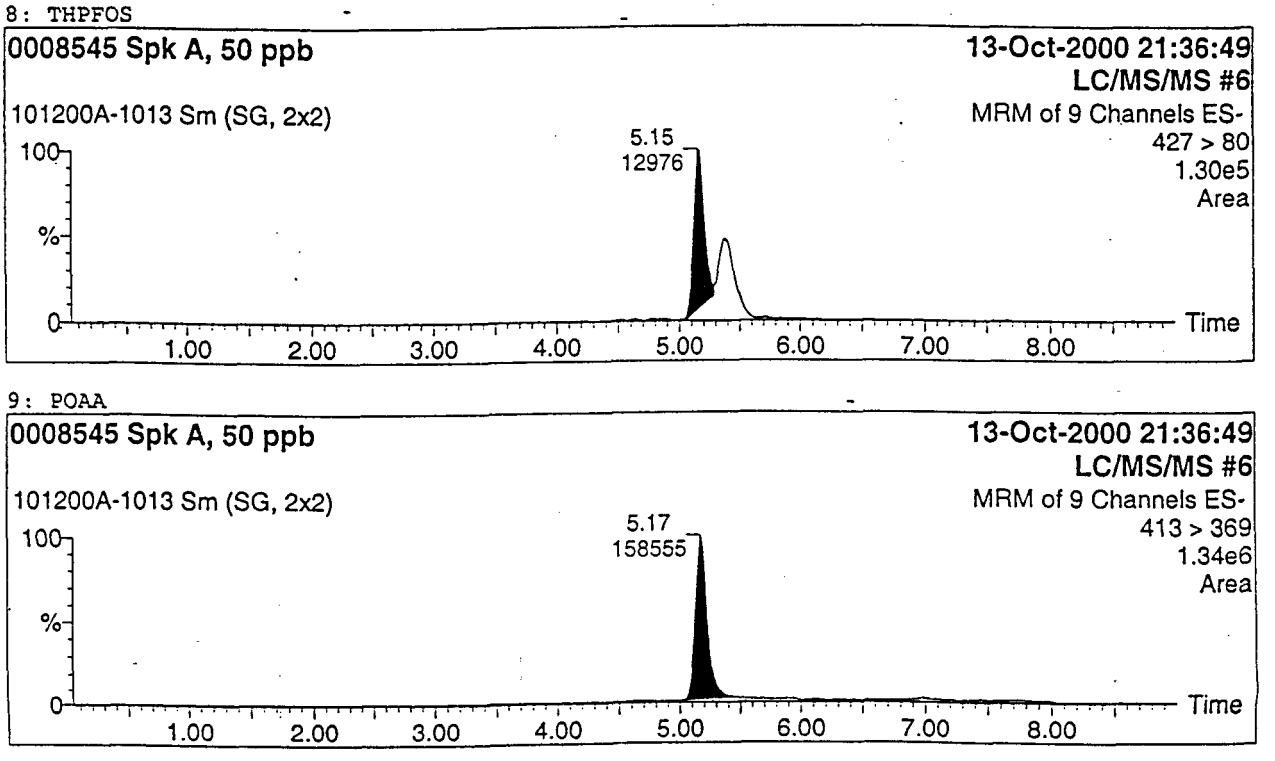
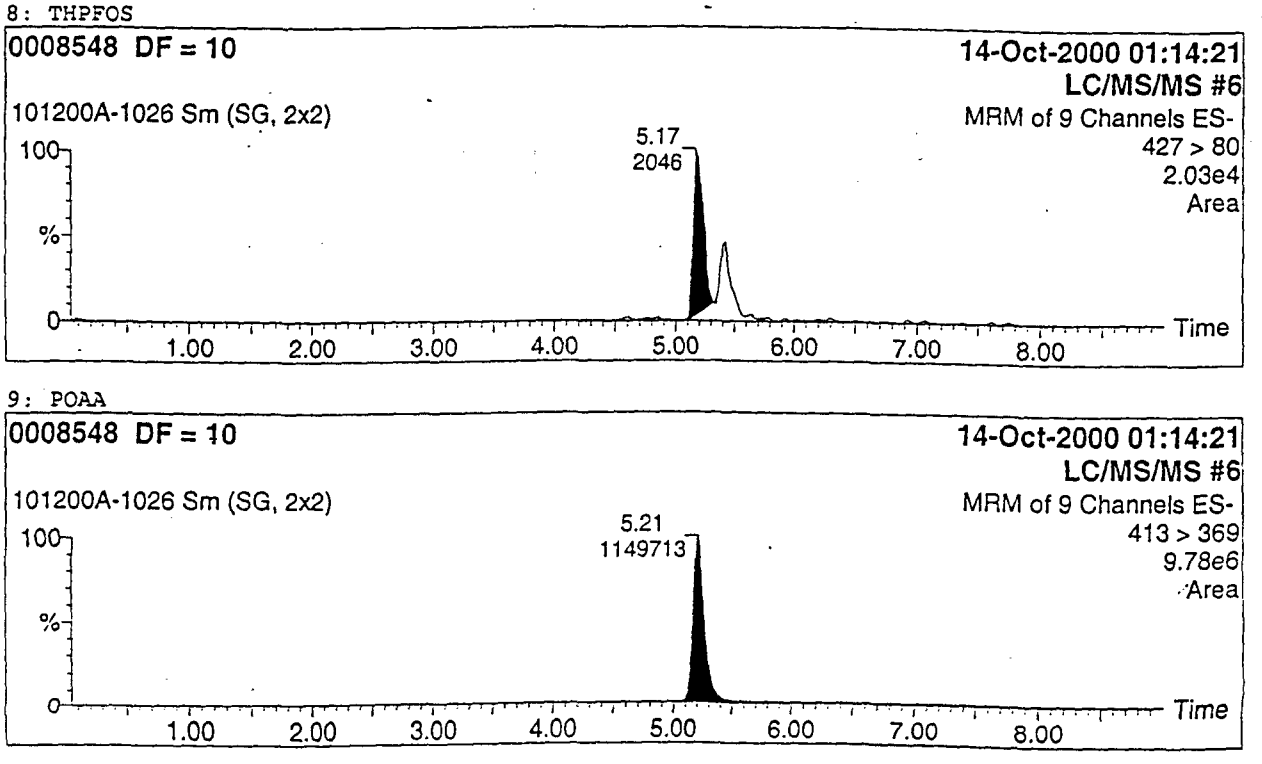


