## *Study Title*

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats (Covance Study No.: 6329-183)

## *Analytical Laboratory Report Title*

Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of Crl:CD®(SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

## *Data Requirement*

Not Applicable

## *Author*

3M Environmental Laboratory

## *Study Completion Date*

At signing

## *Performing Laboratories*

**Liver and Serum Analyses**

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

## *Project Identification*

3M Medical Department Study: T-6295.4
Covance In-Life Study: 6329-183
Analytical Report: FACT TOX-002
3M Laboratory Request No. U2121

## *Total Number of Pages*

181

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## GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of Crl:CD®(SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

Study Identification Number: T-6295.4, FACT TOX-002, LRN-U2121

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions in the bulleted list below.

Exceptions to GLP compliance:

- There were two study directors in this study. This study was designed as two separate studies. The in-life phase study was considered to end at the generation and shipment of specimens. The analytical study was considered to start at the receipt of these specimens for analysis. This resulted in having two separate study directors, one for each phase of the same study. However, since the technical performance of each phase was entirely separate, no effect is expected from this exception.
- Changes in the raw data entries were not all made in accordance with 21 CFR 58.130 (e).
- Specimen stability storage will not be determined.
- One of the control matrices (rabbit liver) was not identified in accordance with 21 CFR 58.120 (a)(2).
- The electronic data systems in use have not been validated and there is not an electronic audit trail of corrections currently available (21 CFR 58.130 (e)). Authenticated hard copies of chromatograms and associated documents will be considered as the original raw data.
- Characterization of the analytical standards is underway, but was not completed at the time this data was assembled (21 CFR 58.105 (a)). When Certificates of Analysis for all remaining lots of PFOS are available, an amendment to this report will be issued. Lot No. 59909 of THPFOS will not be characterized for purity since quantities of this standard are exhausted.

John R. Bartlett — 21 FEB 01
*Study Director* Date

Marvin T. Case — 21 Feb 2001
*Sponsor Representative* Date

## GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Specimens of Crl:CD®(SD) IGS BR Rats Exposed to Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295)

Study Identification Number: T-6295.4, FACT TOX-002, LRN-U2121

This study has been inspected by the 3M Lab 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 |
| 11/11/99 | Extraction | 11/30/99 | 11/30/99 |
| 08/28/00 – 09/18/00 | Data | 09/19/00 | 09/19/00 |
| 10/05/00 – 10/06/00, 10/16/00 – 10/20/00, 10/30/00 – 10/31/00 | Data | 11/02/00 | 11/02/00 |
| 11/01/00 – 11/03/00 | Data | 11/06/00 | 11/06/00 |
| 11/02/00 – 11/03/00, 11/06/00 – 11/07/00 | Data | 11/08/00 | 11/08/00 |
| 01/08/01 – 01/11/01 | Draft Report | 01/12/01 | 01/12/01 |

Casey Howell — *QAU Representative*

Feb 19, 2001 — Date

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## List of Tables

# Study Personnel and Contributors

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### Sponsor

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative*
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### Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test article and analytical reference standard reserve samples, as well as the specimens pertaining to the analytical phase of this study are archived at the 3M Environmental Laboratory.

# Introduction and Purpose

The purpose of the study is to determine the presence and concentration of PFOS in liver and serum specimens collected from Covance Study No.: 6329-183 titled: 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS, T-6295) in Rats, initiated on 22 May 1998.

## Test System

360 males and 360 females were used as the test system. Table 1 outlines the rat population demographics and dosage levels for study 6329-183.

The test system species and strain selected was the Crl:CD®(SD) IGS BR rat received from Charles River Laboratories, Inc., identified using an implanted microchip device. At the initiation of treatment the rats were approximately 6–8 weeks of age and weighed between 100–300 g.

**Table 1. Test System Population Demographics for Study (6329-183)**

| Study Group | Number of Animals | | Dietary Levels (ppm) |
|---|---|---|---|
| | Male | Female | |
| Group 1 Control | 70 | 70 | 0 |
| Group 2 Low | 60 | 60 | 0.5 |
| Group 3 Mid | 60 | 60 | 2.0 |
| Group 4 Mid-High | 60 | 60 | 5.0 |
| Group 5 High | 70 | 70 | 20.0 |
| Group 6 High Recovery | 40 | 40 | 20.0 |

## Specimen Collection and Analysis

Sample specimens were collected by Covance (study 6329-183) and sent to the 3M Environmental Laboratory for analysis. On week 4 and week 14, 50 liver and 50 serum specimens were collected from Groups 1–5 males and females. On week 53, 39 liver and 19 serum specimens were collected from Groups 1 and 5 males and females. On day 719, 9 liver and 9 serum specimens were collected from Group 3 females. At the time of terminal sacrifice week 105), 164 liver and 164 serum specimens were collected from Groups 1–5 males and females. At the time of recovery sacrifice (week 106), 27 liver and 27 serum specimens were collected from Group 6 males and females. 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 5 (through 4/21/00):**
**Serum Specimens**—292 specimens
**Liver Specimens**—312 specimens

**Specimens Collected from Study Group 6 (Recovery) on 4/24/00 and 4/27/00:**
**Serum Specimens**—27 specimens
**Liver Specimens**—27 specimens

Blood specimens were allowed to clot at room temperature and separated by centrifugation. Serum was then harvested and stored in a freezer at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were flash frozen in liquid nitrogen and then stored in a freezer at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver and sera specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice.

Sera and liver specimens were extracted beginning on May 27, 1998 using an ion pairing reagent and either ethyl acetate or methyl-*tert*-butyl ether (MtBE). Liver specimens were homogenized prior to the extraction procedure. Specimen extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ESMSMS) in the multiple reaction monitoring mode. PFOS levels were quantitated by external calibration of extracted curves. Analytical details are included in this report.

## Specimen Receipt and Maintenance

The 3M Environmental Laboratory received liver and serum specimens collected at predetermined time points during and at the end of the *in-life* phase of Covance Study No. 6329-183 from May 1998 through May 2000 from Covance. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-002 were obtained from commercial sources and are presented in Appendix A (see Tables 4 and 5). Specimens analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

## Chemical Characterization

Chemical characterization information on the test article used in this study is presented in Appendix A (see Table 6). Chemical characterization information on the analytical reference materials used in this study is presented in tabular form (Table 2) below.

**Table 2. Characterization of the Analytical Reference Materials Used in Study FACT TOX-002**

| Reference Material / Formula | Acronym | Source | Expiration Date | Storage Conditions | Chemical Lot Number | Physical Description | Purity |
|---|---|---|---|---|---|---|---|
| Potassium Perfluorooctanesulfonate $C_8F_{17}SO_3$-K+ | KPFOS | 3M Specialty Chemicals | 08/31/00 | Ambient temperature | 171 | White crystalline powder | 86.4% |
| | | 3M Specialty Chemicals | 2010 | Ambient temperature | 193[a] | White powder | 88.0%[b] |
| | | 3M Specialty Chemicals | 2010 | Ambient temperature (dry) | 215[a] | White powder | TBD |
| 1H, 1H, 2H, 2H-Tetrahydroperfluorooctanesulfonic acid $C_8H_4F_{13}SO_3H$ | THPFOS | ICN Biomedics, Inc. | 01/01/2010 | Ambient temperature | 53406 | Brown waxy solid | TBD |
| | | ICN | 01/01/2010 | Ambient temperature | 59909 | Brown powder | N/A |

[a] Used for analysis of week 4 and week 14 sera samples.
[b] Samples were not corrected for purity.
TBD—to be determined
N/A—not applicable. This lot is exhausted and cannot be characterized.

### Dose Confirmation Analyses

Dose preparation methods and analysis were performed by Covance, using a validated analytical method provided by the Sponsor (MP-M383-MA), and are reported separately (Reference Covance 6329-183).

## Method Summaries

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

As the present study progressed, more advanced methods evolved and earlier methods were used with deviations until amendments to the protocol were written. Protocol and method deviations are located in Appendix B of this report.

### 3M Environmental Laboratory

#### *PREPARATORY METHODS*

- **FACT-M-1.0**. “Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry”. This method was used for week 4 specimens extracted on 6/11/98 and week 14 specimens extracted on 8/6/98.
- **FACT-M-3.0**, “Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Serum for Analysis using HPLC-Electrospray/Mass

Spectrometry". This method was used for week 4 specimens extracted on 5/27/98 and week 14 specimens extracted on 7/27/98.

- **ETS-8-4.1**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 specimens extracted on 11/10/99, and day 719, week 105, and week 106 specimens extracted on 5/1/00 and 5/2/00.
- **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 specimens extracted on 11/10/99 and 1/25/00, day 719 specimens extracted on 5/16/00 and 5/18/00, week 105 specimens extracted on 5/16-18/00 and 5/22/00, and week 106 specimens extracted on 5/16/00 and 5/18/00.

An ion-pairing reagent was added to the specimen and the analyte ion pair was partitioned into ethyl acetate (FACT-M-1.0 and FACT-M-3.0) or MtBE (ETS-8-4.1 and ETS-8-6.0). 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 then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

***ANALYTICAL METHODS***

- **FACT-M-4.0**, "Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry". With the exception of week 4 sera specimens, data was worked up according to ETS-8-5.1 or ETS-8-7.0 and following control of bias documentation included in study binder.
- **ETS-8-5.1**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry". Sera run 7/15/98 was accepted despite the failure of the continuing calibration checks. Since compliance with method ETS-8-5.1 was in the protocol, deviations from it, including this one are also protocol deviations. A method deviation has been written.
- **ETS-8-7.0**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver 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 PFOS using HPLC-ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3$-) analysis, was fragmented further to produce ion 99 ($FSO_3$-). The characteristic product ion 99 was monitored for quantitative analysis.

### *ANALYTICAL EQUIPMENT*

The actual analytical equipment settings used in the present analytical phase of this study varied slightly during actual data collection. The following is representative of the analytical equipment settings used during the analytical phase of this study.

**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: 13.5 minutes

| *Time (minutes)* | *%B* |
|---|---|
| 0.0 | 40% |
| 8.5 | 90% |
| 11.0 | 90% |
| 12.0 | 40% |
| 13.5 | 40% |

**Mass Spectrometer:** Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.1, 3.3, and 3.4
Cone Voltage: 30–60 V
Collision Gas Energy: 25–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

**Table 3. Negative Ions Monitored in 3M Laboratory Analyses**

| Target Analyte | Primary Ion (AMU) | Product Ion (AMU) |
|---|---|---|
| **PFOS** | 499.0 | 80.0, 99.0, 130.0 |
| **THPFOS** | 427.0 | 80.0 |

## Deviations

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.

## Data Quality Objectives and Data Integrity

The following data quality objectives (DQOs) were indicated in the method performance section of ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry and ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry:

- **Linearity:** The coefficient of determination ($r^2$) equal to or greater than 0.980.
- **Limits of Quantitation (LOQ):** The LOQ for PFOS is 5.55 ng/mL for serum and 26.9 ng/g for liver in the respective method performance sections, however, the LOQ for a given run is based upon the concentration of the lowest acceptable standard in the run.
- **Acceptable Matrix Spike Recoveries:** 70–130% for sera / 50–150% for liver.

## Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Lab methods ETS-8-5.1 and ETS-8-7.0 (see Appendix C) 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 ≥0.985.
- **Calibration Standards:** With the exception of the week 4 sera data, quantitation of the target analytes was based on linear regression analysis of two extracted matrix curves weighted 1/x bracketing each group of samples. Calibration standards were not diluted and were spiked to be within the linear range of the instrument. Week 4 sera data was evaluated using an unweighted curve. 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 may have been deactivated. Quantitation of PFOS was based on the response of one or more 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 extraction blanks. For both liver and sera analysis, the LOQ decreased over the course of the study as methods improved. The highest LOQs in both analyses occurred in the week 4 analysis and were 23.2 ng/mL (sera) and 27.8 ng/g (liver).
- **Blanks:** All blanks were below the lower limit of quantitation for PFOS. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed rat sera and liver, rabbit sera and liver were selected as a suitable surrogate matrix for blanks, curves, and CCVs.
- **Precision:** Not determined for this study.

- **Matrix Spike Recoveries:** Rat sera from 18 control animals were spiked prior to extraction. PFOS was spiked at approximately 75 ng/g, 100 ng/g, and 250 ng/g levels. With two exceptions, all Matrix Spike/Matrix Spike Duplicate (MS/MSD) were recovered at ±30% of expected values. One MS, analyzed with the week 4 samples had a recovery of 167%; one MS analyzed with the week 53 samples was recovered at 131%. These high recoveries were confirmed. Because the remaining 16 spikes were acceptable, the high recoveries do not indicate serious analytical deficiencies.

  Rat liver from 20 control animals were spiked prior to extraction. PFOS was spiked at approximately 100 ng/g and 250 ng/g levels. Thirteen of the 20 spikes were within 50% of the expected value; seven were outside of this range. Four of the deviant spikes, analyzed with the day 719 samples, were mistakenly prepared from G3 and G6 animals instead of from control animals (G1). The high levels of PFOS in the tissue of these test animals obscured the relatively low level of material spiked (spike was <3% of endogenous). This mistake in preparation invalidates these results as indicators of method QC. Two of the remaining deviant spikes were analyzed with the week 4 samples and one deviant MS was analyzed with the week 53 samples. Because 13 of 16 matrix spikes that were prepared correctly were acceptable, the deviant recoveries do not indicate serious analytical deficiencies.

  All matrix spikes were prepared at levels approximate to G1 samples.

- **Surrogates:** The surrogate (THPFOS) was added to all specimens and standards before extraction, as stated in the methods. THPFOS was spiked into samples at concentrations within the linear range when samples were analyzed without dilution. Prior to May 5, 2000, THPFOS was not used for quantitation, but was used to monitor for gross instrument failure (see Appendix C, Analytical Methods). In analyses performed after May 5, 2000, the surrogate response of each analytical run was verified to determine that it did not vary more than ±50% from the mean within each analytical run. At no time was THPFOS used for quantitation as an internal standard. No problems were observed with these data.

### 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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

### Summary of Specimen Results

- **Specimens from Control Animals:** Low levels of PFOS were often detected in the sera and liver of the control animals.
- **Specimens from Dosed Animals:** Detailed specimen data tables are presented in Appendices D and E.

## Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. All specimen results were used to calculate the mean values; the LOQ value was used for specimens with <LOQ results. See Appendix F for example calculations used to generate the liver and serum specimen data in FACT TOX-002.

## Statement of Conclusion

Under the conditions of the present studies, PFOS was observed in the sera and liver of rats dosed with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) during the in-life phase of the study.

## Reference

Covance Study No.: 6329-183, 104-Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS; T-6295) in Rats

# Appendix A: Identification of Test Article and Control Matrices

**Table 4. Characterization of the Control Matrices Used for Sera Analyses in Study FACT TOX-002**

| Control Matrix | Rabbit Serum | Rabbit Serum | Rat Serum | Rat Serum |
|---|---|---|---|---|
| Source | Sigma | Sigma | Sigma | Sigma |
| Expiration Date | 2010 | 2010 | 2010 | 2010 |
| Storage Conditions | Frozen | Frozen | Frozen | Frozen |
| Lot # | 47H4641 | 118H8418 | 17H9306 | 19H89292 |
| Physical Description | Rabbit Serum | Rabbit Serum | Rat Serum | Rat Serum |

**Table 5. Characterization of the Control Matrices Used for Liver Analyses in Study FACT TOX-002**

| Control Matrix | Rabbit Liver | Rabbit Liver | Rabbit Liver | Rabbit Liver |
|---|---|---|---|---|
| Source | CHW | CHW | N/R | CHW |
| Expiration Date | 2010 | 2010 | 2010 | 2010 |
| Storage Conditions | Frozen | Frozen | Frozen | Frozen |
| Lot # | F00014 | F00016 | N/R | F00007 |
| Physical Description | Rabbit Liver | Rabbit Liver | Rabbit Liver | Rabbit Liver |

N/R—not recorded

**Table 6. Identification of the Test Article in Study FACT TOX-002**

| | Test Article |
|---|---|
| **Chemical Name** | **KPFOS**<br>Perfluorooctane sulfonic acid potassium salt |
| **Source** | 3M ICP/PCP Division |
| **Expiration Date** | 08/31/01 |
| **Storage Conditions** | Ambient temperature |
| **Chemical Lot #** | 217 |
| **Physical Description** | White crystalline powder |
| **Purity** | 86.9% |

# Appendix B: Protocol, Amendments, Deviations

# 3M Environmental Laboratory

## Protocol - Analytical Study Phase

## 104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

**In-Vivo Study Reference Number:** Covance # 6329-183

**Study Number:** AMDT – 112296.1
**Test Material:** T-6295

**Name and Address of Sponsor:**
3M Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

**Name and Address of Testing Facility:**
3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55106

**Proposed Experimental Start Date:** May 25, 1998
**Proposed Experimental Termination Date:** December 31, 2001

**Method Numbers and Revisions**

**FACT-M-1.0,** Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry
**FACT-M-2.0,** Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry
**FACT-M-3.0,** Extraction of Perfluorooctanesulfonate from Sera for Analysis using HPLC-Electrospray/Mass Spectrometry
**FACT-M-4.0,** Analysis of Sera Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

Author: Lisa Clemen

5/22/98
Kris J. Hansen, PhD Date
Study Director

7/15/99
Dale Bacon Date
Study Director Management

5/25/99
Andrew M. Seacat, PhD Date
Sponsor Representative

## 1.0 PURPOSE

**1.1** According to this analytical protocol, the 3M Environmental Laboratory will analyze the tissue and fluid samples from the Covance study number 6329-183, "104-Week Dietary Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats." The collected data will be provided to the sponsor for use in the assessment of toxicological effects of the test material when administered in the diets of rats for at least 104 weeks.

**1.2** Data collected in the Environmental Laboratory will be considered non-quantitative screening data until future studies have been conducted to determine absolute recoveries of specific or general fluorochemical compounds.

## 2.0 REGULATORY COMPLIANCE

**2.1** This analytical phase of the study will be conducted in accordance with the FDA Good Laboratory Practices Regulations 21 CFR 58, with the following exceptions:

**2.1.1** The analytical phase is being conducted as a separate study and therefore has a separate Study Director, protocol, and final report, from those listed in the Covance protocol 6329-183.

**2.1.2** The characterization of the reference material, including purity, identity, and stability, are the responsibility of the sponsor.

**2.1.3** Sample storage stability will not be determined.

## 3.0 TEST MATERIALS

**3.1 Control, and reference Materials and Matrices**

**3.1.1 Analytical Reference Material:** T-6295, from 3M ICP/PCP Division

**3.1.2 Analytical Reference Matrix:** Rat liver, from Covance and rat serum, from Sigma Chemical Company.

**3.1.3 Analytical Control Material:** None.

**3.1.4 Analytical Control Matrix:** Rat liver, from Covance and rat serum, from Sigma Chemical Company.

**3.2 Number of Test and Control Samples:** Throughout the course of the study, liver and serum from 580 test animals and 140 control animals will be made available. Samples will be analyzed as requested by the sponsor or the study director. Other biological tissues (kidney, bile, dermal application site, and cellular fraction) will be available for analysis if deemed appropriate.

**3.3 Identification of Test and Control Samples:** The samples will be identified using the Covance animal identification number that consists of a letter and five-digit number, plus the tissue identity and day identity (serum).

**3.4 Purity and Identity of Reference Material:** To be determined by Sponsor.

**3.5 Stability of Reference Material:** To be determined by Sponsor.

**3.6 Storage Conditions for Reference Materials:** Reference materials will be stored at room temperature (3.1.1), and samples will be stored at -20 ± 10°C (3.1.2, 3.1.4). Test and Control samples will be received according to AMDT-S-10-0.

**3.7 Disposition of Specimens:** Biological tissues and fluids will be retained per GLP Regulation for the time period required for studies longer than 28 days.

**3.8 Safety Precautions:** Refer to appropriate MSDS. Wear appropriate laboratory attire. Use caution when handling knives for cutting tissue samples.

## 4.0 EXPERIMENTAL - Overview

**4.1** The tissues from animals dosed as described (Covance# 6329-183), will be available for analysis for fluorine-containing compounds. At the discretion of the Study Director, a series of analytical tests can be performed. All high dose and control sera and livers will be analyzed initially using HPLC-electrospray mass spectrometry to identify fluorine-containing compounds of interest present in the sera and liver (if any). The screening for organic fluoride in liver via combustion may be performed to present definitive data for fluorine in the liver. Based on the findings from these analyses, additional samples, tissues, or fluids may be analyzed at the discretion of the Study Director to determine the presence of fluorochemicals in these matrices.

## 5.0 EXPERIMENTAL - Methods

**5.1 Methods** (attached):

- **5.1.1 FACT-M-1.0,** "Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"
- **5.1.2 FACT-M-2.0,** "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry"
- **5.1.3 FACT-M-3.0,** "Extraction of Perfluorooctanesulfonate from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry"
- **5.1.4 FACT-M-4.0,** "Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry"

## 6.0 DATA ANALYSIS

**6.1 Quality Control:** Matrix spikes will be extracted and analyzed to determine accuracy of the method. Also, continuing calibration checks will be analyzed to determine response bias.

**6.2 Transformations:** Any transformations performed on data collected during the analytical phase of the study will be documented in the final report.

**6.3 Statistics:** At the discretion of the Study Director, statistics used may include regression analysis of serum concentrations with time, averages, and standard deviations of concentrations for the different dose groups. If necessary, simple tests such as the Student's t-test may be applied to determine statistical difference. Any statistical analysis performed will be documented in the final report.

**6.4 Data Reporting:** A final data package will be submitted to 3M Toxicology Services. The data package will include the following with additional data included as deemed appropriate.

**6.4.1** A summary of individual sample results, reported as a concentration (weight/weight, weight/volume) of fluoride per tissue or fluid, or as the mass of a specific fluorochemical (HPLC-electrospray mass spectrometry) per unit of tissue or fluid.

**6.4.2** A summary of quality control results (continuing calibration checks, method blanks, instrument blanks, matrix spikes, and matrix spike duplicates).

**6.4.3** Certified copies or originals of the written validated methods.

**6.4.4** Certified copies or originals of sample identification sheets sent from Covance.

**6.4.5** Certified copies or originals of study specific raw data.

**6.4.6** A summary of key personnel involved with the analytical phase of the study.

**6.4.7** A signed QAU statement listing the dates of inspections and reports of findings to management and Study Director.

## 7.0 MAINTENANCE OF RAW DATA AND RECORDS

**7.1** The following raw data and records (or certified copies thereof) will be maintained in the study folder in the archives according to appropriate SOPs.

**7.1.1** Approved protocol
**7.1.2** Approved methods
**7.1.3** Data summaries
**7.1.4** Study correspondence
**7.1.5** Shipping records
**7.1.6** Raw data
**7.1.7** Electronic copies of data

**7.2** Supporting records to be retained separately from the study folder in the archives according to 3M ET & SS SOPs, will include, but not necessarily be limited to the following:

**7.2.1** Approved validation reports
**7.2.2** Training records
**7.2.3** Calibration records
**7.2.4** Instrument maintenance logs
**7.2.5** Standard operating procedures, equipment procedures, and methods
**7.2.6** Appropriate specimens

## 8.0 REFERENCES

**8.1** Approved AMDT standard operating procedures.

**8.2** Approved ETS standard operating procedures.

## 9.0 ATTACHMENTS

**9.1** **FACT-M-1.0,** Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry

**9.2** **FACT-M-2.0,** Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

**9.3** **FACT-M-3.0,** Extraction of Perfluorooctanesulfonate from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry

**9.4** **FACT-M-4.0,** Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

**3M Environmental Technology and Services**

PO Box 33331
St. Paul, MN 55133-3331
612 778 6442

3M

***Study Title***

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

**PROTOCOL AMENDMENT NO. 1**

***Amendment Date:***

December 13, 1999

***Performing Laboratory***

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

***Laboratory Project Identification***

ET&SS FACT-TOX-002
LIRN U2121

*3M Environmental Laboratory*

*Protocol FACT Tox-002*
*Amendment Number 1*

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

1. ***PROTOCOL READS:***
The following methods will be used:
FACT-M-1.0, Extraction of Perfluorooctanesulfonate from Liver for Analysis usingHPLC-Electrospray/Mass Spectrometry
FACT-M-2.0, Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry
FACT-M-3.0, Extraction of Perfluorooctanesulfonate from Sera for Analysis using HPLC-Electrospray/Mass Spectrometry
FACT-M-4.0, Analysis of Sera Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry

***AMEND TO READ:***
The following methods will be used:
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
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 Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry

***REASON:***
The methods originally listed were superseded during the course of the study.

Note: The methods as stated on page 1, in section 5.1, and section 9.0 will be/were amended. LAC 12/19/00. (Accepted, Andrew M. Seacat 12/22/00)

**Amendment Approval**

Andrew M. Seacat 12/13/99

Andrew M. Seacat, Ph.D., Sponsor Representative — Date

Kristen Hansen 12/20/99

Kristen J. Hansen, Ph.D., Study Director — Date

*3M Environmental Laboratory*

***Study Title***

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

**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-U2121
FACT TOX-002
Covance Study: 6329-183
3M Medical Department Study: T-6295.4

*3M Environmental Laboratory*

*Protocol LRN-U2121*
*Amendment Number 2*

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

1. ***PROTOCOL READS:***
   There is no independent section of the protocol that addresses sample retention.
   ***AMEND TO READ:***
   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.

2. ***PROTOCOL READS:***
   Section 7 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; approved methods; data summaries; study correspondence; shipping records; raw data; and electronic copies of data. Additionally, Section 7 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: Approved validation reports; training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.
   ***AMEND TO READ:***
   Section 7 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis."
   ***REASON:***
   To direct subcontract laboratories in the disposition of the items listed above.

*3M Environmental Laboratory*

*Protocol LRN-U2121*
*Amendment Number 2*

3. ***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 John L. Butenhoff, 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 21 CFR Part 58).

4. ***PROTOCOL READS:***
The sponsor for the present study was identified as Andrew M. Seacat, Ph.D.
***AMEND TO READ:***
The role of sponsor for the present study was reassigned to Marvin T. Case, D.V.M., Ph.D., as of 20 January 2000.
***REASON:***
The change was made at the request of the sponsor.

*3M Environmental Laboratory*

*Protocol LRN-U2121*
*Amendment Number 2*

## Amendment Approval

Andrew M. Seacat 2/10/2000
*Andrew M. Seacat, Ph.D.*, Outgoing Sponsor Representative Date

Kristen Hansen 11-Feb-2000
*Kristen J. Hansen, Ph.D.*, Outgoing Study Director Date

Marvin Case 10 February 2000
*Marvin T. Case, D.V.M., Ph.D.*, Incoming Sponsor Representative Date

John L. Butenhoff February 10, 2000
*John L. Butenhoff, Ph.D.*, Incoming Study Director Date

*3M Environmental Laboratory*

***Study Title***

104-Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats

**PROTOCOL AMENDMENT NO. 3**

***Amendment Date:***

November 21, 2000

***Performing Laboratory***

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

***Laboratory Project Identification***

ET&SS LRN-U2121
FACT TOX-002
Covance 6329-183
3M Medical Department Study: T-6295

*3M Environmental Laboratory*

*Protocol FACT TOX-002*
*Amendment No. 3*

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

***1. PROTOCOL READS:***

1.2 Data Collected in the Environmental Laboratory will be considered non-quantitative screening data until future studies have been conducted to determine absolute recoveries of specific or general fluorochemical compounds.

***AMEND TO READ:***

1.2 If matrix spike studies provide accurate representation of recovery of endogenous levels of PFOS, the 3M Environmental Laboratory will provide semi-quantitative data for sera and liver samples collected from test animals.

***REASON:***

Due to improved analytical methods, the decision was made to change the purpose of the study results from non-quantitative to semi-quantitative.

*3M Environmental Laboratory*

*Protocol FACT TOX-002*
*Amendment No. 3*

## Amendment Approval

Marvin T Case — 27 November 2000

*Marvin T. Case, D.V.M., Ph.D.*, Sponsor Representative — Date

John L. Butenhoff — 28 NOV 2000

*John L. Butenhoff, Ph.D.*, Study Director — Date

*3M Environmental Laboratory*

# Record of Deviation

## I. Identification

Study / Project No.
TOX002 (LIMS #U2121)

Deviation type *(Check one)*: ❒ SOP X Method ❒ Equipment Procedure ❒ Protocol ❒ Other:

Document number ETS-8-7.0

Date(s) of occurrence
7/6/98, 5/25/00, 5/26/00, 6/12/00, 6/14/00, 6/13/00,5/19/00, 5/22/00

## II. Description

Required procedure/process:
Section 10.1.2: Analyze a method blank and a matrix blank prior to each calibration curve.

Actual procedure/process:
On several occasions, the blanks were either a) analyzed after the calibration curves or b) not analyzed at all for a particular MS run

## III. Actions Taken
(such as amendment issued, SOP revision, etc.)

Deviation written.

Recorded by kjh Date 11/14/00

## IV. Impact on Study / Project
*(completed by Study Director or Project Lead)*

All blanks were analyzed at least once during the course of the study. When blanks were run after the calibration curve, all control-of-bias practices were followed. In the instances where blanks were not run at all, the samples being analyzed were well above the typical run LOQ. In addition, the vast majority of samples run without blanks were high level dilutions. The function of the blank is to determine if contamination was introduced during extraction; any low-level contamination would be inconsequential to extracts requiring dilution. This deviation has no adverse affect on the study data.

kjh 11/14/00

Authorized by Marvin T Case 20 Nov 2000 / John Z. Butenhoff 4 Dec 00 Date

Sponsor: Marv Case Study Director: John Butenhoff **Deviation No.** 1
(assigned by Study Director or Project Lead at the end of study or project)

P.E. kjh 11/14/00

Attachment A: Record of Deviation ETS-4-8.0 ~~Page 4 of 4~~

# Record of Deviation

## I. Identification

Study / Project No. FACT-TOX-002

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

| Document Number | Date(s) of occurrence |
|---|---|
| Collected FACT-M-2.0 + reduced ETS-8-7.0 | 07/14/98 original 02/02/00 rework |

## II. Description: Sample list 071498

Required Procedure/process:

Extracts should be diluted into the linear range of the instrument as depicted by the standard curve.
Two consecutive failed CCVs invalidate the remaining portion of the sample list.

Actual Procedure/process:

Despite 1:200 dilutions, some G5/wk4 liver values were above the range of the standard curve. By the time this error was noticed, the sample extracts were over 2 years old. Also, although immediately surrounded by acceptable CCVs, the MS/MSD values were used even though, earlier in the run, 2 consecutive CCVs failed due to extremely high analyte levels in surrounding samples.

## III. Actions Taken:

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

Results will be noted as "tentative" in the final report.

| Recorded By | Date |
|---|---|
| | kjh 10/09/00 |

## IV. Impact on Study / Project

G5/wk4 liver values will be considered to be tentative. kjh 10/03/00

| Authorized By *(Study Director / Project Lead)* | Date |
|---|---|
| kjh 10/03/00 | |
| Marvin T Case 11 Oct 2000 | John E Butenhoff 11 OCT 2000 |

Sponsor Rep - Marvin Case Study Director - John Butenhoff

*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.
FACT-TOX-002 Covance 6329-183

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

Document Number: FACT-M-4.0

Date(s) of occurrence: 07/15/98 (wk 4 sera)

## II. Description:

Required Procedure/process:
The method states a continuing calibration verification will be analyzed after every 10th sample with a minimum of one per batch. Also, continuing calibration verification percent recoveries must be within +/- 30% of the spiked concentration.

Actual Procedure/process:
None of the continuing calibration verifications were within +/- 30% recovery. The recoveries ranged from -32 to -38%.

## III. Actions Taken:
*(such as amendment issued, SOP revision, etc.)*

These data were flagged in the Excel spreadsheet and this deviation was written.

Recorded By: Lisa A Clemens Date: 11/27/00

## IV. Impact on Study / Project

Data is noted as non-compliant.
kjh 11/27/00

Authorized By *(Study Director / Project Lead)*: Marvin T Case 29 Nov 2000; John Butenhoff 30 NOV 2000 Date:

Sponsor: Marv Case Study Director: John Butenhoff
3M Environmental Laboratory
Form ETS-4-8.0

**Deviation No.** 3
(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

8 (RE) LAC 09/08/00

Study / Project No. Covance Study Number: 6329-193/ 3M Project Number Tox002

Deviation Type (Check one): ❒ SOP ✓ Method ❒ Equipment Procedure ❒ Protocol ❒ Other:

Document Number: FACT-M-4.0 | Date(s) of occurrence: 07/23/98

## II. Description:

Required Procedure/process: Method FACT-M.4.0, states

**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.

Calibration checks in this data packet (collected 071598b, on amelia) are not with in the ±30% criteria.

Actual Procedure/process: Calibration checks within ±40% criteria will be accepted for this data set. The 97 ppb standard points at the beginning and end of the run are excluded from the calibration curve due to the analyst adding the wrong amount of standard. This error appears in both curves and is therefore verified. Deletion of the 97 ppb curve point caused the calibration curve to have a greater $r^2$ value and an overall better fit to the points.

## III. Actions Taken:

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

This method deviation, increasing the criteria range for this data set is the only action that will need to be taken.
This new data set will be used for final results.

Recorded By: Harold Johnson 9.5.00 | Date: September 5, 2000

## IV. Impact on Study / Project

Affected data will be noted in final results as meeting +/- 40% criteria.
kjh 09/05/00

Authorized By *(Study Director / Project Lead)*: John Z. Butenhoff 9/13/00 Sharon T. Case 15 Sept 2000 kjh 09/05/00 | Date

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

Sponsor Rep: Mary Case

**Deviation No.** 4
(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.
FACT-TOX-002 Covance 6329-183

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

| Document Number | Date(s) of occurrence |
|---|---|
| 21 CFR 58.120 (a) (11) | 05/22/99 |

## II. Description:

Required Procedure/process:
Sponsor Representative approval of analytical protocol prior to Study Director approval

Actual Procedure/process:
The study Director signed/approved the analytical protocol before the Sponsor Representative.

## III. Actions Taken:
*(such as amendment issued, SOP revision, etc.)*

In the future, analytical protocols will be signed by the Sponsor Representative before the Study Director.

| Recorded By | Date |
|---|---|
| Lisa A Clemen | 07/27/99 |

## IV. Impact on Study / Project

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

| Authorized By (Study Director / Project Lead) | Date |
|---|---|
| Kiten Ha | 7/28/99 |

*3M Environmental Laboratory*
*Form ETS-4-8.0*

**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-002 Covance 6329-183

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

| Document Number | Date(s) of occurrence |
|---|---|
| Protocol FACT-TOX-002 | 11/10/99 |

## II. Description:

Required Procedure/process:
Protocol states method FACT-M-3.0 will be used.

Actual Procedure/process:
Method ETS-8-4.1 was used.

## III. Actions Taken:
*(such as amendment issued, SOP revision, etc.)*

This Deviation was written/filled out.

| Recorded By | Date |
|---|---|
| Lisa A Clemen | 12/01/99 |

## IV. Impact on Study / Project

Method is improved; data will not be affected.

| Authorized By *(Study Director / Project Lead)* | Date |
|---|---|
| kjh | 06 12/02/99 r.e. kjh 12/06/99 |

*3M Environmental Laboratory*
*Form ETS-4-8.0*

**Deviation No.** 6
(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No.
FACT-TOX-002 Covance 6329-183

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

| Document Number | Date(s) of occurrence |
|---|---|
| Protocol FACT-TOX-002 | 11/11/99 |

## II. Description:

Required Procedure/process:
Protocol states method FACT-M-1.0 will be used

Actual Procedure/process:
Method ETS-8-6.0 was used

## III. Actions Taken:
*(such as amendment issued, SOP revision, etc.)*

This deviation was written / filled out.

| Recorded By | Date |
|---|---|
| Lisa A Clemen | 12/01/99 |

## IV. Impact on Study / Project

Data will not be affected.

| Authorized By *(Study Director / Project Lead)* | Date |
|---|---|
| kjn | 12/06/99 |

*3M Environmental Laboratory*
*Form ETS-4-8.0*

**Deviation No.** 7
(assigned by Study Director or Project Lead at the end of study or project)

# Record of Deviation

## I. Identification

Study / Project No. TOX-002

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

| Document Number | Date(s) of occurrence |
|---|---|
| ETS-8-07.0 | 2/00 - 8/00 |

## II. Description:

Required Procedure/process:

14.4.1 Matrix spike percent recoveries must be within +/- 30% of the spiked concentration.

Actual Procedure/process:

Matrix spike recoveries were within +/- 50% of the spiked concentration.

## III. Actions Taken:

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

Deviant recoveries were verified, but were not reanalyzed; deviation will be noted in the final report.

| Recorded By | Date |
|---|---|
| kjh 10/05/00 | |

## IV. Impact on Study / Project

Quality of data for PFOS analysis in liver will be noted in the final report as being accurate to +/- 50%

kjh 10/05/00

| Authorized By (Study Director / Project Lead) | Date |
|---|---|
| Marv T Case 11/7/00 John T. Butenhoff 11/7/00 | |

Sponsor Rep - Marv Case
3M Environmental Laboratory
Form ETS-4-8.0

Study Director - John Butenhoff

**Deviation No.** 8
(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 methods:

**FACT-M-1.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry (8 pages)

**FACT-M-3.0,** Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry (8 pages)

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

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

**FACT-M-2.0**, Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry (8 pages)

**FACT-M-4.0,** Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry (8 pages)

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

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

# 3M ENVIRONMENTAL LABORATORY

## METHOD

## EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER ANIONIC FLUOROCHEMICAL SURFACTANTS FROM LIVER FOR ANALYSIS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

**Method Number:** FACT-M-1.0 **Adoption Date:** 5/26/98

**Revision Date:** N/A

Author: Lisa Clemen

Approved By:

| | |
|---|---|
| [signature] | 5/20/98 |
| Laboratory Manager | Date |
| [signature] | 5/26/98 |
| Group Leader | Date |
| [signature] | 5/27/98 |
| Technical Reviewer | Date |

### 1.0 SCOPE AND APPLICATION

**1.1 Scope:** This method is for the extraction of Potassium Perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver.

**1.2 Applicable Compounds:** Fluorochemical surfactants or other fluorinated 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 how to extract potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver using ion pairing reagent and 5.0 mLs of ethyl acetate. An ion pairing reagent is added to each sample and partitioned into ethyl acetate. Four mLs of extract is removed 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 filter into glass autovials.

## 3.0 Definitions

**3.1** None.

## 4.0 Warnings and Cautions

**4.1 Health and Safety Warnings:**

**4.1.1** Use universal precautions when handling animal livers, they 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 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

## 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** Glass, type A, volumetric flasks
**7.6** 40 mL glass I-CHEM vials
**7.7** Plastic sampule vials, Wheaton, 6 mL
**7.8** Polypropylene centrifuge tubes, 15 mL
**7.9** Labels

**7.10** Syringes, capable of measuring 10 µL to 50 µL

**7.11** Glass, type A, volumetric pipettes

**7.12** Graduated pipettes

**7.13** Electronic pipettor, Eppendorf or equivalent

**7.14** Timer

**7.15** Disposable plastic 3 cc syringes

**7.16** Filters, nylon syringe filters, 0.2 µm, 25 mm

**7.17** 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 Reagents

**8.1.1** Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L nalgene bottle.

**8.1.2** Sodium Hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 1:10. Measure 10 mL of the 10N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL nalgene bottle.

**8.1.3** Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-Q™ water. Adjust to pH 10 using approximately 64 mL 10N NaOH and dilute to volume with Milli-Q™ water. Add NaOH slowly while adding the last 1 mL of NaOH because the pH changes abruptly. Store in a 1 L nalgene bottle.

**8.1.3.1** TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.

**8.1.4** Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker or equivalent), ($Na_2CO_3$/$NaHCO_3$) 0.25M: 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 dilute to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

**8.1.5** PFOS (3M Specialty Chemical Division), molecular weight = 538.

**8.1.6** Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.

**8.1.7** Methanol, Omnisolv, glass distilled or HPLC grade.

**8.1.8** Liver and control liver, received frozen from testing laboratory.

**8.1.9** 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.

### 8.2 Standards

**8.2.1** Prepare PFOS standards for the standard curve.

**8.2.2** Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

**8.2.3** Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/mL).

**8.2.4** Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

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

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

## 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 Spikes

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

**10.1.2** Prepare each spike using liver chosen by the analyst, usually a control liver.

**10.1.3** Expected concentrations will fall in the mid-range of the initial calibration curve.

### 10.2 Continuing Calibration Checks

**10.2.1** Prepare and analyze continuing calibration check samples to determine the continued linearity of the initial calibration curve.

**10.2.2** One check is prepared per group of ten samples. For example, if a sample set = 34, four checks are prepared and extracted.

**10.2.3** Prepare each continuing calibration check from the same liver homogenate used to prep the initial curve.

**10.2.4** The expected concentration will fall within the mid-range of the initial calibration curve.

## 11.0 Calibration and Standardization

### 11.1 Prepare Liver Homogenate to Use for 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 in keeping with a 1:5 ratio.

**11.1.3** See section 13.0 to calculate the actual density of liver.

**11.1.4** Add 1 mL of homogeneous solution to a 15 mL centrifuge tube. Re-suspend homogeneous solution by shaking between aliquots while preparing a total of sixteen 1 mL aliquots of homogeneous solution in 15 mL centrifuge tubes.

**11.1.5** Two 1 mL aliquots serve as matrix blanks. Use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

**Table 1**
**Approximate Spiking Amounts for Calibration Standards**

| Working Standard (Approx. Conc.) | µL | Approx. final conc. of PFOS in liver |
|---|---|---|
| - | - | Blank |
| 0.50 ppm | 4 | 0.010 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 | 1.000 ppm |

**11.1.1** See section 13.0 to calculate actual concentrations of PFOS in calibration standards.

**11.2** Extract spiked liver homogenates following **12.14-12.24** of this method. Use these standards to establish each initial curve on the mass spectrometer.

## 12.0 PROCEDURES

**12.1** Obtain frozen liver samples. In spent tissue, note that the liver has not been packaged with other tissues.

**12.2** Cut approximately 1 g of liver using a dissecting scalpel.

**12.3** Weigh the sample directly into a tared plastic sampule vial.

**12.4** Record the liver weight in the study notebook.

**12.5** Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

**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. Follow AMDT-EP-22.

**12.10** Cap the sample and vortex for 15 seconds.

**12.11** Pipette 1 mL homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. (See Worksheet for documenting the remaining steps.)

**12.12** Spike liver homogenates with the appropriate amount of PFOS standard as described in section 11.1 or Table 1.

**12.13** Pipette two 1 mL aliquots of Milli-Q™ water to centrifuge tubes. These will serve as instrument blanks.

**12.14** Add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

**12.15** Using a volumetric pipette, add 5 mLs ethyl acetate.

**12.16** Cap each sample and put on the shaker for 20 minutes.

**12.17** Centrifuge for 20 to 25 minutes, until layers are well separated. Set power on the centrifuge to approximately 3500 rpm.