Figure 7. Chromatogram of Monkey Feces Sample from Grp 2 in Week 12 (Centre ID: 0008548, Set: 101200A)



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

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

Test Material	Ammonium perfluorooctanoate (APFO/POAA)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 P.O. Box 33220 St. Paul, MN 55133-3220
Sponsor Representative	Paul Lieder, Ph.D., DABT 3M Toxicology Services Building 220-2E-02 651-737-2678
Study Director	Kristen Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Covance Laboratories, Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106
Proposed Study Timetable	
Analytical Start Date	November 12, 1998
Analytical Termination Date	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 °C ± 10 °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 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 N. Lieder 11/3d/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

Exact Copy of Original
LAC 09/26/00
Initial Date

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 °C ± 10 °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 K. Lieder — August 3, 1999
Paul Lieder, Ph.D., DABT, Sponsor Representative — Date

Kris Hansen — 8/4/99
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

Exact Copy of Original
LAC 09/26/00
Initial Date

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.

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

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

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 V. Lieder April 19, 2000

Paul Lieder, Ph.D., Study Director — Date

3M

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 10/02/00
Initial Date

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.

Protocol TOX-026
Amendment 4

Amendment Approval

John Butenhoff 19 SEPT 2000

John Butenhoff, Ph.D. Date
Sponsor Representative, Analytical Phase

Paul N. Lieder 19 SEPT 2000

Paul Lieder, Ph.D., DABT Date
3M Study Director

Enaksha Wickremesinhe 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

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.

Amendment Approval

John L. Butenhoff 10/12/00

John L. Butenhoff, Ph.D. Date
Sponsor Representative, Analytical Phase

Paul Lieder 10/23/00

Paul Lieder, Ph.D., DABT Date
3M Study Director

Emily R Stauffer 11/2/00

Emily Stauffer Date
Centre Analytical Laboratories, Principal Analytical Investigator

3M

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

Exact Copy of Original
LAC 02/12/01
Initial Date

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.

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.

Covance Study 6329-231
Amendment 2

Amendment Approval

John L. Butenhoff, Ph.D., *Sponsor Representative* — Date: 09/FEB/01

Paul Lieder, Ph.D., *Study Director* — Date: 06 Feb 01

Centre Analytical Laboratories, Inc.
3048 Research Drive, State College, PA 16801. Phone: (814) 231-8032, Facsimile: (814)231-1253

PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: 10/3/00

Centre Study Number: 023-011 Sponsor Protocol Number: FACT-TOX-026

DESCRIPTION OF DEVIATION

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.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc.,

1. Protocol deviation issued.

Recorded By/Date: Emily R Stauffer 10/5/00

IMPACT ON THE STUDY

1. No negative impact on the study.

Emily R Stauffer 12/21/00
Principal Investigator Signature Date

John T. Butterbach 10 JAN 2001
Sponsor Representative Signature Date

Paul W. Lucke 20 March 2001
STUDY DIRECTOR DATE

CAL QAU Review MCL 12/18/00

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: 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 12/8/00

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 — Date: 12/21/00

John T. Butterhoff — Sponsor Representative Signature — Date: 10 JAN 2001

Paul W. Luch — STUDY DIRECTOR — Date: 20 March 2001

CAL QAU Review NCL 12/19/00 DATE

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: 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 12/27/00

IMPACT ON THE STUDY

1-6. No negative impact on the study.