**12.18** Remove 4 mLs 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**.

**12.19** Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.

**12.20** Add 1.0 mL of methanol to each centrifuge tube using a graduated pipette.

**12.21** Vortex mix for 30 seconds.

**12.22** 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.

**12.23** 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.

**12.24** Cap and hold for electrospray mass spectrometry analysis.

**12.25** Complete the worksheet and tape to page of study notebook.

## 13.0 DATA ANALYSIS AND CALCULATIONS

### 13.1 Calculations:

**13.1.1** Calculate the density of liver (mg) in 1.0 mL homogenate using the following equation:

$$\frac{\text{g of Liver} \times \text{Average weight of ten 1 mL aliquots (mg)}}{(\text{g of Liver} + \text{g of Water})}$$

**13.1.2** Calculate actual concentrations of PFOS in calibration standards using the following equation:

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

*Average weight of liver in solution as determined in **13.1.1**, by weighing ten 1 mL homogenates of approximately 40 mg liver in 200 mL of Milli-Q water.

## 14.0 METHOD PERFORMANCE

**14.1** The method detection limit is equal to half the lowest standard in the 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 and tape into the study notebook.

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

**17.1** The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

## 18.0 REFERENCES

**18.1** AMDT-EP-22, "Routine Maintenance of Ultra-Turrax T-25"

## 19.0 AFFECTED DOCUMENTS

**19.1** FACT-M-2, "Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## 20.0 REVISIONS

| Revision Number. | Reason For Revision | Revision Date |
|---|---|---|

# Extraction Worksheet for FACT-M-1

| Study # | Sample Number set # | PFOS approx. 0.5 ppm actual ppm #W_____ | PFOS approx. 5 ppm actual ppm #W_____ | PFOS approx. 50 ppm actual ppm #W_____ | Date and Initials for Std. |
|---|---|---|---|---|---|
| - | $H_2O$ Blank | - | - | - | |
| - | Liver Blank | - | - | - | |
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[1] Study number where the original worksheet is located.

Blank Liver Homogenate: Std.# Liver amount = g

| **Liver Extraction Method** : | **Date & Initials** |
|---|---|
| Vortex 15 sec. | |
| Pipette 1 mL of Liver Solution | |
| 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 1.0 mL of Methanol 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 homogenate as for std curve.

FACT-M-1.0
Extraction of PFOS from Liver

Page 8 of 8

# 3M ENVIRONMENTAL LABORATORY

## METHOD

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

**Method Number:** FACT-M-3.0

**Adoption Date:** 4/22/98

**Revision Date:** N/A

Author: Lisa Clemen

Approved By:

4/22/98
Laboratory Manager — Date

4/22/98
Group Leader — Date

4/22/98
Technical Reviewer — Date

### 1.0 SCOPE AND APPLICATION

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

**1.2 Applicable Compounds:** Fluorochemical surfactants or other fluorinated compounds.

**1.3 Matrices:** Rabbit, rat, and bovine serum or other sera as designated in the validation report.

## 2.0 Summary of Method

**2.1** This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other anionic fluorochemical surfactants from serum using an ion pairing reagent and 5.0 mL of ethyl acetate. 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** None.

## 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 serum, 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** 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 gm)

## 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** Glass, type A, volumetric flasks
**7.5** 40 mL glass I-CHEM vials
**7.6** Polypropylene centrifuge tubes, 15 mL
**7.7** Labels
**7.8** Syringes, capable of measuring 10 µL to 50 µL
**7.9** Glass, type A, volumetric pipettes
**7.10** Graduated pipettes

**7.11** Electronic pipettor, Eppendorf or equivalent

**7.12** Timer

**7.13** Disposable plastic 3 cc syringes

**7.14** Filters, nylon syringe filters, 0.2 µm, 25 mm

**7.15** 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 Reagents

**8.1.1** Sodium hydroxide (J.T Baker or equivalent), (NaOH) 10N: weigh approximately 200 grams NaOH. Pour into a 1000 mL beaker containing 500 liters (L) Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

**8.1.2** Sodium hydroxide (J.T Baker or equivalent), (NaOH) 1N. Dilute 10N 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.

**8.1.3** Tetrabutylammonium hydrogen sulfate (Kodak or equivalent), (TBA) 0.5M: Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 L Milli-Q™ water. Adjust to pH 10 using approximately 64 mL of 10N NaOH and dilute to volume with Milli-Q™ water. Add NaOH slowly while adding the last mL of NaOH because the pH changes abruptly. Store in a 1 L Nalgene bottle.

**8.1.3.1** TBA requires a check prior to each use to ensure pH = 10. Adjust as needed using 1N NaOH solution.

**8.1.4** Sodium carbonate/sodium bicarbonate buffer (J.T. Baker or equivalent), ($Na_2CO_3$/$NaHCO_3$) 0.25M: 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.

**8.1.5** PFOS (3M Specialty Chemical Division), molecular weight = 538.

**8.1.6** Other fluorochemicals, as appropriate.

**8.1.7** Ethyl Acetate, Omnisolv, glass distilled or HPLC grade.

**8.1.8** Methanol, Omnisolv, glass distilled or HPLC grade.

**8.1.9** Serum, frozen liquid from Sigma.

**8.1.10** Control serum received with each sample set.

**8.1.11** 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.

### 8.2 Standards

**8.2.1** Prepare PFOS standards for the standard curve.

**8.2.2** Prepare other fluorochemical standards, as appropriate.

**8.2.3** Weigh approximately 100 mg of PFOS into a 100 mL volumetric flask and record the actual weight.

**8.2.4** Bring to volume with methanol for a stock standard of approximately 1000 ppm (μg/mL).

**8.2.5** Dilute the stock solution with methanol for a working standard 1 solution of approximately 50 ppm.

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

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

## 9.0 Sample Handling

**9.1** All sera 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** Two 1.0 mL aliquots of the serum are extracted following this procedure and used as matrix blanks. See section 11.1.2.

**10.1.2** Two 1.0 mL aliquots of Milli-Q™ water are extracted following this procedure and used 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 serum chosen by the analyst, usually control serum 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.

### 10.3 Continuing Calibration Checks

**10.3.1** Prepare and analyze continuing calibration check samples to determine the continued linearity of the initial calibration curve.

**10.3.2** One check is prepared per group of ten 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 serum used to prep the initial curve.

**10.3.4** The expected concentration will fall within the mid-range of the initial calibration curve.

## 11.0 CALIBRATION AND STANDARDIZATION

### 11.1 Prepare Serum Standards

**11.1.1** Transfer 1 mL of serum to a 15 mL centrifuge tube.

**11.1.2** If the majority of serum sample volumes are less than 1.0 mL, extract standards using serum volumes in the standards equal to the serum volumes in samples. Do not extract below 0.50 mL of serum. Record the serum volume on the extraction sheet.

**11.1.3** Mix or shake between aliquots while preparing a total of sixteen aliquots of serum in 15 mL centrifuge tubes.

**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 to spike, in duplicate, two standard curves for a total of fourteen samples.

**11.1.5** Refer to the validation report FACT-M-3.0-V-1 and FACT-M-4.0-V-1 which lists the working ranges for calibration curves.

**Table 1**
**Approximate Spiking Amounts for Standards and Spikes Using 1.0 mL of Serum**

| Working Standard (Approx. Conc.) | µL | Approx. final conc. of PFOS in serum |
|---|---|---|
| - | - | Blank |
| 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 |

**11.1.4** See section 13.0 to calculate actual concentrations of PFOS in calibration standards.

**11.2** Extract spiked serum standards following **12.6-12.16** of this method. Use these standards to establish each initial curve on the mass spectrometer.

## 12.0 PROCEDURES

**12.1** Obtain frozen serum samples and allow to thaw.

**12.2** Vortex mix for 15 seconds then remove 1.0 mL or appropriate volume to a 15 mL polypropylene centrifuge tube.

**12.3** Return serum samples to freezer after extraction amount has been removed.

**12.4** Record the serum volume on the extraction worksheet. The final methanol volume will equal the initial serum volume.

**12.5** Label the tube with the study number, serum ID, date and analyst initials. See attached worksheet for documenting the remaining steps.

**12.6** Spike serum with the appropriate amount of PFOS standard as described in section 11.1 or Table I for the calibration curve standards. Also spike matrix spikes and continuing calibration standards.

**12.7** Vortex mix the standard curve samples, matrix spike samples, and continuing calibration samples for 15 seconds.

**12.8** Add 1 mL 0.5 M TBA and 2 mL of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

**12.9** Using a volumetric pipette, add 5 mL ethyl acetate.

**12.10** Cap each sample and put on the shaker for 20 minutes.

**12.11** Centrifuge for 20 to 25 minutes, until layers are well separated. Set power on the centrifuge to approximately 3500 rpm.

**12.12** 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**.

**12.13** Put each sample on the analytical nitrogen evaporator until dry, approximately 2 to 3 hours.

**12.14** Add 1.0 mL or appropriate volume of methanol to each centrifuge tube using a graduated pipette. (This volume equals the initial volume of serum used for the extraction.)

**12.15** Vortex mix for 30 seconds.

**12.16** 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.

**12.17** 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.

**12.18** Cap and hold for HPLC-electrospray/mass spectrometry analysis. Extracts may be stored at 4° C until analysis.

**12.19** Complete the extraction worksheet, attached to this document, and tape to page of study notebook.

## 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:

$$\frac{\text{mL of Standard x Concentration (µg /mL)}}{\text{mL of Standard + Initial Serum Volume (mL)}} = \text{Final Concentration (µg/mL) of PFOS in Serum}$$

## 14.0 Method Performance

**14.1** The method detection limit is equal to half the lowest standard in the 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.

## 17.0 Tables, Diagrams, Flowcharts, and Validation Data

**17.1** The validation report associated with this method is FACT-M-3.0 & 4.0-V-1.

## 18.0 References

**18.1** None

## 19.0 Affected Documents

**19.1** FACT-M-4, "Analysis of Serum Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

## 20.0 Revisions

| Revision Number. | Reason For Revision | Revision Date |
|---|---|---|

# Extraction Worksheet for FACT-M-3

| Study # | Sample Number<br>set # | PFOS approx. 0.5 ppm<br>actual ppm<br>#W_____ | PFOS approx. 5 ppm<br>actual ppm<br>#W_____ | PFOS approx. 50 ppm<br>actual ppm<br>#W_____ | Date and Initials for Std. or Comments |
|---|---|---|---|---|---|
| - | $H_2O$ Blank | - | - | - | |
| - | Serum Blank | - | - | - | |
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[1] Study number where the original worksheet is located.

Blank Serum Std # Serum amount = g

| **Serum Extraction Method** : | **Date & Initials** |
|---|---|
| Vortex 15 sec. | |
| Pipette Serum 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 serum as for std curve.

FACT-M-3.0
Extraction of PFOS from Serum

# 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 |

### 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<br>Approximate spiking amounts for standards and spikes<br>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.<br>Section 12.13 Added the shaker speed.<br>Section 12.17 Final volume is 1.0 mL; not adjusted for initial volumes less than 1.0 mL. | 04/02/99 |

# Extraction Worksheet ETS-8-4.1

| Study #_____<br>Matrix_____<br>Box #_____<br>Wk/Day_____<br>DateSpiked/Analyst | Surrogate Std<br>approx. ppm<br>actual ppm<br>#_____ | FC-Mix<br>approx. 0.5 pm<br>actual ppm<br>#_____ | FC-Mix<br>approx. 5 ppm<br>actual ppm<br>#_____ | FC-Mix<br>approx. 50 ppm<br>actual ppm<br>#_____ | Comments |
|---|---|---|---|---|---|
| CCV | | | | | |
| MS | | | | | |
| MSD | | | | | |
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|---|---|---|---|
| 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):**
**Analyte(s):**
**Sample matrix:**

**Standard number:**
**Equipment number:**
**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 ng/mL | |
| 97.6 | 98.9 | 104 | 97.8 | 99.3 | 102 | 500 | |
| 248 | 251 | 263 | 248 | 252 | 258 | | |
| 493 | 500 | 524 | 494 | 501 | 513 | | |
| 735 | 746 | 782 | 737 | 749 | 766 | | |
| 976 | 989 | 1038 | 978 | 993 | 1017 | | |

**Validated ranges – approximate concentrations**

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

# 3M ENVIRONMENTAL LABORATORY

## METHOD

## EXTRACTION OF POTASSIUM PERFLUOROOCTANESULFONATE OR OTHER 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

### 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:

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

* 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 #_____<br>Matrix_____<br>Box #_____<br>Wk/Day_____<br>Date Spiked/Analyst | Surrogate Std<br>approx. ppm<br>actual ppm<br>#_____ | FC Mix Std<br>approx. 0.5 ppm<br>actual ppm<br>#_____ | FC Mix Std<br>approx. 5 ppm<br>actual ppm<br>#_____ | FC Mix Std<br>approx. 50 ppm<br>actual ppm<br>#_____ | Comments |
|---|---|---|---|---|---|
| CCV | | | | | |
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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 ng/mL | |
| 120 | 120 | 120 | 120 | 120 | 120 | | 0.500 | |
| 299 | 299 | 299 | 299 | 299 | 299 | | | |
| 599 | 599 | 599 | 599 | 599 | 599 | | | |
| 898 | 898 | 898 | 898 | 898 | 898 | | | |
| 1198 | 1198 | 1198 | 1198 | 1198 | 1198 | | | |

**Validated ranges – approximate concentrations**

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

# 3M ENVIRONMENTAL LABORATORY

## METHOD

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

**Method Number:** FACT-M-2.0

**Adoption Date:** 5/26/98

**Revision Date:** N/A

Author: Lisa Clemen

Approved By:

D J [illegible] 5/26/98
Laboratory Manager Date

[illegible] 5/26/98
Group Leader Date

Lisa A Clemen 5/27/98
Technical Reviewer Date

### 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** None.

## 4.0 WARNINGS AND CAUTIONS

**4.1 Health and Safety Warnings:**

**4.1.1** Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

**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** 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 changed 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** Nitrogen gas, refrigerated liquid, 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** 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, HPLC grade or equivalent.

**8.2 Standards**

**8.2.1** Typically one $H_2O$ blank, one liver blank, and seven liver standards are prepared during the extraction procedure. See FACT-M-1.

## 9.0 Sample Handling

**9.1** Fresh liver 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 refrigerated 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.

**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.

**10.4 System Suitability**

**10.4.1** System suitability (e.g. peak area, retention time and peak shape, etc.) will be assessed for each run.

## 11.0 Calibration and Standardization

**11.1** Analyze the extracted liver 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 for the calibration curve using MassLynx or other suitable software.

**11.2** The $r^2$ value for the data should be 0.98 or greater. Lower values may be acceptable at the discretion of the analyst.

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

## 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. Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A scan is usually collected along with the SIRs. Save method.

**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 = 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 Sep-up

**12.3.1** Refer to AMDT-EP-31 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.

**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** 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. 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** The method detection limit is equal to at least three times the baseline noise in the matrix blank.

**14.2** The practical quantitation limit is equal to the lowest standard in the 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 glass pipette waste is disposed in broken glass containers. All containers are located in the laboratory.

## 16.0 Records

**16.1** Store chromatograms in the study folder. Each chromatogram should have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).

**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 tape into appropriate study notebook.

**16.6** Summarize data using suitable software and store in the study folder.

**16.7** Back up electronic data to appropriate media. 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 Data reporting spreadsheet

**17.2** The validation report associated with this method is FACT-M-1.0 & 2.0-V-1.

## 18.0 References

**18.1** AMDT-EP-31, "Operation of VG Platform Electrospray Mass Spectrometer"

## 19.0 AFFECTED DOCUMENTS

**19.1** FACT-M-1.0, "Extraction of Potassium Perfluorooctanesulfonate from Liver 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 |
|---|---|---|---|---|---|
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| | | | | | |

**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 FLUOROCHEMICALS IN SERUM EXTRACTS USING HPLC-ELECTROSPRAY/MASS SPECTROMETRY

**Method Number:** FACT-M-4.0

**Adoption Date:** 4/22/98

**Revision Date:** N/A

Author: Lisa Clemen

Approved By:

Laboratory Manager — Date: 4/22/98

Group Leader — Date: 4/22/98

Lisa Clemen
Technical Reviewer — Date: 4/14/98

### 1.0 SCOPE AND APPLICATION

**1.1 Scope:** This method is for the analysis of extracts of serum or tissue 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, and bovine serum or other sera as designated in the validation report.

## 2.0 Summary of Method

**2.1** This method describes the analysis of fluorochemical surfactants extracted from serum 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** None.

## 4.0 Warnings and Cautions

**4.1 Health and Safety Warnings:**

**4.1.1** Use caution with the voltage cable for the probe. When the voltage cable is plugged into the probe DO NOT TOUCH THE PROBE, there is risk of electrical shock.

**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** 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 changed 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** Nitrogen gas, refrigerated liquid, 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** 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, HPLC grade or equivalent.

**8.2 Standards**

**8.2.1** Typically one $H_2O$ blank, one serum blank, and seven serum standards are prepared during the extraction procedure. See FACT-M-3.

## 9.0 Sample Handling

**9.1** Fresh serum 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 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 a 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.

**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.

**10.4 System Suitability**

**10.4.1** System suitability (e.g., peak area, retention time, peak shape, etc.) will be assessed for each run.

## 11.0 Calibration and Standardization

**11.1** Analyze the extracted serum 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 for the calibration curve using MassLynx or other suitable software.

**11.2** The $r^2$ value for the data should be 0.98 or greater. Lower values may be acceptable at the discretion of the analyst.

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

## 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 scan is usually collected along with the SIRs. Save method.

**12.1.3** Typically the sample list begins with the first set of serum 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 = 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 AMDT-EP-31 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.

**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. 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, anion in serum (μg/mL):

$$\frac{\text{μg of PFO calc. from std. Curve}}{\text{Initial Volume of serum (mL)}} \times \text{Dilution Factor} \times \text{Final Volume (mL)}$$

## 14.0 Method Performance

**14.1** The method detection limit is equal to half the lowest standard in the calibration curve.

**14.2** The practical quantitation limit is equal to the lowest standard in the calibration curve.

## 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 folder. Each chromatogram must have the following information included either in the header or hand written on the chromatogram: study number, sample name, extraction date, and dilution factor (if applicable).

**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 tape into appropriate study notebook.

**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 Data reporting spreadsheet

**17.2** The validation report associated with this method is FACT-M-3.0 & 4.0-V-1.

## 18.0 References

**18.1** AMDT-EP-31, "Operation of VG Platform Electrospray Mass Spectrometer"

## 19.0 Affected Documents

**19.1** FACT-M-3.0, "Extraction of Fluorochemical Anions from Serum 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 |
|---|---|---|---|---|---|
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**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 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 |

### 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.<br>Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve.<br>Section 12.2.2.4 Clarification of solvent ramp.<br>Section 17.1 Changed from attachment B to A. | 04/02/99 |

# Laboratory Study #

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

| Group 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

Study #: FACT-TOX-002

# 3M Environmental Lab -- Method Modification

Method:

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

Section modified: 10.3.2, 14.5.1, add sections 14.3.2-14.3.6

Effective date of modifications: April 26, 1999

**Section 10.3.2**

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

**Section 14.5.1**

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

**Section 14.3.2**

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

Study #: FACT-TOX-002

**Section 14.3.3**

Method reads:

NA

Modify method to read:

14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

**Section 14.3.4**

Method reads:

NA

Modify method to read:

14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

**Section 14.3.5**

Method reads:

NA

Modify method to read:

14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

**Section 14.3.6**

Method reads:

NA

Modify method to read:

14.3.6 A valid calibration curve must contain at least 5 active points.

Kristen He 11/29/00

Signature of PAI and date

Marvin J Case 13 Dec 2000

Signature of Sponsor and date

John Z. Butterfield 13 DEC 2000

Signature of Study Director and date

# 3M ENVIRONMENTAL LABORATORY

## METHOD

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

**Method Number:** ETS-8-7.0

**Adoption Date:** 07/22/99

**Revision Date:** NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

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

## 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):

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

## 14.0 Method Performance

**14.1** Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. 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

Study #: FACT-TOX-002

# 3M Environmental Lab -- Method Modification

Method:

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

Section modified: 10.3.2, 14.5.1, add sections 14.3.2-14.3.6

Effective date of modifications: July 22, 1999

## Section 10.3.2

Method reads:

10.3.2 Analyze a mid-range calibration standard after every tenth sample, with a minimum of one per batch.

Modify method to read:

10.3.2 Analyze a mid-range calibration standard **at least** after every ten samples, with a minimum of one per batch.

## Section 14.5.1

Method reads:

14.5.1 Continuing calibration verification percent recoveries must be within ± 30% of the spiked concentration.

Modify method to read:

14.5.1 At least one continuing calibration verification per ten samples must show a percent recovery within +/-30% of the spiked concentration.

## Section 14.3.2

Method reads:

NA

Modify method to read:

14.3.2 The second (bracketing) calibration curve may be deactivated if instrumental drift affects the data. The first curve and acceptable calibration checks shall bracket usable data.

Study #: FACT-TOX-002

**Section 14.3.3**

Method reads:

NA

Modify method to read:

14.3.3 Calibration standards with peak areas less than 2 times the curve matrix blank should be deactivated to disqualify a data range that may be affected by background levels of the analyte.

**Section 14.3.4**

Method reads:

NA

Modify method to read:

14.3.4 Low or high curve points may be deactivated to optimize a linear range appropriate to the data.

**Section 14.3.5**

Method reads:

NA

Modify method to read:

14.3.5 A curve point may be deactivated if it deviates more than 30% from the theoretical value when the curve is evaluated over a linear range appropriate to the data.

**Section 14.3.6**

Method reads:

NA

Modify method to read:

14.3.6 A valid calibration curve must contain at least 5 active points.

11/29/00
Signature of PAI and date

13 Dec 2000
Signature of Sponsor and date

13 DEC 2000
Signature of Study Director and date

# Appendix D: Data Summary Tables

## Table 7. FACT TOX-002 Data Summary of PFOS Concentration—Serum (µg/mL)

| Timepoint | Sex | Group 1 Control Average ±SD | Group 2 Low Average ±SD | Group 3 Mid Average ±SD | Group 4 Mid-High Average ±SD | Group 5 High Average ±SD | Group 6 High Recovery Average ±SD |
|---|---|---|---|---|---|---|---|
| Week 4[a] | Male | <LOQ[b] (n = 5) | 0.907 ±0.0619 (n = 5) | 4.33 ±1.16 (n = 5) | 7.57 ±2.17 (n = 5) | 41.8 ±7.92 (n = 5) | |
| | Female | 0.0259 ±0.00663 (n = 5) | 1.61 ±0.207 (n = 5) | 6.62 ±0.499 (n = 5) | 12.6 ±1.73 (n = 5) | 54.0 ±7.34 (n = 5) | |
| Week 14[a] | Male | <LOQ[c] (n = 5) | 4.04 ±0.801 (n = 5) | 17.1 ±1.22 (n = 5) | 43.9 ±4.90 (n = 5) | 148 ±13.8 (n = 5) | |
| | Female | 2.67 ±4.58 (n = 5) | 6.96 ±0.993 (n = 4[d]) | 27.3 ±2.34 (n = 5) | 64.4 ±5.48 (n = 5) | 223 ±22.4 (n = 5) | |
| Week 53 | Male | 0.0249 ±0.0182 (n = 5) | | | | 146 ±33.5 (n = 4) | |
| | Female | 0.395 ±0.777 (n = 5) | | | | 220 ±44.0 (n = 5) | |
| Day 719 | Male | | | | | | |
| | Female | | | 20.2 ±13.3 (n = 9) | | | |
| Week 105 | Male | 0.0118 ±0.0104 (n = 11) | 1.31 ±1.30 (n = 10) | 7.60 ±8.60 (n = 17) | 22.5 ±23.5 (n = 25) | 69.3 ±57.9 (n = 22) | |
| | Female | 0.0836 ±0.134 (n = 24) | 4.35 ±2.78 (n = 15) | | 75.0 ±45.7 (n = 15) | 233 ±124 (n = 25) | |
| Week 106 | Male | | | | | | 2.42 ±5.09 (n = 10) |
| | Female | | | | | | 9.51 ±8.70 (n = 17) |

[a] Not corrected for purity of the standard material.
[b] LOQ—Limit of Quantitation = 0.00910 µg/mL
[c] LOQ—Limit of Quantitation = 0.0457 µg/mL
[d] C92987F sample spilled during extraction, no sample remaining for analysis.
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 8. FACT TOX-002 Data Summary of PFOS Concentration—Liver (µg/g)

| Timepoint | Sex | Group 1 Control Average ±SD | Group 2 Low Average ±SD | Group 3 Mid Average ±SD | Group 4 Mid-High Average ±SD | Group 5 High Average ±SD | Group 6 High Recovery Average ±SD |
|---|---|---|---|---|---|---|---|
| Week 4[a] | Male | 0.104 ±0.0673 (n = 5) | 11.0 ±2.31 (n = 5) | 31.3 ±5.84 (n = 5) | 47.6 ±12.5 (n = 5) | 282 ±45.3[b] (n = 5) | |
| | Female | 0.107 ±0.0486 (n = 5) | 8.71 ±0.552 (n = 5) | 25.0 ±6.11 (n = 5) | 83.0 ±14.1 (n = 5) | 373 ±44.1[b] (n = 5) | |
| Week 14[a] | Male | 0.459 ±0.0573 (n = 5) | 23.8 ±3.45 (n = 5) | 74.0 ±6.16 (n = 5) | 358 ±28.8 (n = 5) | 568 ±107 (n = 5) | |
| | Female | 12.0 ±22.4 (n = 5) | 19.2 ±3.77 (n = 5) | 69.2 ±3.46 (n = 5) | 370 ±22.3 (n = 5) | 635 ±49.0 (n = 5) | |
| Week 53 | Male | 0.635 ±1.04 (n = 10) | | | | 435 ±96.9 (n = 9) | |
| | Female | 0.923 ±1.77 (n = 10) | | | | 560 ±180 (n = 10) | |
| Day 719 | Male | | | | | | |
| | Female | | | 55.1 ±31.5 (n = 9) | | | |
| Week 105 | Male | 0.114 ±0.148 (n = 11) | 7.83 ±7.34 (n = 10) | 26.4 ±20.4 (n = 17) | 70.5 ±63.1 (n = 25) | 189 ±141 (n = 22) | |
| | Female | 0.185 ±0.184 (n = 24) | 12.9 ±6.81 (n = 15) | | 131 ±61.4 (n = 15) | 381 ±176 (n = 25) | |
| Week 106 | Male | | | | | | 3.12 ±5.97 (n = 10) |
| | Female | | | | | | 12.9 ±10.4 (n = 17) |

[a] Not corrected for purity of the standard material.
[b] The values in this mean were tentative
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 9. Week 4 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92506M | <LOQ |
| | C92524M | <LOQ |
| | C92526M | <LOQ |
| | C92529M | <LOQ |
| | C92546M | <LOQ |
| Group 2 Low | C92574M | 0.910* |
| | C92575M | 0.989* |
| | C92610M | 0.944* |
| | C92613M | 0.852* |
| | C92618M | 0.842* |
| Group 3 Mid | C92646M | 3.97* |
| | C92650M | 2.85* |
| | C92652M | 4.80* |
| | C92668M | 5.98* |
| | C92678M | 4.04* |
| Group 4 Mid-High | C92715M | 8.46* |
| | C92718M | 9.89* |
| | C92731M | 4.03* |
| | C92734M | 7.69* |
| | C92744M | 7.81* |
| Group 5 High | C92752M | 29.0* |
| | C92759M | 40.7* |
| | C92779M | 49.5* |
| | C92793M | 42.7* |
| | C92798M | 47.0* |

Note: Not corrected for purity of the standard material.
LOQ—Limit of Quantitation = 0.00910 µg/mL

* Continuing calibration verifications did not meet ±30% criteria; data may be biased low.

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 10. Week 4 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002**

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92861F | 0.0227 |
| | C92864F | 0.0309 |
| | C92914F | 0.0347 |
| | C92919F | 0.0193 |
| | C92926F | 0.0216 |
| Group 2 Low | C92982F | 1.69* |
| | C92941F | 1.67* |
| | C92945F | 1.89* |
| | C92950F | 1.38* |
| | C92969F | 1.44* |
| Group 3 Mid | C92996F | 6.84* |
| | C93006F | 5.75* |
| | C93008F | 6.85* |
| | C93031F | 6.65* |
| | C93045F | 6.99* |
| Group 4 Mid-High | C93058F | 15.3* |
| | C93067F | 13.2* |
| | C93070F | 11.6* |
| | C93075F | 10.8* |
| | C93084F | 12.1* |
| Group 5 High | C93114F | 62.8* |
| | C93123F | 52.4* |
| | C93142F | 56.4* |
| | C93144F | 42.7* |
| | C93159F | 55.8* |

Note: Not corrected for purity of the standard material.

* Continuing calibration verifications did not meet ±30% criteria; data may be biased low.

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 11. Week 14 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92509M | <LOQ |
| | C92511M | <LOQ |
| | C92521M | <LOQ |
| | C92528M | <LOQ |
| | C92532M | <LOQ |
| Group 2 Low | C92593M | 4.70 |
| | C92600M | 4.58 |
| | C92616M | 4.09 |
| | C92621M | 4.13 |
| | C92627M | 2.69 |
| Group 3 Mid | C92640M | 15.9 |
| | C92645M | 16.9 |
| | C92662M | 16.1 |
| | C92676M | 17.9 |
| | C92684M | 18.8 |
| Group 4 Mid-High | C92713M | 49.1 |
| | C92714M | 37.2 |
| | C92719M | 41.6 |
| | C92722M | 43.3 |
| | C92728M | 48.2 |
| Group 5 High | C92765M | 139 |
| | C92777M | 145 |
| | C92789M | 149 |
| | C92799M | 136 |
| | C92812M | 171 |

Note: Not corrected for purity of the standard material.

LOQ—Limit of Quantitation = 0.0457 µg/mL

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 12. Week 14 Reported PFOS Levels in Sera Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92880F | 0.332 |
| | C92887F | 10.8 |
| | C92898F | 0.252 |
| | C92903F | 1.62 |
| | C92905F | 0.333 |
| Group 2 Low | C92944F | 5.90 |
| | C92962F | 8.21 |
| | C92967F | 7.23 |
| | C92978F | 6.50 |
| | C92987F | * |
| Group 3 Mid | C92993F | 28.7 |
| | C93000F | 26.7 |
| | C93018F | 25.1 |
| | C93023F | 25.4 |
| | C93035F | 30.6 |
| Group 4 Mid-High | C93051F | 56.7 |
| | C93064F | 64.5 |
| | C93071F | 71.3 |
| | C93085F | 62.3 |
| | C93109F | 67.3 |
| Group 5 High | C93111F | 229 |
| | C93127F | 210 |
| | C93143F | 258 |
| | C93155F | 220 |
| | C93169F | 199 |

Note: Not corrected for purity of the standard material.

* Sample spilled during extraction, no sample remaining for analysis.

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 13. Week 53 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92504M | 0.0278 |
| | C92512M | 0.0149 |
| | C92534M | 0.0251 |
| | C92562M | 0.0529 |
| | C92570M | <LOQ |
| Group 5 High | C92778M | 96.6 |
| | C92795M | 162 |
| | C92796M | 171 |
| | C92817M | 154 |

LOQ—Limit of Quantitation = 0.00395 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 14. Week 53 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92876F | 0.0310 |
| | C92889F | 1.78 |
| | C92899F | 0.0656 |
| | C92923F | 0.0891 |
| | C92930F | <LOQ |
| Group 5 High | C93112F | 240 |
| | C93120F | 144 |
| | C93154F | 223 |
| | C93163F | 245 |
| | C93171F | 250 |

LOQ—Limit of Quantitation = 0.00395 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 15. Day 719 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 3 Mid | C92991F G3 Day 719 | 15.1 |
| | C93003F G3 Day 719 | 13.0 |
| | C93007F G3 Day 719 | 30.9 |
| | C93011F G3 Day 719 | 3.57 |
| | C93022F G3 Day 719 | 2.02 |
| | C93039F G3 Day 719 | 27.6 |
| | C93040F G3 Day 719 | 43.8 |
| | C93047F G3 Day 719 | 21.9 |
| | C93048F G3 Day 719 | 24.3 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92503M | 0.0148 |
| | C92505M | 0.0161 |
| | C92510M | 0.0285 |
| | C92513M | 0.0127 |
| | C92514M | 0.0325 |
| | C92518M | <LOQ |
| | C92541M | <LOQ |
| | C92542M | <LOQ |
| | C92544M | <LOQ |
| | C92551M | <LOQ |
| | C92569M | <LOQ |
| Group 2 Low | C92577M | 0.309 |
| | C92583M | 0.212 |
| | C92590M | 2.55 |
| | C92597M | 1.95 |
| | C92598M | 0.230 |
| | C92601M | 3.94 |
| | C92602M | 1.39 |
| | C92619M | 0.461 |
| | C92623M | 2.05 |
| | C92626M | 1.48 |
| Group 3 Mid | C92631M | 8.98 |
| | C92633M | 14.3 |
| | C92637M | 1.81 |
| | C92641M | 15.1 |
| | C92642M | 6.05 |
| | C92643M | 2.75 |
| | C92644M | 1.21 |
| | C92649M | 8.63 |
| | C92653M | 0.487 |
| | C92657M | 5.20 |
| | C92659M | 14.4 |
| | C92660M | 1.66 |
| | C92669M | 3.13 |
| | C92674M | 35.3 |
| | C92682M | 4.77 |
| | C92683M | 2.72 |
| | C92690M | 2.83 |

LOQ—Limit of Quantitation = 0.00428 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 4 Mid-High | C92691M | 4.48 |
| | C92693M | 7.71 |
| | C92694M | 74.2 |
| | C92695M | 75.6 |
| | C92696M | 38.7 |
| | C92698M | 12.1 |
| | C92699M | 73.3 |
| | C92700M | 28.8 |
| | C92702M | 3.14 |
| | C92704M | 10.9 |
| | C92705M | 23.0 |
| | C92708M | 9.72 |
| | C92716M | 13.5 |
| | C92717M | 3.01 |
| | C92724M | 12.8 |
| | C92726M | 38.8 |
| | C92730M | 6.72 |
| | C92735M | 24.4 |
| | C92736M | 4.10 |
| | C92739M | 5.72 |
| | C92740M | 35.7 |
| | C92742M | 2.28 |
| | C92747M | 45.7 |
| | C92748M | 3.66 |
| | C92750M | 3.40 |

LOQ—Limit of Quantitation = 0.00428 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 16. Week 105 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 5 High | C92753M | 35.8 |
| | C92757M | 196 |
| | C92761M | 27.3 |
| | C92762M | 130 |
| | C92774M | 10.4 |
| | C92775M | 11.1 |
| | C92776M | 108 |
| | C92780M | 67.4 |
| | C92782M | 16.7 |
| | C92786M | 27.5 |
| | C92788M | 118 |
| | C92791M | 21.4 |
| | C92792M | 18.0 |
| | C92800M | 64.2 |
| | C92802M | 169 |
| | C92803M | 141 |
| | C92804M | 127 |
| | C92808M | 37.2 |
| | C92809M | 6.02 |
| | C92814M | 89.2 |
| | C92815M | 11.9 |
| | C92818M | 90.1 |

LOQ—Limit of Quantitation = 0.00428 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 1 Control | C92866F | 0.624 |
| | C92867F | 0.225 |
| | C92868F | 0.0467 |
| | C92869F | 0.306 |
| | C92871F | 0.00650 |
| | C92873F | 0.0602 |
| | C92874F | 0.0427 |
| | C92877F | 0.100 |
| | C92881F | 0.0258 |
| | C92882F | 0.0483 |
| | C92885F | <LOQ (1) |
| | C92890F | 0.0675 |
| | C92892F | 0.0140 |
| | C92895F | 0.0233 |
| | C92896F | 0.0407 |
| | C92897F | 0.0443 |
| | C92906F | 0.0795 |
| | C92907F | 0.0176 |
| | C92909F | 0.0402 |
| | C92911F | 0.0687 |
| | C92912F | 0.0311 |
| | C92913F | 0.0575 |
| | C92925F | <LOQ (2) |
| | C92927F | 0.0229 |
| Group 2 Low | C92936F | 8.72 |
| | C92938F | 0.349 |
| | C92946F | 9.08 |
| | C92953F | 6.64 |
| | C92957F | 5.67 |
| | C92960F | 1.70 |
| | C92964F | 5.97 |
| | C92970F | 1.73 |
| | C92973F | 1.34 |
| | C92974F | 4.06 |
| | C92975F | 3.72 |
| | C92983F | 6.81 |
| | C92984F | 2.78 |
| | C92986F | 5.24 |
| | C92988F | 5.76 |

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 4 Mid-High | C93059F | 103 |
| | C93060F | 89.5 |
| | C93062F | 102 |
| | C93063F | 171 |
| | C93072F | 114 |
| | C93077F | 38.0 |
| | C93078F | 25.9 |
| | C93079F | 72.8 |
| | C93080F | 73.6 |
| | C93081F | 70.5 |
| | C93090F | 99.1 |
| | C93098F | 5.17 |
| | C93101F | 117 |
| | C93102F | 11.4 |
| | C93107F | 33.2 |

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 17. Week 105 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 5 High | C93113F | 403 |
| | C93115F | 286 |
| | C93124F | 160 |
| | C93125F | 353 |
| | C93133F | 242 |
| | C93135F | 274 |
| | C93137F | 143 |
| | C93139F | 375 |
| | C93140F | 3.85 |
| | C93146F | 51.5 |
| | C93149F | 432 |
| | C93151F | 139 |
| | C93153F | 121 |
| | C93157F | 28.6 |
| | C93158F | 287 |
| | C93161F | 103 |
| | C93162F | 446 |
| | C93164F | 321 |
| | C93165F | 165 |
| | C93166F | 189 |
| | C93168F | 339 |
| | C93174F | 304 |
| | C93177F | 248 |
| | C93178F | 216 |
| | C93179F | 190 |

LOQ—Limit of Quantitation = (1) 0.00428 µg/mL, (2) 0.00848 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 18. Week 106 Reported PFOS Levels in Sera of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 6 High Recovery | C92821M | 8.32 |
| | C92823M | 0.533 |
| | C92827M | 0.0303 |
| | C92829M | 0.0243 |
| | C92833M | 0.0795 |
| | C92834M | 0.0138 |
| | C92848M | 14.9 |
| | C92851M | 0.0331 |
| | C92853M | 0.210 |
| | C92856M | <LOQ |

LOQ—Limit of Quantitation = 0.00482 µg/mL
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 19. Week 106 Reported PFOS Levels in Sera of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/mL |
|---|---|---|
| Group 6 High Recovery | C93181F | 5.76 |
| | C93182F | 1.69 |
| | C93183F | 5.68 |
| | C93184F | 1.64 |
| | C93185F | 11.8 |
| | C93190F | 31.6 |
| | C93192F | 16.8 |
| | C93197F | 9.35 |
| | C93198F | 8.63 |
| | C93201F | 27.0 |
| | C93202F | 10.1 |
| | C93205F | 0.0594 |
| | C93209F | 6.57 |
| | C93211F | 12.3 |
| | C93216F | 2.85 |
| | C93219F | 2.39 |
| | C93220F | 7.44 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 20. Week 4 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92506M | <LOQ |
| | C92524M | 0.169 |
| | C92526M | 0.186 |
| | C92529M | <LOQ |
| | C92546M | <LOQ |
| Group 2 Low | C92574M | 12.4 |
| | C92575M | 9.67 |
| | C92610M | 8.54 |
| | C92613M | 14.2 |
| | C92618M | 9.94 |
| Group 3 Mid | C92646M | 40.7 |
| | C92650M | 26.1 |
| | C92652M | 31.2 |
| | C92668M | 32.0 |
| | C92678M | 26.7 |
| Group 4 Mid-High | C92715M | 53.5 |
| | C92718M | 59.6 |
| | C92731M | 26.7 |
| | C92734M | 49.8 |
| | C92744M | 48.3 |
| Group 5 High | C92752M | 219* |
| | C92759M | 264* |
| | C92779M | 342* |
| | C92793M | 286* |
| | C92798M | 298* |

Note: Not corrected for purity of the standard material.
LOQ—Limit of Quantitation = 0.0552µg/g
* Results were outside the range of the curve and should be considered estimates.
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 21. Week 4 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92861F | 0.0817 |
| | C92864F | 0.0738 |
| | C92914F | 0.184 |
| | C92919F | 0.0700 |
| | C92926F | 0.126 |
| Group 2 Low | C92982F | 9.09 |
| | C92941F | 7.99 |
| | C92945F | 9.40 |
| | C92950F | 8.43 |
| | C92969F | 8.67 |
| Group 3 Mid | C92996F | 28.9 |
| | C93006F | 14.6 |
| | C93008F | 29.7 |
| | C93031F | 27.1 |
| | C93045F | 24.8 |
| Group 4 Mid-High | C93058F | 107 |
| | C93067F | 83.9 |
| | C93070F | 77.0 |
| | C93075F | 70.7 |
| | C93084F | 76.7 |
| Group 5 High | C93114F | 435* |
| | C93123F | 360* |
| | C93142F | 395* |
| | C93144F | 317* |
| | C93159F | 359* |

Note: Not corrected for purity of the standard material.
* Results were outside the range of the curve and should be considered estimates.
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 22. Week 14 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92509M | 0.440 |
| | C92511M | 0.542 |
| | C92521M | 0.492 |
| | C92528M | 0.403 |
| | C92532M | 0.418 |
| Group 2 Low | C92593M | 24.6 |
| | C92600M | 19.3 |
| | C92616M | 27.2 |
| | C92621M | 21.2 |
| | C92627M | 26.6 |
| Group 3 Mid | C92640M | 82.7 |
| | C92645M | 73.5 |
| | C92662M | 68.3 |
| | C92676M | 68.3 |
| | C92684M | 77.4 |
| Group 4 Mid-High | C92713M | 375 |
| | C92714M | 372 |
| | C92719M | 377 |
| | C92722M | 308 |
| | C92728M | 356 |
| Group 5 High | C92765M | 722 |
| | C92777M | 618 |
| | C92789M | 559 |
| | C92799M | 456 |
| | C92812M | 485 |