Emily R Stauffer — Principal Investigator Signature — Date: 12/21/00

John P. Butenhoff — Sponsor Representative Signature — Date: 10 JAN 2001

Paul H Finch — Study Director — CAL QAU Review — Date: 20 March 2001

cc: EHS 3/15/01 — QAU 12/18/00

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 — 12/21/00
Principal Investigator Signature — Date

John T. Bruchalski — 10 JAN 2001
Sponsor Representative Signature — Date

David W. Fish 20 March 2001 — CAL QAU Review NCL 12/20/00
STUDY DIRECTOR DATE

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 Wickremesinhe, 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

TOTAL NUMBER OF PAGES

20

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 Wickremesinhe, 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.

06/06/00

Kris Hansen, Ph.D. Date
Group Leader
3M Environmental Laboratory

TABLE OF CONTENTS

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).

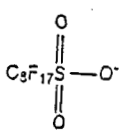
The proposed limit of quantitation (LOQ) for this method is 10 ppb each for PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA. This is based on studies conducted during the development of this method using control monkey feces.

2. FLUOROCHEMICAL 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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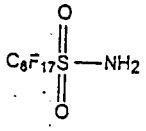
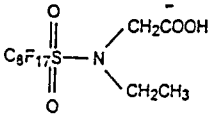
PFOSA
Molecular weight: 499



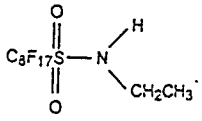
Chemical Name = Perfluorooctane sulfonylamide

PFOSAA
Molecular weight: 585



Chemical Name = perfluorooctane sulfonylamidoethylacetate

PFOSEA
Molecular weight: 527



Chemical Name = Perfluorooctanesulfonylethylamide

POAA
Molecular weight: 413

$C_7F_{15}COO^-$

Chemical Name = Perfluorooctanoate

Note: The neutral molecule and standard form that the POAA (anion) is derived from is ammonium perfluoroctanoate [$C_7F_{15}COONH_4$], molecular weight 431.

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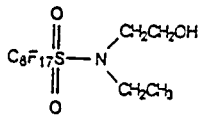
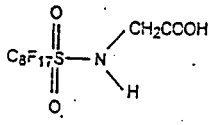
Et-FOSE-OH
Molecular weight: 571



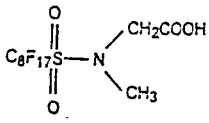
Chemical Name = 2(N-ethylperfluorooctanesulfonamido)-ethyl alcohol

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, $C_8F_{13}SO_3H$

Chemical Name = 4-H, perfluorooctane sulfonic acid

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

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 µL.	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.

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.

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 Mixed Fortification Solution (µg/ml)	Fortification Volume (µL)	Weight of Control Sample (g)	Conc. of Extracted Calibration Standard (ppb)	Vol. of Surrogate Standard* 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.

4. METHOD

4.1. FLOW DIAGRAM

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

Method Flow Diagram

Weigh 1.0 g of sample
(fortify samples designated as calibration standards and QC recoveries)
↓
Extract with ACN and filter
↓
Carbon Solid-Phase Extraction
↓
Concentrate to almost dryness
↓
Adjust final volume (2.0 mL)
↓
LC/MS/MS

4.2. SAMPLE PROCESSING

All samples are received frozen and will be kept frozen (below -10°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).

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 (± 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.

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.

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_8 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in 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

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_{18} etc.) can be used provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Parent Ion	Product Ion	Approximate Retention Time (min)
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.

Analyte	Dwell (s)	Collision Energy (eV)	Cone (V)
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

Source	Set
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

Analyzer	Set
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

Gas Flows	Set
Cone Gas	~ 150 L/hr
Desolvation	~ 700 L/hr

Pressures	Read back
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

a. 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.

b. 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.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.98 (R^2≥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), martrix zero blanks (with the surrogate standard), a set of extracted standards, and QC recovery samples.

b. Inject an aliquot (10 µL) 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 ± 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% (± 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.

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:

$$\text{Analyte found (ppb)} = \frac{(\text{peak area} - \text{intercept}) \times DF}{\text{slope}}$$

Where DF = dilution factor, if samples were diluted.

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.

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ATTACHMENT 1.