Note: Not corrected for purity of the standard material.
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 23. Week 14 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92880F | 1.02 |
| | C92887F | 52.0 |
| | C92898F | 0.697 |
| | C92903F | 5.26 |
| | C92905F | 1.18 |
| Group 2 Low | C92944F | 18.9 |
| | C92962F | 17.7 |
| | C92967F | 24.8 |
| | C92978F | 14.5 |
| | C92987F | 19.8 |
| Group 3 Mid | C92993F | 69.0 |
| | C93000F | 71.1 |
| | C93018F | 67.4 |
| | C93023F | 64.9 |
| | C93035F | 73.9 |
| Group 4 Mid-High | C93051F | 355 |
| | C93064F | 361 |
| | C93071F | 355 |
| | C93085F | 373 |
| | C93109F | 408 |
| Group 5 High | C93111F | 692 |
| | C93127F | 619 |
| | C93143F | 621 |
| | C93155F | 675 |
| | C93169F | 569 |

Note: Not corrected for purity of the standard material.
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 24. Week 53 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92501M | 0.0326 |
| | C92504M | 0.358 |
| | C92507M | 0.301 |
| | C92512M | 0.283 |
| | C92517M | 0.761 |
| | C92523M | 0.529 |
| | C92534M | 0.359 |
| | C92540M | 0.106 |
| | C92562M | 3.52 |
| | C92570M | 0.103 |
| Group 5 High | C92751M | 559 |
| | C92754M | 486 |
| | C92755M | 486 |
| | C92778M | 279 |
| | C92790M | 405 |
| | C92795M | 554 |
| | C92796M | 427 |
| | C92817M | 403 |
| | C92820M | 316 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 25. Week 53 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92862F | 5.66 |
| | C92876F | 0.131 |
| | C92889F | 2.08 |
| | C92894F | 0.276 |
| | C92899F | 0.217 |
| | C92910F | 0.198 |
| | C92917F | 0.246 |
| | C92922F | 0.0580 |
| | C92923F | 0.154 |
| | C92930F | 0.215 |
| Group 5 High | C93112F | 296 |
| | C93116F | 534 |
| | C93117F | 514 |
| | C93120F | 430 |
| | C93130F | 504 |
| | C93138F | 565 |
| | C93147F | 619 |
| | C93154F | 424 |
| | C93163F | 883 |
| | C93171F | 830 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 26. Day 719 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 3 Mid | C92991F | 43.6 |
| | C93003F | 51.7 |
| | C93007F | 97.2 |
| | C93011F | 13.2 |
| | C93022F | 9.02 |
| | C93039F | 79.7 |
| | C93040F | 94.1 |
| | C93047F | 53.3 |
| | C93048F | 53.8 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92503M | 0.152 |
| | C92505M | 0.106 |
| | C92510M | 0.511 |
| | C92513M | 0.0663 |
| | C92514M | 0.233 |
| | C92518M | <LOQ |
| | C92541M | 0.0283 |
| | C92542M | 0.0342 |
| | C92544M | 0.0291 |
| | C92551M | <LOQ |
| | C92569M | 0.0690 |
| Group 2 Low | C92577M | 1.60 |
| | C92583M | 1.64 |
| | C92590M | 3.89 |
| | C92597M | 17.2 |
| | C92598M | 2.02 |
| | C92601M | 21.6 |
| | C92602M | 10.0 |
| | C92619M | 1.39 |
| | C92623M | 13.4 |
| | C92626M | 5.61 |
| Group 3 Mid | C92631M | 41.5 |
| | C92633M | 51.2 |
| | C92637M | 3.82 |
| | C92641M | 3.35 |
| | C92642M | 23.9 |
| | C92643M | 18.5 |
| | C92644M | 4.32 |
| | C92649M | 54.0 |
| | C92653M | 2.85 |
| | C92657M | 19.8 |
| | C92659M | 61.4 |
| | C92660M | 10.6 |
| | C92669M | 17.4 |
| | C92674M | 59.0 |
| | C92682M | 38.2 |
| | C92683M | 16.9 |
| | C92690M | 21.5 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 4 Mid-High | C92691M | 27.7 |
| | C92693M | 30.5 |
| | C92694M | 208 |
| | C92695M | 195 |
| | C92696M | 120 |
| | C92698M | 18.9 |
| | C92699M | 164 |
| | C92700M | 69.1 |
| | C92702M | 13.1 |
| | C92704M | 65.3 |
| | C92705M | 67.7 |
| | C92708M | 51.6 |
| | C92716M | 85.8 |
| | C92717M | 13.7 |
| | C92724M | 46.1 |
| | C92726M | 95.0 |
| | C92730M | 18.0 |
| | C92735M | 73.7 |
| | C92736M | 15.1 |
| | C92739M | 35.6 |
| | C92740M | 155 |
| | C92742M | 7.16 |
| | C92747M | 170 |
| | C92748M | 11.5 |
| | C92750M | 6.80 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 27. Week 105 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002 (continued)

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 5 High | C92753M | 113 |
| | C92757M | 329 |
| | C92761M | 80.2 |
| | C92762M | 175 |
| | C92774M | 39.4 |
| | C92775M | 35.0 |
| | C92776M | 320 |
| | C92780M | 163 |
| | C92782M | 53.1 |
| | C92786M | 98.6 |
| | C92788M | 385 |
| | C92791M | 69.0 |
| | C92792M | 54.8 |
| | C92800M | 159 |
| | C92802M | 409 |
| | C92803M | 411 |
| | C92804M | 330 |
| | C92808M | 119 |
| | C92809M | 23.3 |
| | C92814M | 358 |
| | C92815M | 72.0 |
| | C92818M | 352 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 1 Control | C92866F | 0.907 |
| | C92867F | 0.367 |
| | C92868F | 0.108 |
| | C92869F | 0.250 |
| | C92871F | <LOQ |
| | C92873F | 0.254 |
| | C92874F | 0.151 |
| | C92877F | 0.271 |
| | C92881F | 0.0904 |
| | C92882F | 0.228 |
| | C92885F | <LOQ |
| | C92890F | 0.248 |
| | C92892F | 0.0490 |
| | C92895F | 0.113 |
| | C92896F | 0.116 |
| | C92897F | 0.158 |
| | C92906F | 0.328 |
| | C92907F | 0.0346 |
| | C92909F | 0.113 |
| | C92911F | 0.206 |
| | C92912F | 0.0667 |
| | C92913F | 0.183 |
| | C92925F | <LOQ |
| | C92927F | 0.161 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 2 Low | C92936F | 10.5 |
| | C92938F | 1.72 |
| | C92946F | 15.3 |
| | C92953F | 20.1 |
| | C92957F | 14.5 |
| | C92960F | 4.94 |
| | C92964F | 20.1 |
| | C92970F | 6.69 |
| | C92973F | 5.08 |
| | C92974F | 15.8 |
| | C92975F | 13.5 |
| | C92983F | 27.0 |
| | C92984F | 8.58 |
| | C92986F | 13.2 |
| | C92988F | 17.2 |
| Group 4 Mid-High | C93059F | 183 |
| | C93060F | 133 |
| | C93062F | 242 |
| | C93063F | 133 |
| | C93072F | 167 |
| | C93077F | 152 |
| | C93078F | 61.7 |
| | C93079F | 113 |
| | C93080F | 149 |
| | C93081F | 118 |
| | C93090F | 186 |
| | C93098F | 19.2 |
| | C93101F | 193 |
| | C93102F | 40.0 |
| | C93107F | 79.8 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

**Table 28. Week 105 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002 (continued)**

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 5 High | C93113F | 373 |
| | C93115F | 378 |
| | C93124F | 174 |
| | C93125F | 444 |
| | C93133F | 403 |
| | C93135F | 582 |
| | C93137F | 281 |
| | C93139F | 446 |
| | C93140F | 9.20 |
| | C93146F | 131 |
| | C93149F | 575 |
| | C93151F | 292 |
| | C93153F | 234 |
| | C93157F | 76.8 |
| | C93158F | 617 |
| | C93161F | 212 |
| | C93162F | 695 |
| | C93164F | 510 |
| | C93165F | 411 |
| | C93166F | 394 |
| | C93168F | 462 |
| | C93174F | 493 |
| | C93177F | 623 |
| | C93178F | 396 |
| | C93179F | 328 |

LOQ—Limit of Quantitation = 0.0107 µg/g
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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 29. Week 106 Reported PFOS Levels in Liver of Male Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 6 High Recovery | C92821M | 9.68 |
| | C92823M | 1.91 |
| | C92827M | 0.144 |
| | C92829M | 0.117 |
| | C92833M | 0.382 |
| | C92834M | 0.203 |
| | C92848M | 17.9 |
| | C92851M | 0.363 |
| | C92853M | 0.431 |
| | C92856M | 0.0332 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

## Table 30. Week 106 Reported PFOS Levels in Liver of Female Rats in Study FACT TOX-002

| Dosage Group | Specimen ID | PFOS µg/g |
|---|---|---|
| Group 6 High Recovery | C93181F | 4.32 |
| | C93182F | 2.10 |
| | C93183F | 9.08 |
| | C93184F | 1.69 |
| | C93185F | 15.9 |
| | C93190F | 22.8 |
| | C93192F | 15.0 |
| | C93197F | 8.65 |
| | C93198F | 6.18 |
| | C93201F | 19.2 |
| | C93202F | 17.0 |
| | C93205F | 0.132 |
| | C93209F | 14.9 |
| | C93211F | 42.6 |
| | C93216F | 14.7 |
| | C93219F | 4.96 |
| | C93220F | 19.5 |

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, indicated that the sera data are accurate to ±30%; liver data are accurate to ±50%.

# Appendix E: Data Spreadsheets

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum - Curve linear regression, no weighting |
| Method/Revision: | FACT-M-3.0 & FACT-M-4.0 - Reworked x dropping 97 ppb std |
| 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: | 05/27/98 OK |
| Date of Analysis/Analyst: | 06/26/98, 07/15/98 HOJ/MEE/KJH |
| Date of Data Reduction/Analyst: | 07/02/98, 07/23/98 HOJ |

**Blks**
**Grp 1**
**Grp 2**
**Grp 3**
**Grp 4**
**Grp 5**
**MS, MSD**

**Sample Data**

**WEEK 4 RAT SERA** Lot 215

| Group Dose | Sample # | Initial Vol. mL | PFOS Std Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | Concentration of PFOS ug/mL or % Rec. | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-1 | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.00453 ug/mL) * | | |
| | H2O Blk-2 | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.00453 ug/mL) * | <LOQ (0.00453 ug/mL) | NA |
| Matrix Blk | Rat Serum Blk-1 | 1 | 0.9275 | ND | 1 | 1.75 | <LOQ (0.00453 ug/mL) * | | |
| | Rat Serum Blk-2 | 1 | 0.9275 | ND | 1 | 1.73 | <LOQ (0.00453 ug/mL) * | <LOQ (0.00453 ug/mL) | NA |
| QC-75 ppb | MS | 1 | NA | NA | 1 | 122 | 167% * | | |
| | MSD | 1 | NA | NA | 1 | 92.6 | 127% * | 147% | 27% |
| **Group 1** | C92506M | 1 | 0.9275 | ND | 1 | 1.57 | <LOQ (0.00910 ug/mL) | | |
| Control | C92524M | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.00910 ug/mL) | | |
| 0.0 mg/kg | C92526M | 1 | 0.9275 | ND | 1 | 4.31 | <LOQ (0.00910 ug/mL) | | |
| | C92529M | 1 | 0.9275 | ND | 1 | 4.54 | <LOQ (0.00910 ug/mL) | | NA |
| | C92546M | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.00910 ug/mL) | <LOQ (0.00910 ug/mL) | NA |
| | C92861F | 1 | 0.9275 | ND | 1 | 24.5 | 0.0227 | | |
| | C92864F | 1 | 0.9275 | ND | 1 | 33.4 | 0.0309 | | |
| | C92914F | 1 | 0.9275 | ND | 1 | 37.5 | 0.0347 | | |
| | C92919F | 1 | 0.9275 | ND | 1 | 20.8 | 0.0193 | | 25.7 |
| | C92926F | 1 | 0.9275 | ND | 1 | 23.3 | 0.0216 | 0.0259 | 0.00663 |
| **Group 2** | C92574M | 1 | 0.9275 | ND | 20 | 49.0 | 0.910 * | | |
| Low Dose | C92575M | 1 | 0.9275 | ND | 20 | 53.3 | 0.989 * | | |
| 0.5 mg/kg | C92610M | 1 | 0.9275 | ND | 20 | 50.9 | 0.944 * | | |
| | C92613M | 1 | 0.9275 | ND | 20 | 46.0 | 0.852 * | | 6.83 |
| | C92618M | 1 | 0.9275 | ND | 20 | 45.4 | 0.842 * | 0.907 | 0.0619 |
| | C92982F | 1 | 0.9275 | ND | 20 | 91.1 | 1.69 * | | |
| | C92941F | 1 | 0.9275 | ND | 20 | 89.8 | 1.67 * | | |
| | C92945F | 1 | 0.9275 | ND | 20 | 102 | 1.89 * | | |
| | C92950F | 1 | 0.9275 | ND | 20 | 74.6 | 1.38 * | | 12.8 |
| | C92969F | 1 | 0.9275 | ND | 20 | 77.5 | 1.44 * | 1.61 | 0.207 |
| **Group 3** | C92646M | 1 | 0.9275 | ND | 50 | 85.5 | 3.97 * | | |
| Mid Dose | C92650M | 1 | 0.9275 | ND | 50 | 61.4 | 2.85 * | | |
| 2.0 mg/kg | C92652M | 1 | 0.9275 | ND | 50 | 103 | 4.80 * | | |
| | C92668M | 1 | 0.9275 | ND | 50 | 129 | 5.98 * | | 26.7 |
| | C92678M | 1 | 0.9275 | ND | 50 | 87.2 | 4.04 * | 4.33 | 1.16 |
| | C92996F | 1 | 0.9275 | ND | 50 | 147 | 6.84 * | | |
| | C93006F | 1 | 0.9275 | ND | 50 | 124 | 5.75 * | | |
| | C93008F | 1 | 0.9275 | ND | 50 | 148 | 6.85 * | | |
| | C93031F | 1 | 0.9275 | ND | 50 | 143 | 6.65 * | | 7.54 |
| | C93045F | 1 | 0.9275 | ND | 50 | 151 | 6.99 * | 6.62 | 0.499 |
| **Group 4** | C92715M | 1 | 0.9275 | ND | 100 | 91.2 | 8.46 * | | |
| Mid-High Dose | C92718M | 1 | 0.9275 | ND | 100 | 107 | 9.89 * | | |
| 5.0 mg/kg | C92731M | 1 | 0.9275 | ND | 100 | 43.4 | 4.03 * | | |
| | C92734M | 1 | 0.9275 | ND | 100 | 82.9 | 7.69 * | | 28.6 |
| | C92744M | 1 | 0.9275 | ND | 100 | 84.2 | 7.81 * | 7.57 | 2.17 |
| | C93058F | 1 | 0.9275 | ND | 100 | 165 | 15.3 * | | |
| | C93067F | 1 | 0.9275 | ND | 100 | 142 | 13.2 * | | |
| | C93070F | 1 | 0.9275 | ND | 100 | 125 | 11.6 * | | |
| | C93075F | 1 | 0.9275 | ND | 100 | 116 | 10.8 * | | 13.8 |
| | C93084F | 1 | 0.9275 | ND | 100 | 131 | 12.1 * | 12.6 | 1.73 |
| **Group 5** | C92752M | 1 | 0.9275 | ND | 200 | 157 | 29.0 * | | |
| High Dose | C92759M | 1 | 0.9275 | ND | 200 | 219 | 40.7 * | | |
| 20 mg/kg | C92779M | 1 | 0.9275 | ND | 200 | 267 | 49.5 * | | |
| | C92793M | 1 | 0.9275 | ND | 200 | 230 | 42.7 * | | 19.0 |
| | C92798M | 1 | 0.9275 | ND | 200 | 253 | 47.0 * | 41.8 | 7.92 |
| | C93114F | 1 | 0.9275 | ND | 200 | 338 | 62.8 * | | |
| | C93123F | 1 | 0.9275 | ND | 200 | 282 | 52.4 * | | |
| | C93142F | 1 | 0.9275 | ND | 200 | 304 | 56.4 * | | |
| | C93144F | 1 | 0.9275 | ND | 200 | 230 | 42.7 * | | 13.6 |
| | C93159F | 1 | 0.9275 | ND | 200 | 301 | 55.8 * | 54.0 | 7.34 |

Original PFOS LOQ (4.88 ng/mL, 9.81 ng/mL) updated to reflect K+ information on 10/04/00. LAC 10/04/00

PFOS = Perfluorooctanesulfonate

Correction factors not applicable for MS/MSD QC data

* Data entered, Continuing calibration verifications did not meet +/- 30% criteria. LAC 09/05/00

ND = Not Determined

NA= Components were not integrated because they were not detected in the group 5 samples.

Date Entered/By: 07/30/98, 09/05/00 LAC

Date Verified/ By: 08/24/00 KJH

Purity Entered/Verified: 09/12/00 LAC

FACT-M-4.0
Excel 97

TOX-002-sera183-3N

2/13/01

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum - Curve linear regression, 1/X weighted |
| Method/Revision: | FACT-M-3.0 & ETS-8-5.1 |
| Analytical Equipment System Number: | Amelia 062498 |
| Instrument Software/Version: | MassLynx 3.3 |
| Filename: | See Attachments |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 07/28/98 IAS |
| Date of Analysis/Analyst: | 07/29/98, 08/14/98, 08/17/98 HOJ/MEE |
| Date of Data Reduction/Analyst: | 02/01/00, 02/02/00 IAS |

Filenames

| | PFOS | Dilutions |
|---|---|---|
| **Blks** | A072998002-3 & 71-72 | 1/1 |
| **Grp 1 M** | 072998013-17 | 1/1 |
| **Grp 1 F** | 072998021, 24, 25 | 1/1 |
| **Grp 1 F** | 81498012-13 | 1/25 |
| **Grp 2** | 81498016-26 | 1/25 |
| **Grp 3** | 81498029-40 | 1/50 |
| **Grp 4** | 81498043-54 | 1/100 |
| **Grp 5 M** | 81498064-68 | 1/250 |
| **Grp 5 F** | 08179825-29 | 1/500 |
| **MS, MSD** | 81498071-72 | 1/1 |

**Sample Data**

**WEEK 14 RAT SERA REWORK** Lot 193

| Group Dose | Sample # | Initial Vol. mL | PFOS Std Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | Concentration of PFOS ug/mL or % Rec. | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-1 | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.0457 ug/mL) | | |
| | H2O Blk-2 | 1 | 0.9275 | ND | 1 | 7.82 | <LOQ (0.0457 ug/mL) | <LOQ (0.0457 ug/mL) | NA |
| Matrix Blk | Rabbit Serum Blk-1 | 1 | 0.9275 | ND | 1 | 0.00 | <LOQ (0.0457 ug/mL) | | |
| | Rabbit Serum Blk-2 | 1 | 0.9275 | ND | 1 | 6.79 | <LOQ (0.0457 ug/mL) | <LOQ (0.0457 ug/mL) | NA |
| QC-100 ppb | TN-A-1902-MS | 1 | NA | NA | 1 | 105 | 107% | | |
| Rat Serum | TN-A-1902-MSD | 1 | NA | NA | 1 | 105 | 108% | 108% | 1% |
| **Group 1** | C92509M | 1 | 0.9275 | ND | 1 | 18.0 | <LOQ (0.0457 ug/mL) | | |
| Control | C92511M | 1 | 0.9275 | ND | 1 | 17.4 | <LOQ (0.0457 ug/mL) | | |
| 0.0 ppm | C92521M | 1 | 0.9275 | ND | 1 | 17.2 | <LOQ (0.0457 ug/mL) | | |
| in Diet | C92528M | 1 | 0.9275 | ND | 1 | 18.9 | <LOQ (0.0457 ug/mL) | | NA |
| | C92532M | 1 | 0.9275 | ND | 1 | 17.5 | <LOQ (0.0457 ug/mL) | <LOQ (0.0457 ug/mL) | NA |
| | C92880F | 1 | 0.9275 | ND | 1 | 360 | 0.332 | | |
| | C92887F | 1 | 0.9275 | ND | 25 | 468 | 10.8 | | |
| | C92898F | 1 | 0.9275 | ND | 1 | 273 | 0.252 | | |
| | C92903F | 1 | 0.9275 | ND | 25 | 70.0 | 1.62 | | 172 |
| | C92905F | 1 | 0.9275 | ND | 1 | 361 | 0.333 | 2.67 | 4.58 |
| **Group 2** | C92593M | 1 | 0.9275 | ND | 25 | 204 | 4.70 | | |
| Low Dose | C92600M | 1 | 0.9275 | ND | 25 | 198 | 4.58 | | |
| 0.5 ppm | C92616M | 1 | 0.9275 | ND | 25 | 177 | 4.09 | | |
| in Diet | C92621M | 1 | 0.9275 | ND | 25 | 179 | 4.13 | | 19.8 |
| | C92627M | 1 | 0.9275 | ND | 25 | 117 | 2.69 | 4.04 | 0.801 |
| | C92944F | 1 | 0.9275 | ND | 25 | 256 | 5.90 | | |
| | C92962F | 1 | 0.9275 | ND | 25 | 356 | 8.21 | | |
| | C92967F | 1 | 0.9275 | ND | 25 | 314 | 7.23 | | |
| | C92978F | 1 | 0.9275 | ND | 25 | 282 | 6.50 | | 14.3 |
| | C92987F | 1 | 0.9275 | ND | 25 | ** | ** | 6.96 | 0.993 |
| **Group 3** | C92640M | 1 | 0.9275 | ND | 50 | 344 | 15.9 | | |
| Mid Dose | C92645M | 1 | 0.9275 | ND | 50 | 367 | 16.9 | | |
| 2.0 ppm | C92662M | 1 | 0.9275 | ND | 50 | 350 | 16.1 | | |
| in Diet | C92676M | 1 | 0.9275 | ND | 50 | 387 | 17.9 | | 7.13 |
| | C92684M | 1 | 0.9275 | ND | 50 | 408 | 18.8 | 17.1 | 1.22 |
| | C92993F | 1 | 0.9275 | ND | 50 | 621 | 28.7 | | |
| | C93000F | 1 | 0.9275 | ND | 50 | 579 | 26.7 | | |
| | C93018F | 1 | 0.9275 | ND | 50 | 543 | 25.1 | | |
| | C93023F | 1 | 0.9275 | ND | 50 | 549 | 25.4 | | 8.57 |
| | C93035F | 1 | 0.9275 | ND | 50 | 663 | 30.6 | 27.3 | 2.34 |
| **Group 4** | C92713M | 1 | 0.9275 | ND | 100 | 532 | 49.1 | | |
| Mid-High Dose | C92714M | 1 | 0.9275 | ND | 100 | 403 | 37.2 | | |
| 5.0 ppm | C92719M | 1 | 0.9275 | ND | 100 | 451 | 41.6 | | |
| in Diet | C92722M | 1 | 0.9275 | ND | 100 | 469 | 43.3 | | 11.2 |
| | C92728M | 1 | 0.9275 | ND | 100 | 522 | 48.2 | 43.9 | 4.90 |
| | C93051F | 1 | 0.9275 | ND | 100 | 614 | 56.7 | | |
| | C93064F | 1 | 0.9275 | ND | 100 | 699 | 64.5 | | |
| | C93071F | 1 | 0.9275 | ND | 100 | 773 | 71.3 | | |
| | C93085F | 1 | 0.9275 | ND | 100 | 675 | 62.3 | | 8.51 |
| | C93109F | 1 | 0.9275 | ND | 100 | 729 | 67.3 | 64.4 | 5.48 |
| **Group 5** | C92765M | 1 | 0.9275 | ND | 250 | 602 | 139 | | |
| High Dose | C92777M | 1 | 0.9275 | ND | 250 | 629 | 145 | | |
| 20 ppm | C92789M | 1 | 0.9275 | ND | 250 | 645 | 149 | | |
| in Diet | C92799M | 1 | 0.9275 | ND | 250 | 588 | 136 | | 9.35 |
| | C92812M | 1 | 0.9275 | ND | 250 | 740 | 171 | 148 | 13.8 |
| | C93111F | 1 | 0.9275 | ND | 500 | 496 | 229 | | |
| | C93127F | 1 | 0.9275 | ND | 500 | 456 | 210 | | |
| | C93143F | 1 | 0.9275 | ND | 500 | 559 | 258 | | |
| | C93155F | 1 | 0.9275 | ND | 500 | 477 | 220 | | 10.0 |
| | C93169F | 1 | 0.9275 | ND | 500 | 432 | 199 | 223 | 22.4 |

Original PFOS LOQ (49.3 ng/mL) updated to reflect K+ information on 10/04/00. LAC 10/04/00
Correction factors not applicable for MS/MSD QC data ND = Not Determined
**PFOS** = Perfluorooctanesulfonate
** Sample spilled during extraction, no sample remaining for analysis.
Date Entered/By: 02/09/00, 09/07/00 LAC
Date Verified/ By: 08/24/00 KJH
Purity Entered/Verified: 09/12/00 LAC, 9/12/00 MMH
Original PFOS LOQ = 25 ng/mL updated to reflect purity information on 10/04/00. LAC 10/04/00

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum |
| Method/Revision: | ETS-8-4.1 and ETS-8-5.1 |
| Analytical Equipment System Number: | Soup 020199 |
| Instrument Software/Version: | MassLynx 3.3 |
| Filename: | See Attachments |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 11/10/99 SEE |
| Date of Analysis/Analyst: | 11/15/99, 11/19/99, 11/22/99, 11/24/99 IAS, 12/15/99 IAS |
| Date of Data Reduction/Analyst: | 11/16/99, 11/22/99, 11/23/99, 11/29/99 IAS, 12/13/99 MMH |

Filenames

| | PFOS | Dilutions |
|---|---|---|
| **Blanks** | S121499043-45,63-65 | 1/1 |
| **Grp 1 M** | 111599019-25 | 1/1 |
| **Grp 1 F** | 111599026,28-32 | 1/1 |
| **Grp 1 F** | 121499057 | 1/2 |
| **Grp 5 M** | 112299017-20 | 1/200&2000 |
| **Grp 5 F** | 112499016-19 | 1/1 |
| **MS, MSD** | 121499058-59 | 1/1 |
| **Box** 99-220 | | |

**Sample Data**

**WEEK 53 RAT SERA** Lot 171

| Group Dose | Sample # | Initial Vol. mL | PFOS Std Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | Concentration of PFOS ug/mL or % Rec. | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-11 | 1.0 | 0.9275 | 0.8640 | 1 | 9.75 | <LOQ (0.00782 ug/mL) | | |
| | H2O Blk-12 | 1.0 | 0.9275 | 0.8640 | 1 | 5.11 | <LOQ (0.00782 ug/mL) | <LOQ (0.00782 ug/mL) | NA |
| Matrix Blk | Rabbit Serum Blk-11 | 1.0 | 0.9275 | 0.8640 | 1 | 6.61 | <LOQ (0.00782 ug/mL) | | |
| | Rabbit Serum Blk-12 | 1.0 | 0.9275 | 0.8640 | 1 | 3.62 | <LOQ (0.00782 ug/mL) | <LOQ (0.00782 ug/mL) | NA |
| Matrix Blk | Rat Serum Blk-11 | 1.0 | 0.9275 | 0.8640 | 1 | 46.6 | 0.0373 | | |
| | Rat Serum Blk-12 | 1.0 | 0.9275 | 0.8640 | 1 | 476 | 0.3814 * | 0.209 | 0.243 |
| QC-250 ppb | RTS11109-250ppb-MS-11 | 1.0 | NA | NA | 1 | 189 | 76% | | |
| | RTS11109-250ppb-MSD-11 | 1.0 | NA | NA | 1 | 224 | 90% | 83% | 17% |
| **Group 1** | C92504M | 0.600 | 0.9275 | 0.8640 | 1 | 20.8 | 0.0278 | | |
| Control | C92512M | 0.700 | 0.9275 | 0.8640 | 1 | 13.1 | 0.0149 | | |
| 0.0 ppm | C92534M | 0.700 | 0.9275 | 0.8640 | 1 | 21.9 | 0.0251 | | |
| in Diet | C92562M | 0.700 | 0.9275 | 0.8640 | 1 | 46.2 | 0.0529 | | 73.2 |
| | C92570M | 0.700 | 0.9275 | 0.8640 | 1 | 4.71 | <LOQ (0.00395 ug/mL) | 0.0249 | 0.0182 |
| | C92876F | 0.800 | 0.9275 | 0.8640 | 1 | 31.0 | 0.0310 | | |
| | C92889F | 0.700 | 0.9275 | 0.8640 | 2 | 779 | 1.78 | | |
| | C92899F | 0.800 | 0.9275 | 0.8640 | 1 | 65.5 | 0.0656 | | |
| | C92923F | 0.700 | 0.9275 | 0.8640 | 1 | 77.9 | 0.0891 | | 197 |
| | C92930F | 0.700 | 0.9275 | 0.8640 | 1 | 3.93 | <LOQ (0.00395 ug/mL) | 0.395 | 0.777 |
| **Group 5** | C92778M | 0.800 | 0.9275 | 0.8640 | 200 | 482 | 96.6 | | |
| High Dose | C92795M | 0.800 | 0.9275 | 0.8640 | 200 | 806 | 162 | | |
| 20 ppm | C92796M | 0.600 | 0.9275 | 0.8640 | 200 | 640 | 171 | | 23.0 |
| in Diet | C92817M | 0.800 | 0.9275 | 0.8640 | 200 | 767 | 154 | 146 | 33.5 |
| | C93112F | 1.000 | 0.9275 | 0.8640 | 2000 | 150 | 240 | | |
| | C93120F | 1.000 | 0.9275 | 0.8640 | 200 | 897 | 144 | | |
| | C93154F | 0.900 | 0.9275 | 0.8640 | 2000 | 125 | 223 | | |
| | C93163F | 0.900 | 0.9275 | 0.8640 | 2000 | 138 | 245 | | 20.0 |
| | C93171F | 0.900 | 0.9275 | 0.8640 | 2000 | 140 | 250 | 220 | 44.0 |

Original PFOS LOQ (4.93 ng/mL, 9.76 ng/mL) updated to reflect K+ and purity information on 10/04/00. LAC 10/04/00
NA = Not Appicable
**PFOS** = Perfluorooctanesulfonate
* Second analysis confirmed high result. LAC 08/24/00
Date Entered/By: 11/22/99, 11/24/99 GML/LAC, 12/1/99 GML, 12/30/99 MMH
Date Verified/ By: 08/31/00 KJH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum |
| Method/Revision: | ETS-8-4.1 & ETS-8-5.1 |
| ANAlytical Equipment System Number: | Ruby 100699 |
| Instrument Software/Version: | MassLynx 3.3, 3.4 |
| FileNAme: | See listing to the right |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Dates of Extraction/ANAlyst: | 05/01/00, 05/02/00 SAL/KJK/RWW |
| Dates of ANAlysis/ANAlyst: | 05/02/00 05/04/00, 05/10/00 05/26/00, 05/30/00 MMH/IAS |
| Date of Data Reduction/ANAlyst: | 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH |

**Sample Data**

**DAY 719 RAT SERA**

| Group Dose | Sample # | Initial Vol. mL | Surrogate Verified | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | FileNAme (optional) | Concentration of PFOS ug/mL or % Rec | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 3 mg/kg/day | C92991F G3 Day719 | 1.00 | NA | 0.9949 | 0.8640 | 50 | 348 | D0510000054 | 15.1 | | |
| | C93003F G3 Day719 | 1.00 | NA | 0.9949 | 0.8640 | 50 | 299 | D0510000055 | 13.0 | | |
| | C93007F G3 Day719 | 0.80 | NA | 0.9949 | 0.8640 | 50 | 569 | D0510000058 | 30.9 | | |
| | C93011F G3 Day719 | 0.75 | NA | 0.9949 | 0.8640 | 50 | 61.7 | D0510000059 | 3.57 | | |
| | C93022F G3 Day719 | 0.85 | NA | 0.9949 | 0.8640 | 50 | 39.5 | D0510000060 | 2.02 | | |
| | C93039F G3 Day719 | 1.00 | NA | 0.9949 | 0.8640 | 50 | 637 | D0510000061 | 27.6 | | |
| | C93040F G3 Day719 | 0.80 | NA | 0.9949 | 0.8640 | 200 | 201 | R0531000030 | 43.6 | | |
| | C93047F G3 Day719 | 0.85 | NA | 0.9949 | 0.8640 | 50 | 429 | D0510000065 | 21.9 | | 65.9 |
| | C93048F G3 Day719 | 0.75 | NA | 0.9949 | 0.8640 | 50 | 420 | D0510000066 | 24.3 | 20.2 | 13.3 |

Original PFOS LOQ (24.8 ng/mL, 4.93 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

| | |
|---|---|
| Date Entered/By: | 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/17/00 MMH/CSH/LAC |
| Date Verified/ By: | 08/31/00 KJH, 11/21/00 MMH |
| Purity Entered/Verified: | 10/04/00 LAC, 10/04/00 KJH |

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum |
| Method/Revision: | ETS-8-4.1 & ETS-8-5.1 |
| ANAlytical Equipment System Number: | Davey 070799, Ruby 100699, Amelia 062498 |
| Instrument Software/Version: | MassLynx 3.3, 3.4 |
| FileNAme: | See listing to the right |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Dates of Extraction/Analyst: | 05/01/00, 05/02/00 SAL/KJK/RWW |
| Dates of Analysis/Analyst: | 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00 MMH/IAS |
| Date of Data Reduction/Analyst: | 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH |

**Sample Data**

**WEEK 105 RAT SERA** Lot 171

| Group Dose | Sample # | Initial Vol. mL | Surrogate Verified | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | Filename (optional) | Concentration of PFOS ug/mL or % Rec | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | C92503M | 0.64 | NA | 0.9949 | 0.8640 | 1 | 10.9 | A051000043 | 0.0148 | | |
| mg/kg/day | C92505M | 0.85 | NA | 0.9949 | 0.8640 | 1 | 15.8 | D050200028 | 0.0161 | | |
| | C92510M | 0.84 | NA | 0.9949 | 0.8640 | 1 | 27.6 | A051000060 | 0.0285 | | |
| | C92513M | 0.82 | NA | 0.9949 | 0.8640 | 1 | 12.0 | A051000061 | 0.0127 | | |
| | C92514M | 0.85 | NA | 0.9949 | 0.8640 | 1 | 31.8 | A051000046 | 0.0325 | | |
| | C92518M | 0.70 | NA | 0.9949 | 0.8640 | 1 | 0.00 | D050200029 | < LOQ (0.00428 ug/mL) | | |
| | C92541M | 0.74 | NA | 0.9949 | 0.8640 | 1 | 3.28 | R053100019 | < LOQ (0.00428 ug/mL) | | |
| | C92542M | 0.84 | NA | 0.9949 | 0.8640 | 1 | 3.10 | R053100028 | < LOQ (0.00428 ug/mL) | | |
| | C92544M | 0.78 | NA | 0.9949 | 0.8640 | 1 | 0.360 | R053100020 | < LOQ (0.00428 ug/mL) | | |
| | C92551M | 0.70 | NA | 0.9949 | 0.8640 | 1 | 0.820 | D050200030 | < LOQ (0.00428 ug/mL) | | 87.6 |
| | C92569M | 0.90 | NA | 0.9949 | 0.8640 | 1 | 3.06 | D050200033 | < LOQ (0.00428 ug/mL) | 0.0118 | 0.0104 |
| | C92866F | 0.98 | NA | 0.9949 | 0.8640 | 1 | 705 | A051000048 | 0.624 | | |
| | C92867F | 0.76 | NA | 0.9949 | 0.8640 | 1 | 197 | A051000064 | 0.225 | | |
| | C92868F | 0.90 | NA | 0.9949 | 0.8640 | 1 | 48.4 | D050200034 | 0.0467 | | |
| | C92869F | 0.87 | NA | 0.9949 | 0.8640 | 1 | 307 | A051000067 | 0.306 | | |
| | C92871F | 0.90 | NA | 0.9949 | 0.8640 | 1 | 6.74 | D050200035 | 0.00650 | | |
| | C92873F | 0.98 | NA | 0.9949 | 0.8640 | 1 | 68.0 | A051000049 | 0.0602 | | |
| | C92874F | 0.85 | NA | 0.9949 | 0.8640 | 1 | 41.8 | D050200036 | 0.0427 | | |
| | C92877F | 0.85 | NA | 0.9949 | 0.8640 | 1 | 97.9 | A051000068 | 0.100 | | |
| | C92881F | 0.87 | NA | 0.9949 | 0.8640 | 1 | 25.9 | A051000050 | 0.0258 | | |
| | C92882F | 0.95 | NA | 0.9949 | 0.8640 | 1 | 52.8 | A051000053 | 0.0483 | | |
| | C92885F | 0.76 | NA | 0.9949 | 0.8640 | 1 | 0.00 | R053100029 | < LOQ (0.00428 ug/mL) | | |
| | C92890F | 0.89 | NA | 0.9949 | 0.8640 | 1 | 69.2 | A051000055 | 0.0675 | | |
| | C92892F | 0.96 | NA | 0.9949 | 0.8640 | 1 | 15.5 | A051000069 | 0.0140 | | |
| | C92895F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 26.9 | A051000070 | 0.0233 | | |
| | C92896F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 46.8 | D050200037 | 0.0407 | | |
| | C92897F | 0.85 | NA | 0.9949 | 0.8640 | 1 | 43.3 | D050200040 | 0.0443 | | |
| | C92906F | 0.85 | NA | 0.9949 | 0.8640 | 1 | 77.9 | D050200041 | 0.0795 | | |
| | C92907F | 0.73 | NA | 0.9949 | 0.8640 | 1 | 14.8 | A051000056 | 0.0176 | | |
| | C92909F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 46.3 | A051000071 | 0.0402 | | |
| | C92911F | 0.81 | NA | 0.9949 | 0.8640 | 1 | 64.1 | A051000074 | 0.0687 | | |
| | C92912F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 35.8 | A051000075 | 0.0311 | | |
| | C92913F | 0.90 | NA | 0.9949 | 0.8640 | 1 | 59.6 | D050200042 | 0.0575 | | |
| | C92925F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 3.62 | A051000057 | <LOQ (0.00848 ug/mL) | | 161 |
| | C92927F | 1.00 | NA | 0.9949 | 0.8640 | 1 | 26.4 | D050200043 | 0.0229 | 0.0836 | 0.134 |
| Group 2 | C92577M | 0.90 | NA | 0.9949 | 0.8640 | 1 | 320 | R052600022 | 0.309 | | |
| mg/kg/day | C92583M | 1.00 | NA | 0.9949 | 0.8640 | 1 | 244 | D050200044 | 0.212 | | |
| | C92590M | 0.43 | NA | 0.9949 | 0.8640 | 10 | 127 | D051000017 | 2.55 | | |
| | C92597M | 1.00 | NA | 0.9949 | 0.8640 | 10 | 225 | R050400017 | 1.95 | | |
| | C92598M | 0.85 | NA | 0.9949 | 0.8640 | 1 | 226 | D050200048 | 0.230 | | |
| | C92601M | 0.81 | NA | 0.9949 | 0.8640 | 10 | 367 | D051000018 | 3.94 | | |
| | C92602M | 0.40 | NA | 0.9949 | 0.8640 | 10 | 64.0 | D051000019 | 1.39 | | |
| | C92619M | 0.81 | NA | 0.9949 | 0.8640 | 10 | 43.0 | D051000020 | 0.461 | | |
| | C92623M | 0.75 | NA | 0.9949 | 0.8640 | 10 | 177 | R051000023 | 2.05 | | 99.0 |
| | C92626M | 1.00 | NA | 0.9949 | 0.8640 | 10 | 171 | R051000024 | 1.48 | 1.31 | 1.30 |
| | C92936F | 1.00 | NA | 0.9949 | 0.8640 | 50 | 201 | R052500106 | 8.72 | | |
| | C92938F | 0.92 | NA | 0.9949 | 0.8640 | 10 | 37.0 | D0510000026 | 0.349 | | |
| | C92946F | 0.83 | NA | 0.9949 | 0.8640 | 50 | 174 | R052500107 | 9.08 | | |
| | C92953F | 0.70 | NA | 0.9949 | 0.8640 | 10 | 535 | R051000030 | 6.64 | | |
| | C92957F | 1.00 | NA | 0.9949 | 0.8640 | 10 | 653 | R051000031 | 5.67 | | |
| | C92960F | 1.00 | NA | 0.9949 | 0.8640 | 10 | 196 | R051000032 | 1.70 | | |
| | C92964F | 0.75 | NA | 0.9949 | 0.8640 | 10 | 515 | R050400018 | 5.97 | | |
| | C92970F | 0.85 | NA | 0.9949 | 0.8640 | 10 | 169 | R050400019 | 1.73 | | |
| | C92973F | 0.95 | NA | 0.9949 | 0.8640 | 10 | 147 | R050400020 | 1.34 | | |
| | C92974F | 0.90 | NA | 0.9949 | 0.8640 | 10 | 421 | R051000033 | 4.06 | | |
| | C92975F | 0.95 | NA | 0.9949 | 0.8640 | 10 | 407 | R051000034 | 3.72 | | |
| | C92983F | 1.00 | NA | 0.9949 | 0.8640 | 10 | 784 | R050400023 | 6.81 | | |
| | C92984F | 0.98 | NA | 0.9949 | 0.8640 | 10 | 313 | R051000037 | 2.78 | | |
| | C92986F | 1.00 | NA | 0.9949 | 0.8640 | 10 | 603 | R051000038 | 5.24 | | 63.8 |
| | C92988F | 0.85 | NA | 0.9949 | 0.8640 | 10 | 563 | R050400024 | 5.76 | 4.35 | 2.78 |
| Group 3 | C92631M | 0.75 | NA | 0.9949 | 0.8640 | 10 | 776 | R050400025 | 8.98 | | |
| mg/kg/day | C92633M | 0.33 | NA | 0.9949 | 0.8640 | 50 | 109 | R051000039 | 14.3 | | |
| | C92637M | 0.85 | NA | 0.9949 | 0.8640 | 10 | 178 | R050400026 | 1.81 | | |
| | C92641M | 0.74 | NA | 0.9949 | 0.8640 | 50 | 257 | R052500108 | 15.1 | | |
| | C92642M | 0.90 | NA | 0.9949 | 0.8640 | 10 | 627 | R050400027 | 6.05 | | |
| | C92643M | 0.75 | NA | 0.9949 | 0.8640 | 50 | 47.5 | R051000041 | 2.75 | | |
| | C92644M | 1.00 | NA | 0.9949 | 0.8640 | 10 | 139 | R050400030 | 1.21 | | |
| | C92649M | 0.82 | NA | 0.9949 | 0.8640 | 50 | 163 | R051000044 | 8.63 | | |
| | C92653M | 1.00 | NA | 0.9949 | 0.8640 | 1 | 561 | R052600026 | 0.487 | | |
| | C92657M | 0.90 | NA | 0.9949 | 0.8640 | 50 | 108 | R051000046 | 5.20 | | |
| | C92659M | 0.78 | NA | 0.9949 | 0.8640 | 50 | 258 | R051000047 | 14.4 | | |
| | C92660M | 0.96 | NA | 0.9949 | 0.8640 | 50 | 36.7 | R051000048 | 1.66 | | |
| | C92669M | 0.35 | NA | 0.9949 | 0.8640 | 50 | 25.2 | R051000051 | 3.13 | | |
| | C92674M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 406 | R050400031 | 35.3 | | |
| | C92682M | 0.90 | NA | 0.9949 | 0.8640 | 10 | 495 | R050400032 | 4.77 | | |
| | C92683M | 0.85 | NA | 0.9949 | 0.8640 | 50 | 53.3 | R051000052 | 2.72 | | 113 |
| | C92690M | 0.80 | NA | 0.9949 | 0.8640 | 50 | 52.1 | R051000053 | 2.83 | 7.60 | 8.60 |