Centre Analytical Laboratories, Inc.
3048 Research Drive, State College, PA 16801. Phone: (814)231-8032 Fax: (814)231-1253

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PREPARATION OF SAMPLES FOR EXTRACTION AND ANALYSIS

Protocol No.: NA Centre Study No.: 023-003 Matrix: Feces

Method No.: NA Analytes: PFOS, PFOSA, POAA, Et-FOSE-OH, PFOSEA, PFOSAA, M 570, M 556, THPFOS

Sponsor Sample ID	Centre Sample ID	Sample Description	Sample Weight (g)	Surrogate Fort. Vol. (µL)	Fortification Standard ID	Fort. Std. Conc. (µg/mL)	Fort. Vol. (µL)	Fort. Level (ppb)
		Matrix blank	1.0	-	-	-	-	-
		Matrix zero blank	1.0	200		-	-	-
		Std 1	1.0	200		0.1	100	10
		Std 2	1.0	200		0.1	200	20
		Std 3	1.0	200		0.5	100	50
		Std 4	1.0	200		0.5	200	100
		Std 5	1.0	200		2.5	100	250
		Std 6	1.0	200		2.5	200	500
		Std 7	1.0	200		10.0	100	1000
				Fortified with: _____ Capacity: _____ µL				
				Initials/Date: ____ / ____				

If a sample is not fortified, draw a line through the fortification section. Vertical arrows in a column indicate identical values.

Samples removed from freezer # _____ Time:_____ Initials/Date: ____/____

Samples were weighed on Balance # _____ Time:_____ Initials/Date: ____/____

Samples returned to freezer # _____ Time:_____ Initials/Date: ____/____

* Surrogate Std ID : _____ Initials/Date: ____/____

Samples extracted with ACN and filtered : Initials/Date: ____/____

Carbon SPE clean-up and rotovap : Initials/Date: ____/____

Final volume adjusted to 2 mL with methanol : Initials/Date: ____/____

Extracts placed in refrigerator # _____ Initials/Date: ____/____

Reagents	Lot #
Acetonitrile	
Glass Acrodisc	
Carbon 120/400	
Ascorbic Acid	
Methanol	
1-Octanol	
$C_8H_4Cl_2Si$	
Toluene	

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METHOD DEVIATION

Deviation Number: 1

Date of Occurrence: 10/04-05/00

Centre Study Number: 023-011 Sponsor Protocol Number: FACT-TOX-026

DESCRIPTION OF DEVIATION

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 60%, 54%, 65%, 66%, 57%, and 60% were accepted for EtFOSE-OH in Set 100200AR.

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: Emily R Stauffer 10/5/00

IMPACT ON THE STUDY

1. No negative impact

Emily R Stauffer 12/21/00
Principal Investigator Signature Date

Paul N Keith 2 CAL QAU Review NRL 12/19/00 21 March 2001
STUDY DIRECTOR DATE February 12, 1998/2

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METHOD DEVIATION

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

DESCRIPTION OF DEVIATION

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.

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: Emily P Stauffer 12/19/00

IMPACT ON THE STUDY

1. No negative impact

Emily R Stauffer 12/21/00
Principal Investigator Signature Date

Paul N Lush 21 March 2001
Study Director DATE

CAL QAU Review NCL 12/22/00

February 12, 1998/2

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METHOD DEVIATION

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, (5) 10/24-25/00, (6) 10/26/00

Centre Study Number: 023-011 Sponsor Protocol Number: FACT-TOX-026

DESCRIPTION OF DEVIATION

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.

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1-6. Method deviation issued.

Recorded By/Date: Emily R Stauffer 12/19/00

IMPACT ON THE STUDY

1-6. No negative impact

Emily R. Stauffer 12/21/00
Principal Investigator Signature Date

STUDY DIRECTOR CAL QAU Review DATE 12/22/00

February 12, 1998/2

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METHOD DEVIATION

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

DESCRIPTION OF DEVIATION

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.
2. Section 4.3.5: Only prepared 2 QC samples for sets that contained more than 20 samples.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1-2. Method deviation issued

Recorded By/Date: Emily R Stauffer 12/8/00

IMPACT ON THE STUDY

1-2. No negative impact

Emily R Stauffer 12/12/00
Principal Investigator Signature Date

Paul W Lieb 5/4/01 CAL QAU Review MLL 12/15/00
STUDY DIRECTOR DATE

February 12, 1998/2

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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 ammounium acetate.
2. . Section 4.5.4 Sample Analysis (Item e.)
   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:

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

   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):

$$\text{Recovery (\%)} = \frac{\text{((anal. found (ng/mL) - (anal. found in corresponding sample (ng/mL)/DF)} \times \text{FV (mL)} \times \text{DF)} \times 100}{\text{amount added (ng)}}$$

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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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 (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 T. Fitzsimmons 10 JAN 2001
Date

Paul H 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

Appendix I: Report Signature Page

Paul Lieder, Ph.D., DABT, *Study Director* — June 11, 2001
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

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

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