Original PFOS LOQ (4.93 ng/mL, 9.76 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

| | |
|---|---|
| Date Entered/By: | 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/14/00, 11/17/00 MMH/CSH/LAC |
| Date Verified/ By: | 08/31/00 KJH, 11/21/00 MMH |
| Purity Entered/Verified: | 10/04/00 LAC / 10/04/00 KJH |

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum |
| Method/Revision: | ETS-8-4.1 & ETS-8-5.1 |
| Analytical Equipment System Number: | Ruby 100699, Davey 070799 |
| Instrument Software/Version: | MassLynx 3.3, 3.4 |
| Filename: | See listing to the right |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Dates of Extraction/Analyst: | 05/01/00, 05/02/00 SAL/KJK/RWW |
| Dates of Analysis/Analyst: | 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00 MMH/IAS |
| Date of Data Reduction/Analyst: | 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/01/00, 11/16/00 IAS/MMH |

**Sample Data**

**WEEK 105 RAT SERA**

Lot 171

| Group Dose | Sample # | Initial Vol. mL | Surrogate Verified | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | Filename (optional) | Concentration of PFOS ug/mL or % Rec | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 4 mg/kg/day | C92691M | 0.90 | NA | 0.9949 | 0.8640 | 20 | 232 | R050400033 | 4.48 | | |
| | C92693M | 0.85 | NA | 0.9949 | 0.8640 | 100 | 75.5 | D051100016 | 7.71 | | |
| | C92694M | 0.75 | NA | 0.9949 | 0.8640 | 100 | 640 | R050400034 | 74.2 | | |
| | C92695M | 0.85 | NA | 0.9949 | 0.8640 | 100 | 740 | R050400037 | 75.6 | | |
| | C92696M | 0.95 | NA | 0.9949 | 0.8640 | 100 | 423 | R050400038 | 38.7 | | |
| | C92698M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 140 | D051100017 | 12.1 | | |
| | C92699M | 0.70 | NA | 0.9949 | 0.8640 | 100 | 591 | R050400039 | 73.3 | | |
| | C92700M | 0.75 | NA | 0.9949 | 0.8640 | 100 | 248 | D051100018 | 28.8 | | |
| | C92702M | 0.90 | NA | 0.9949 | 0.8640 | 100 | 32.6 | D051100019 | 3.14 | | |
| | C92704M | 0.79 | NA | 0.9949 | 0.8640 | 100 | 98.9 | D051100020 | 10.9 | | |
| | C92705M | 0.73 | NA | 0.9949 | 0.8640 | 200 | 96.8 | R053100024 | 23.0 | | |
| | C92708M | 0.67 | NA | 0.9949 | 0.8640 | 20 | 375 | R053100025 | 9.72 | | |
| | C92716M | 0.75 | NA | 0.9949 | 0.8640 | 20 | 584 | R053100018 | 13.5 | | |
| | C92717M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 34.7 | D051100024 | 3.01 | | |
| | C92724M | 0.85 | NA | 0.9949 | 0.8640 | 20 | 626 | R050400044 | 12.8 | | |
| | C92726M | 0.75 | NA | 0.9949 | 0.8640 | 100 | 335 | D051100025 | 38.8 | | |
| | C92730M | 0.70 | NA | 0.9949 | 0.8640 | 100 | 54.1 | D051100026 | 6.72 | | |
| | C92735M | 0.70 | NA | 0.9949 | 0.8640 | 200 | 98.5 | R050900029 | 24.4 | | |
| | C92736M | 0.75 | NA | 0.9949 | 0.8640 | 100 | 35.5 | D051100027 | 4.10 | | |
| | C92739M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 65.8 | D051100030 | 5.72 | | |
| | C92740M | 0.85 | NA | 0.9949 | 0.8640 | 100 | 349 | D051100031 | 35.7 | | |
| | C92742M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 26.3 | D051100032 | 2.28 | | |
| | C92747M | 0.57 | NA | 0.9949 | 0.8640 | 100 | 300 | D051100033 | 45.7 | | |
| | C92748M | 1.00 | NA | 0.9949 | 0.8640 | 100 | 42.1 | D051100034 | 3.66 | | 105 |
| | C92750M | 0.85 | NA | 0.9949 | 0.8640 | 100 | 33.3 | D051100037 | 3.40 | 22.5 | 23.5 |
| | C93059F | 0.80 | NA | 0.9949 | 0.8640 | 500 | 189 | R052500109 | 103 | | |
| | C93060F | 0.85 | NA | 0.9949 | 0.8640 | 500 | 175 | R052600028 | 89.5 | | |
| | C93062F | 1.00 | NA | 0.9949 | 0.8640 | 1000 | 117 | R050900030 | 102 | | |
| | C93063F | 1.00 | NA | 0.9949 | 0.8640 | 1000 | 197 | R050900031 | 171 | | |
| | C93072F | 0.85 | NA | 0.9949 | 0.8640 | 500 | 223 | R052500109 | 114 | | |
| | C93077F | 1.00 | NA | 0.9949 | 0.8640 | 100 | 438 | D051100041 | 38.0 | | |
| | C93078F | 0.94 | NA | 0.9949 | 0.8640 | 50 | 562 | R050400048 | 25.9 | | |
| | C93079F | 1.00 | NA | 0.9949 | 0.8640 | 500 | 168 | R052500113 | 72.8 | | |
| | C93080F | 0.90 | NA | 0.9949 | 0.8640 | 500 | 153 | R052600029 | 73.6 | | |
| | C93081F | 0.78 | NA | 0.9949 | 0.8640 | 500 | 127 | R052500114 | 70.5 | | |
| | C93090F | 1.00 | NA | 0.9949 | 0.8640 | 200 | 570 | R050400051 | 99.1 | | |
| | C93098F | 1.00 | NA | 0.9949 | 0.8640 | 100 | 59.5 | D051100047 | 5.17 | | |
| | C93101F | 0.75 | NA | 0.9949 | 0.8640 | 500 | 202 | R052500115 | 117 | | |
| | C93102F | 0.80 | NA | 0.9949 | 0.8640 | 100 | 105 | R051100051 | 11.4 | | 60.9 |
| | C93107F | 0.83 | NA | 0.9949 | 0.8640 | 50 | 635 | R050400052 | 33.2 | 75.0 | 45.7 |
| Group 5 mg/kg/day | C92753M | 0.80 | NA | 0.9949 | 0.8640 | 2000 | 16.5 | D051100052 | 35.8 | | |
| | C92757M | 0.87 | NA | 0.9949 | 0.8640 | 1000 | 196 | R050900032 | 196 | | |
| | C92761M | 0.65 | NA | 0.9949 | 0.8640 | 2000 | 10.2 | D051100053 | 27.3 | | |
| | C92762M | 1.00 | NA | 0.9949 | 0.8640 | 200 | 751 | R050400054 | 130 | | |
| | C92774M | 1.00 | NA | 0.9949 | 0.8640 | 50 | 239 | R052600033 | 10.4 | | |
| | C92775M | 0.85 | NA | 0.9949 | 0.8640 | 50 | 217 | R052500117 | 11.1 | | |
| | C92776M | 0.96 | NA | 0.9949 | 0.8640 | 2000 | 59.9 | D051100058 | 108 | | |
| | C92780M | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 38.8 | D051100059 | 67.4 | | |
| | C92782M | 0.57 | NA | 0.9949 | 0.8640 | 50 | 220 | R052500120 | 16.7 | | |
| | C92786M | 0.91 | NA | 0.9949 | 0.8640 | 50 | 577 | R050400055 | 27.5 | | |
| | C92788M | 0.80 | NA | 0.9949 | 0.8640 | 2000 | 54.4 | D051100061 | 118 | | |
| | C92791M | 0.93 | NA | 0.9949 | 0.8640 | 50 | 459 | R050400058 | 21.4 | | |
| | C92792M | 0.64 | NA | 0.9949 | 0.8640 | 2000 | 6.62 | D051100062 | 18.0 | | |
| | C92800M | 0.91 | NA | 0.9949 | 0.8640 | 200 | 336 | R050400059 | 64.2 | | |
| | C92802M | 0.92 | NA | 0.9949 | 0.8640 | 1000 | 179 | R050900033 | 169 | | |
| | C92803M | 0.75 | NA | 0.9949 | 0.8640 | 2000 | 60.7 | D051100065 | 141 | | |
| | C92804M | 0.88 | NA | 0.9949 | 0.8640 | 200 | 645 | R050400061 | 127 | | |
| | C92808M | 0.86 | NA | 0.9949 | 0.8640 | 50 | 736 | R050400062 | 37.2 | | |
| | C92809M | 0.65 | NA | 0.9949 | 0.8640 | 50 | 90.1 | R052600034 | 6.02 | | |
| | C92814M | 0.85 | NA | 0.9949 | 0.8640 | 2000 | 43.7 | D051100067 | 89.2 | | |
| | C92815M | 0.70 | NA | 0.9949 | 0.8640 | 2000 | 4.79 | D051100068 | 11.9 | | 83.6 |
| | C92818M | 0.85 | NA | 0.9949 | 0.8640 | 2000 | 44.1 | D051100069 | 90.1 | 69.3 | 57.9 |
| | C93113F | 0.94 | NA | 0.9949 | 0.8640 | 2000 | 218 | R050900036 | 403 | | |
| | C93115F | 0.90 | NA | 0.9949 | 0.8640 | 2000 | 148 | D051100072 | 286 | | |
| | C93124F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 91.9 | D051100073 | 160 | | |
| | C93125F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 203 | R050900037 | 353 | | |
| | C93133F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 139 | D051100074 | 242 | | |
| | C93135F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 158 | R050900038 | 274 | | |
| | C93137F | 0.95 | NA | 0.9949 | 0.8640 | 2000 | 78.0 | D051100075 | 143 | | |
| | C93139F | 0.75 | NA | 0.9949 | 0.8640 | 2000 | 162 | D051100076 | 375 | | |
| | C93140F | 0.67 | NA | 0.9949 | 0.8640 | 50 | 59.5 | R052500123 | 3.85 | | |
| | C93146F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 29.6 | D051100080 | 51.5 | | |
| | C93149F | 0.80 | NA | 0.9949 | 0.8640 | 2000 | 199 | R050900039 | 432 | | |
| | C93151F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 80.1 | D051100081 | 139 | | |
| | C93153F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 69.7 | D051100082 | 121 | | |
| | C93157F | 0.85 | NA | 0.9949 | 0.8640 | 2000 | 14.0 | D051100083 | 28.6 | | |
| | C93158F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 165 | R050900040 | 287 | | |
| | C93161F | 0.84 | NA | 0.9949 | 0.8640 | 200 | 499 | R050400072 | 103 | | |
| | C93162F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 257 | R050900043 | 446 | | |
| | C93164F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 185 | R050900044 | 321 | | |
| | C93165F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 94.7 | D051100086 | 165 | | |
| | C93166F | 0.70 | NA | 0.9949 | 0.8640 | 2000 | 76.0 | D051100087 | 189 | | |
| | C93168F | 0.92 | NA | 0.9949 | 0.8640 | 2000 | 180 | D051100088 | 339 | | |
| | C93174F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 175 | R050900045 | 304 | | |
| | C93177F | 0.90 | NA | 0.9949 | 0.8640 | 2000 | 128 | D051100089 | 248 | | |
| | C93178F | 1.00 | NA | 0.9949 | 0.8640 | 2000 | 124 | D051100090 | 216 | | 53.2 |
| | C93179F | 0.99 | NA | 0.9949 | 0.8640 | 2000 | 109 | D051100093 | 190 | 233 | 124 |

Original PFOS LOQ updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

| | |
|---|---|
| Date Entered/By: | 5/11/00, 5/12/00, 5/18/00, 5/19/00, 6/8/00, 6/19/00, 11/14/00, 11/17/00 MMH/CSH/LAC |
| Date Verified/ By: | 08/31/00 kjh, 11/21/00 MMH |
| Purity Entered/Verified: | 10/04/00 LAC, 10/04/00 KJH |

**AMDT# 112296.1**
**Covance# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 (PFOS) |
| Matrix: | Rat Serum |
| Method/Revision: | ETS-8-4.1 & ETS-8-5.1 |
| ANAlytical Equipment System Number: | Ruby 100699, Davey 070799 |
| Instrument Software/Version: | MassLynx 3.3, 3.4 |
| Filename: | See listing to the right |
| R-Squared Value: | See Attachments |
| Slope: | See Attachments |
| Y-Intercept: | See Attachments |
| Dates of Extraction/Analyst: | 05/01/00, 05/02/00 SAL/KJK/RWW |
| Dates of Analysis/Analyst: | 05/02/00, 05/04/00, 05/10/00, 05/26/00, 05/30/00, 07/28/00 MMH/IAS |
| Date of Data Reduction/Analyst: | 05/04/00, 05/05/00, 05/11/00, 05/30/00, 06/1/00, 07/31/00, 11/16/00 IAS/MMH |

**Sample Data**

**WEEK 106 RAT SERA** Lot 171

| Group Dose | Sample # | Initial Vol. mL | Surrogate Verified | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Dilution Factor | PFOS Conc. ng/mL | FileNAme (optional) | Concentration of PFOS ug/mL or % Rec | Mean PFOS ug/mL | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 6 mg/kg/day | C92821M | 0.82 | NA | 0.9949 | 0.8640 | 20 | 393 | R052600036 | 8.32 | | |
| | C92823M | 0.84 | NA | 0.9949 | 0.8640 | 20 | 25.8 | R052600037 | 0.533 | | |
| | C92827M | 0.96 | NA | 0.9949 | 0.8640 | 1 | 33.6 | R053100031 | 0.0303 | | |
| | C92829M | 0.93 | NA | 0.9949 | 0.8640 | 1 | 26.0 | R053100032 | 0.0243 | | |
| | C92833M | 0.66 | NA | 0.9949 | 0.8640 | 1 | 60.4 | R053100035 | 0.0795 | | |
| | C92834M | 0.69 | NA | 0.9949 | 0.8640 | 1 | 11.0 | R053100036 | 0.0138 | | |
| | C92848M | 0.80 | NA | 0.9949 | 0.8640 | 20 | 686 | R052600044 | 14.9 | | |
| | C92851M | 0.84 | NA | 0.9949 | 0.8640 | 1 | 32.0 | R053100037 | 0.0331 | | |
| | C92853M | 0.84 | NA | 0.9949 | 0.8640 | 1 | 203 | D072800017 | 0.210 | | 211 |
| | C92856M | 0.90 | NA | 0.9949 | 0.8640 | 1 | 3.65 | R053100038 | < LOQ (0.00428 ug/mL) | 2.42 | 5.09 |
| | C93181F | 0.86 | NA | 0.9949 | 0.8640 | 20 | 285 | R052600050 | 5.76 | | |
| | C93182F | 0.86 | NA | 0.9949 | 0.8640 | 20 | 83.5 | R052600051 | 1.69 | | |
| | C93183F | 1.00 | NA | 0.9949 | 0.8640 | 20 | 327 | R052600054 | 5.68 | | |
| | C93184F | 0.82 | NA | 0.9949 | 0.8640 | 20 | 77.6 | R052600055 | 1.64 | | |
| | C93185F | 0.95 | NA | 0.9949 | 0.8640 | 20 | 647 | R052600056 | 11.8 | | |
| | C93190F | 0.76 | NA | 0.9949 | 0.8640 | 50 | 553 | D072800018 | 31.6 | | |
| | C93192F | 0.95 | NA | 0.9949 | 0.8640 | 20 | 918 | R052600058 | 16.8 | | |
| | C93197F | 1.00 | NA | 0.9949 | 0.8640 | 20 | 538 | R052600061 | 9.35 | | |
| | C93198F | 0.81 | NA | 0.9949 | 0.8640 | 20 | 403 | R052600062 | 8.63 | | |
| | C93201F | 0.79 | NA | 0.9949 | 0.8640 | 50 | 492 | D072800019 | 27.0 | | |
| | C93202F | 0.77 | NA | 0.9949 | 0.8640 | 20 | 447 | R052600064 | 10.1 | | |
| | C93205F | 0.78 | NA | 0.9949 | 0.8640 | 1 | 53.3 | R053100039 | 0.0594 | | |
| | C93209F | 0.87 | NA | 0.9949 | 0.8640 | 20 | 329 | R052600068 | 6.57 | | |
| | C93211F | 0.88 | NA | 0.9949 | 0.8640 | 20 | 623 | R052600069 | 12.3 | | |
| | C93216F | 1.00 | NA | 0.9949 | 0.8640 | 20 | 164 | R052600070 | 2.85 | | |
| | C93219F | 0.85 | NA | 0.9949 | 0.8640 | 20 | 117 | R052600071 | 2.39 | | 91.5 |
| | C93220F | 1.00 | NA | 0.9949 | 0.8640 | 20 | 428 | R052600072 | 7.44 | 9.51 | 8.70 |

Original PFOS LOQ (4.93 ng/mL) updated to reflect purity information on 10/04/00. LAC 10/04/00

NA = Not Applicable

| | |
|---|---|
| Date Entered/By: | 06/08/00, 06/19/00, 08/14/00, 11/14/00, 11/17/00 CSH/LAC |
| Date Verified/ By: | 08/31/00 KJH, 11/21/00 MMH |
| Purity Entered/Verified: | 10/04/00 LAC, 10/04/00 KJH |

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 (PFOS) | | |
| Matrix: | Rat Liver | Filename: | See List to Right |
| Method/Revision: | FACT-M-1.0 & ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Madeline 041098, Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | MassLynx 3.3 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 6/11/98 IAS/RWW | | |
| Date of Analysis/Analyst: | 07/06/98, 07/14/98 KJH/MEE | | |
| Date of Data Reduction/Analyst: | 02/02/00 IAS | | |

**Sample Data**

**WEEK 4 RAT LIVER REWORK** Lot 193

| Group Dose | Sample # | Initial Wt. g | Total Mass of Liver g | PFOS Std Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Concentration of PFOS ug/g or % Rec. | | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-1 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | | |
| | H2O Blk-2 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | <LOQ (0.0552 ug/g) | NA |
| Matrix Blk | Rabbit Liver Blk-1 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | | |
| | Rabbit Liver Blk-2 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | <LOQ (0.0552 ug/g) | NA |
| QC - 100 ppb | C92506M-MS | 1.0108 | NA | NA | 231 | 1 | 207 | 174% | | | |
| | C92506M-MSD | 1.0108 | NA | NA | 311 | 1 | 286 | 241% | | 207% | 32.15% |
| **Group 1** | C92506M | 1.0108 | NA | 0.9275 | 21.8 | 1 | 20.0 | <LOQ (0.0552 ug/g) | | | |
| Control | C92524M | 0.9998 | NA | 0.9275 | 182 | 1 | 169 | 0.169 | | | |
| 0.0 ppm | C92526M | 1.0287 | NA | 0.9275 | 207 | 1 | 186 | 0.186 | | | |
| in Diet | C92529M | 1.0117 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | | 64.6 |
| | C92546M | 1.0034 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ (0.0552 ug/g) | | 0.104 | 0.0673 |
| | C92861F | 1.0140 | NA | 0.9275 | 89.4 | 1 | 81.7 | 0.0817 | | | |
| | C92864F | 1.0172 | NA | 0.9275 | 80.9 | 1 | 73.8 | 0.0738 | | | |
| | C92914F | 1.0186 | NA | 0.9275 | 202 | 1 | 184 | 0.184 | | | |
| | C92919F | 1.0145 | NA | 0.9275 | 76.6 | 1 | 70.0 | 0.0700 | | | 45.4 |
| | C92926F | 1.0092 | NA | 0.9275 | 137 | 1 | 126 | 0.126 | | 0.107 | 0.0486 |
| **Group 2** | C92574M | 0.9928 | NA | 0.9275 | 398 | 33.3 | 12376 | 12.4 | | | |
| Low Dose | C92575M | 1.0187 | NA | 0.9275 | 319 | 33.3 | 9670 | 9.67 | | | |
| 0.5 ppm | C92610M | 1.0060 | NA | 0.9275 | 278 | 33.3 | 8539 | 8.54 | | | |
| in Diet | C92613M | 1.0075 | NA | 0.9275 | 465 | 33.3 | 14248 | 14.2 | | | 21.1 |
| | C92618M | 1.0133 | NA | 0.9275 | 326 | 33.3 | 9937 | 9.94 | | 11.0 | 2.31 |
| | C92982F | 0.9971 | NA | 0.9275 | 293 | 33.3 | 9089 | 9.09 | | | |
| | C92941F | 1.0104 | NA | 0.9275 | 261 | 33.3 | 7988 | 7.99 | | | |
| | C92945F | 1.0161 | NA | 0.9275 | 309 | 33.3 | 9398 | 9.40 | | | |
| | C92950F | 1.0132 | NA | 0.9275 | 277 | 33.3 | 8429 | 8.43 | | | 6.33 |
| | C92969F | 1.0038 | NA | 0.9275 | 282 | 33.3 | 8669 | 8.67 | | 8.71 | 0.552 |
| **Group 3** | C92646M | 1.0116 | NA | 0.9275 | 444 | 100 | 40692 | 40.7 | | | |
| Mid Dose | C92650M | 1.0072 | NA | 0.9275 | 283 | 100 | 26094 | 26.1 | | | |
| 2.0 ppm | C92652M | 1.0030 | NA | 0.9275 | 337 | 100 | 31167 | 31.2 | | | |
| in Diet | C92668M | 1.0036 | NA | 0.9275 | 346 | 100 | 31975 | 32.0 | | | 18.7 |
| | C92678M | 1.0010 | NA | 0.9275 | 289 | 100 | 26736 | 26.7 | | 31.3 | 5.84 |
| | C92996F | 1.0047 | NA | 0.9275 | 313 | 100 | 28866 | 28.9 | | | |
| | C93006F | 0.9943 | NA | 0.9275 | 157 | 100 | 14601 | 14.6 | | | |
| | C93008F | 1.0158 | NA | 0.9275 | 325 | 100 | 29691 | 29.7 | | | |
| | C93031F | 1.0166 | NA | 0.9275 | 297 | 100 | 27099 | 27.1 | | | 24.4 |
| | C93045F | 1.0076 | NA | 0.9275 | 270 | 100 | 24847 | 24.8 | | 25.0 | 6.11 |
| **Group 4** | C92715M | 1.0003 | NA | 0.9275 | 577 | 100 | 53503 | 53.5 | | | |
| Mid-High Dose | C92718M | 1.0026 | NA | 0.9275 | 645 | 100 | 59632 | 59.6 | | | |
| 5.0 ppm | C92731M | 1.0139 | NA | 0.9275 | 292 | 100 | 26740 | 26.7 | | | |
| in Diet | C92734M | 0.9930 | NA | 0.9275 | 533 | 100 | 49826 | 49.8 | | | 26.2 |
| | C92744M | 1.0150 | NA | 0.9275 | 528 | 100 | 48285 | 48.3 | | 47.6 | 12.5 |
| | C93058F | 1.0083 | NA | 0.9275 | 1161 | 100 | 106760 | 107 | | | |
| | C93067F | 1.0038 | NA | 0.9275 | 908 | 100 | 83858 | 83.9 | | | |
| | C93070F | 0.9957 | NA | 0.9275 | 826 | 100 | 76985 | 77.0 | | | |
| | C93075F | 1.0068 | NA | 0.9275 | 767 | 100 | 70660 | 70.7 | | | 17.0 |
| | C93084F | 1.0133 | NA | 0.9275 | 838 | 100 | 76723 | 76.7 | | 83.0 | 14.1 |
| **Group 5** | C92752M | 1.0100 | NA | 0.9275 | 1190 | 200 | 218558 | 219 | * | | |
| High Dose | C92759M | 1.0122 | NA | 0.9275 | 1439 | 200 | 263670 | 264 | * | | |
| 20 ppm | C92779M | 1.0082 | NA | 0.9275 | 1858 | 200 | 341904 | 342 | * | | |
| in Diet | C92793M | 0.9949 | NA | 0.9275 | 1533 | 200 | 285921 | 286 | * | | 16.1 |
| | C92798M | 1.0158 | NA | 0.9275 | 1630 | 200 | 297613 | 298 | * | 282 | 45.3 |
| | C93114F | 1.0043 | NA | 0.9275 | 2355 | 200 | 434951 | 435 | * | | |
| | C93123F | 1.0010 | NA | 0.9275 | 1943 | 200 | 360089 | 360 | * | | |
| | C93142F | 1.0076 | NA | 0.9275 | 2144 | 200 | 394644 | 395 | * | | |
| | C93144F | 1.0102 | NA | 0.9275 | 1728 | 200 | 317269 | 317 | * | | 11.8 |
| | C93159F | 1.0130 | NA | 0.9275 | 1959 | 200 | 358782 | 359 | * | 373 | 44.1 |

Original PFOS LOQ (59.5 ng/g) updated to reflect k+ information on 10/04/00. LAC 10/04/00

NA=Not Applicable

**PFOS** = Perfluorooctanesulfonate

Date Entered/Analyst: 02/08/00 LAC
Date Verified/Analyst: 10/03/00 KJH
Purity Entered/Verified: 10/04/00 LAC

Correction Factors not applicable to MS/MSD QC data

* Results were outside the range of the curve and should be considered to be estimates. LAC 10/04/00

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 | | |
| Matrix: | Rat Liver | Filename: | See List to Right |
| Method/Revision: | FACT-M-1.0 & ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Madeline 041098, Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | MassLynx 3.3 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 08/06/98 SAH | | |
| Date of Analysis/Analyst: | 08/10/98, 08/14/98, 08/17/98 MEE/HOJ | | |
| Date of Data Reduction/Analyst: | 02/03/00 IAS | | |

**Sample Data**

**WEEK 14 RAT LIVER REWORK** Lot 193

| Group Dose | Sample # | Initial Wt. g | Total Mass of Liver g | PFOS Std Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Concentration of PFOS ug/g or % Rec. | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-1 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ(0.0569 ug/g) | | |
| | H2O Blk-2 | 1.0000 | NA | 0.9275 | 60.9 | 1 | 56.5 | <LOQ(0.0569 ug/g) | <LOQ(0.0569 ug/g) | NA |
| Matrix Blk | Rabbit Liver Blk-1 | 1.0000 | NA | 0.9275 | 0.00 | 1 | 0.00 | <LOQ(0.0569 ug/g) | | |
| | Rabbit Liver Blk-2 | 1.0000 | NA | 0.9275 | 12.1 | 1 | 11.2 | <LOQ(0.0569 ug/g) | <LOQ(0.0569 ug/g) | NA |
| QC - 100 ppb | C92509M-MS | 0.9979 | NA | NA | 533 | 1 | 59.6 | 50% | | |
| | C92509M-MSD | 0.9979 | NA | NA | 658 | 1 | 184 | 154% | 102% | 102% |
| **Group 1** | C92509M | 0.9979 | NA | 0.9275 | 473 | 1 | 440 | 0.440 | | |
| Control | C92511M | 1.0044 | NA | 0.9275 | 587 | 1 | 542 | 0.542 | | |
| 0.0 ppm | C92521M | 0.9960 | NA | 0.9275 | 529 | 1 | 492 | 0.492 | | |
| in Diet | C92528M | 1.0131 | NA | 0.9275 | 440 | 1 | 403 | 0.403 | | 12.5 |
| | C92532M | 1.0140 | NA | 0.9275 | 457 | 1 | 418 | 0.418 | 0.459 | 0.0573 |
| | C92880F | 1.0008 | NA | 0.9275 | 110 | 10 | 1017 | 1.02 | | |
| | C92887F | 1.0047 | NA | 0.9275 | 563 | 100 | 51996 | 52.0 | | |
| | C92898F | 1.0165 | NA | 0.9275 | 76.4 | 10 | 697 | 0.697 | | |
| | C92903F | 1.0078 | NA | 0.9275 | 572 | 10 | 5263 | 5.26 | | 186 |
| | C92905F | 0.9945 | NA | 0.9275 | 126 | 10 | 1175 | 1.18 | 12.0 | 22.4 |
| **Group 2** | C92593M | 0.9975 | NA | 0.9275 | 265 | 100 | 24625 | 24.6 | | |
| Low Dose | C92600M | 1.0058 | NA | 0.9275 | 209 | 100 | 19274 | 19.3 | | |
| 0.5 ppm | C92616M | 1.0112 | NA | 0.9275 | 296 | 100 | 27191 | 27.2 | | |
| in Diet | C92621M | 1.0082 | NA | 0.9275 | 230 | 100 | 21166 | 21.2 | | 14.5 |
| | C92627M | 0.9940 | NA | 0.9275 | 285 | 100 | 26634 | 26.6 | 23.8 | 3.45 |
| | C92944F | 1.0005 | NA | 0.9275 | 204 | 100 | 18933 | 18.9 | | |
| | C92962F | 0.9994 | NA | 0.9275 | 191 | 100 | 17718 | 17.7 | | |
| | C92967F | 1.0132 | NA | 0.9275 | 271 | 100 | 24839 | 24.8 | | |
| | C92978F | 1.0013 | NA | 0.9275 | 156 | 100 | 14461 | 14.5 | | 19.7 |
| | C92987F | 1.0095 | NA | 0.9275 | 216 | 100 | 19842 | 19.8 | 19.2 | 3.77 |
| **Group 3** | C92640M | 1.0095 | NA | 0.9275 | 180 | 500 | 82666 | 82.7 | | |
| Mid Dose | C92645M | 0.9974 | NA | 0.9275 | 158 | 500 | 73468 | 73.5 | | |
| 2.0 ppm | C92662M | 0.9977 | NA | 0.9275 | 147 | 500 | 68263 | 68.3 | | |
| in Diet | C92676M | 0.9926 | NA | 0.9275 | 146 | 500 | 68329 | 68.3 | | 8.32 |
| | C92684M | 0.9987 | NA | 0.9275 | 167 | 500 | 77352 | 77.4 | 74.0 | 6.16 |
| | C92993F | 1.0006 | NA | 0.9275 | 149 | 500 | 69020 | 69.0 | | |
| | C93000F | 0.9994 | NA | 0.9275 | 153 | 500 | 71061 | 71.1 | | |
| | C93018F | 1.0081 | NA | 0.9275 | 146 | 500 | 67380 | 67.4 | | |
| | C93023F | 0.9965 | NA | 0.9275 | 139 | 500 | 64869 | 64.9 | | 4.99 |
| | C93035F | 1.0019 | NA | 0.9275 | 160 | 500 | 73916 | 73.9 | 69.2 | 3.46 |
| **Group 4** | C92713M | 1.0048 | NA | 0.9275 | 406 | 1000 | 374738 | 375 | | |
| Mid-High Dose | C92714M | 1.0044 | NA | 0.9275 | 403 | 1000 | 372357 | 372 | | |
| 5.0 ppm | C92719M | 1.0050 | NA | 0.9275 | 409 | 1000 | 377202 | 377 | | |
| in Diet | C92722M | 1.0095 | NA | 0.9275 | 336 | 1000 | 308422 | 308 | | 8.05 |
| | C92728M | 1.0011 | NA | 0.9275 | 384 | 1000 | 356019 | 356 | 358 | 28.8 |
| | C93051F | 0.9944 | NA | 0.9275 | 381 | 1000 | 355218 | 355 | | |
| | C93064F | 1.0103 | NA | 0.9275 | 393 | 1000 | 360819 | 361 | | |
| | C93071F | 1.0001 | NA | 0.9275 | 383 | 1000 | 354845 | 355 | | |
| | C93085F | 1.0038 | NA | 0.9275 | 404 | 1000 | 373393 | 373 | | 6.03 |
| | C93109F | 1.0022 | NA | 0.9275 | 441 | 1000 | 408111 | 408 | 370 | 22.3 |
| **Group 5** | C92765M | 0.9983 | NA | 0.9275 | 777 | 1000 | 722350 | 722 | | |
| High Dose | C92777M | 1.0067 | NA | 0.9275 | 670 | 1000 | 617695 | 618 | | |
| 20 ppm | C92789M | 0.9920 | NA | 0.9275 | 598 | 1000 | 559277 | 559 | | |
| in Diet | C92799M | 1.0046 | NA | 0.9275 | 494 | 1000 | 456225 | 456 | | 18.8 |
| | C92812M | 1.0064 | NA | 0.9275 | 526 | 1000 | 485214 | 485 | 568 | 107 |
| | C93111F | 0.9982 | NA | 0.9275 | 745 | 1000 | 692038 | 692 | | |
| | C93127F | 1.0056 | NA | 0.9275 | 672 | 1000 | 619486 | 619 | | |
| | C93143F | 1.0136 | NA | 0.9275 | 679 | 1000 | 621011 | 621 | | |
| | C93155F | 1.0125 | NA | 0.9275 | 737 | 1000 | 674725 | 675 | | 7.72 |
| | C93169F | 0.9981 | NA | 0.9275 | 612 | 1000 | 569017 | 569 | 635 | 49.0 |

Original PFOS LOQ (61.3 ng/mL) updated to reflect k+ information on 10/04/00. LAC 10/04/00

NA = Not Applicable

**PFOS** = Perfluorooctanesulfonate
**PFOSA** = Perfluorooctanesulfonamide
**PFOSAA** = Perfluorooctanesulfonamidoacetate
**EtFOSE** = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
Correction Factors not applicable to MS/MSD QC data

Date Entered/Analyst: 02/09/00 LAC
Date Verified/Analyst: 10/03/00 KJH
Purity Entered/Verified: 10/04/00 LAC

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | | |
|---|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | | |
| Product Number(Test Substance): | T-6295 | | | |
| Matrix: | Rat Liver | Filename: | See List to Right | |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 | R-Squared Value: | See Attachments | |
| Analytical Equipment System Number: | Soup 020199 | Slope: | See Attachments | |
| Instrument Software/Version: | MassLynx 3.3 | Y-Intercept: | See Attachments | |
| Date of Extraction/Analyst: | 11/11/99, 01/25/00 MCH/SAL | | | |
| Date of Analysis/Analyst: | 11/15/99, 11/19/99 11/22/99 IAS/MMH, 11/24/99 GML, 12/16/99 IAS | | | |
| Date of Data Reduction/Analyst: | 11/16/99, 11/22/99, 11/23/99 IAS, 12/1/99 MMH, 12/17/99, 11/20/00 IAS/KJH | | | |

Filenames

| | PFOS | PFOSA |
|---|---|---|
| Blanks | 121699046-47,92-93 | NA |
| Grp 1 M | 111999056-58, 121699067-76 | NA |
| Grp 1 F | 111999059-, 121699063,77-87 | NA |
| Grp 5 M | 112499088-99 | NA |
| Grp 5 F | 112499100-115 | NA |
| MS, MSD | 121699060 | NA |
| Box 99-222 | | |

**Sample Data**

**WEEK 53 RAT LIVER** Lot 171

| Group Dose | Sample # | Initial Wt. g | Total Mass of Liver g | PFOS Std Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Concentration of PFOS ug/g or % Rec. | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | H2O Blk-9 | 1.0000 | NA | 0.9275 | 0.8640 | 24.4 | 1 | 19.6 | <LOQ (0.0491 ug/g) | | |
| | H2O Blk-10 | 1.0000 | NA | 0.9275 | 0.8640 | 21.4 | 1 | 17.1 | <LOQ (0.0491 ug/g) | <LOQ (0.0491 ug/g) | NA |
| Matrix Blk | Rabbit Liver Blk-9 | 1.0000 | NA | 0.9275 | 0.8640 | 29.0 | 1 | 23.2 | <LOQ (0.0491 ug/g) | | |
| | Rabbit Liver Blk-10 | 1.0000 | NA | 0.9275 | 0.8640 | 13.7 | 1 | 11.0 | <LOQ (0.0491 ug/g) | <LOQ (0.0491 ug/g) | NA |
| QC | C92517M-250 ppb-MS9 | 0.9979 | NA | NA | NA | 109 | 10 | 119 | 40% | | |
| | C92876F-250 ppb-MSD10 | 0.9937 | NA | NA | NA | 338 | 1 | 176 | 58% | 49% | 39% |
| **Group 1** | C92501M | 1.0185 | NA | 0.9275 | 0.8640 | 41.5 | 1 | 32.6 | 0.0326 | | |
| Control | C92504M | 1.0095 | NA | 0.9275 | 0.8640 | 451 | 1 | 358 | 0.358 | | |
| 0.0 ppm | C92507M | 0.9916 | NA | 0.9275 | 0.8640 | 372 | 1 | 301 | 0.301 | | |
| in Diet | C92512M | 0.9982 | NA | 0.9275 | 0.8640 | 352 | 1 | 283 | 0.283 | | |
| | C92517M | 1.0192 | NA | 0.9275 | 0.8640 | 96.8 | 10 | 761 | 0.761 | | |
| **Males** | C92523M | 0.9909 | NA | 0.9275 | 0.8640 | 65.4 | 10 | 529 | 0.529 | | |
| | C92534M | 1.0065 | NA | 0.9275 | 0.8640 | 451 | 1 | 359 | 0.359 | | |
| | C92540M | 1.0043 | NA | 0.9275 | 0.8640 | 133 | 1 | 106 | 0.106 | | |
| | C92562M | 1.0017 | NA | 0.9275 | 0.8640 | 440 | 10 | 3519 | 3.52 | | 163 |
| | C92570M | 1.0099 | NA | 0.9275 | 0.8640 | 130 | 1 | 103 | 0.103 | 0.635 | 1.04 |
| **Group 1** | C92862F | 1.0085 | NA | 0.9275 | 0.8640 | 712 | 10 | 5657 | 5.66 | | |
| Control | C92876F | 0.9937 | NA | 0.9275 | 0.8640 | 163 | 1 | 131 | 0.131 | | |
| 0.0 ppm | C92889F | 0.9965 | NA | 0.9275 | 0.8640 | 259 | 10 | 2081 | 2.08 | | |
| in Diet | C92894F | 1.0128 | NA | 0.9275 | 0.8640 | 349 | 1 | 276 | 0.276 | | |
| | C92899F | 0.9979 | NA | 0.9275 | 0.8640 | 271 | 1 | 217 | 0.217 | | |
| **Females** | C92910F | 1.0171 | NA | 0.9275 | 0.8640 | 251 | 1 | 198 | 0.198 | | |
| | C92917F | 1.0066 | NA | 0.9275 | 0.8640 | 309 | 1 | 246 | 0.246 | | |
| | C92922F | 1.0083 | NA | 0.9275 | 0.8640 | 73.0 | 1 | 58.0 | 0.0580 | | |
| | C92923F | 1.0118 | NA | 0.9275 | 0.8640 | 195 | 1 | 154 | 0.154 | | 191 |
| | C92930F | 1.0028 | NA | 0.9275 | 0.8640 | 269 | 1 | 215 | 0.215 | 0.923 | 1.77 |
| **Group 5** | C92751M | 1.0034 | NA | 0.9275 | 0.8640 | 280 | 2500 | 558532 | 559 | | |
| High Dose | C92754M | 0.9907 | NA | 0.9275 | 0.8640 | 240 | 2500 | 485592 | 486 | | |
| 20 ppm | C92755M | 1.0139 | NA | 0.9275 | 0.8640 | 246 | 2500 | 485843 | 486 | | |
| in Diet | C92778M | 0.9951 | NA | 0.9275 | 0.8640 | 139 | 2500 | 279139 | 279 | | |
| | C92790M | 1.0111 | NA | 0.9275 | 0.8640 | 204 | 2500 | 404861 | 405 | | |
| **Males** | C92795M | 1.0179 | NA | 0.9275 | 0.8640 | 282 | 2500 | 554138 | 554 | | |
| | C92796M | 0.9930 | NA | 0.9275 | 0.8640 | 212 | 2500 | 426888 | 427 | | |
| | C92817M | 0.9950 | NA | 0.9275 | 0.8640 | 200 | 2500 | 403197 | 403 | | 22.3 |
| | C92820M | 1.0019 | NA | 0.9275 | 0.8640 | 158 | 2500 | 315897 | 316 | 435 | 96.9 |
| **Group 5** | C93112F | 1.0040 | NA | 0.9275 | 0.8640 | 148 | 2500 | 296060 | 296 | | |
| High Dose | C93116F | 0.9931 | NA | 0.9275 | 0.8640 | 265 | 2500 | 533843 | 534 | | |
| 20 ppm | C93117F | 0.9943 | NA | 0.9275 | 0.8640 | 255 | 2500 | 513715 | 514 | | |
| in Diet | C93120F | 1.0004 | NA | 0.9275 | 0.8640 | 215 | 2500 | 430218 | 430 | | |
| | C93130F | 0.9912 | NA | 0.9275 | 0.8640 | 250 | 2500 | 504367 | 504 | | |
| **Females** | C93138F | 0.9945 | NA | 0.9275 | 0.8640 | 280 | 2500 | 564538 | 565 | | |
| | C93147F | 1.0040 | NA | 0.9275 | 0.8640 | 310 | 2500 | 619098 | 619 | | |
| | C93154F | 0.9967 | NA | 0.9275 | 0.8640 | 211 | 2500 | 423936 | 424 | | |
| | C93163F | 1.0141 | NA | 0.9275 | 0.8640 | 447 | 2500 | 883325 | 883 | | 32.2 |
| | C93171F | 0.9000 | NA | 0.9275 | 0.8640 | 373 | 2500 | 830454 | 830 | 560 | 180 |

Original PFOS LOQ (26.9 ng/g, 61.3 ng/g, 12.3 ng/g) updated to reflect K+ and purity information on 10/04/00. LAC 10/04/00
Correction Factors not applicable to MS/MSD QC data
**PFOS** = Perfluorooctanesulfonate
NA = Not Applicable

Date Entered/Analyst: 11/24/99 LAC, 12/2/99 GML, 01/24/00 MMH, 11/20/00 LAC
Date Verified/Analyst: 10/03/00 KJH, 11/21/00 MMH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 | | |
| Matrix: | Rat Liver | Filename: | See Below |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.4 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 05/16/00, 05/18/00 SAL/KJK | | |
| Date of Analysis/Analyst: | 05/22/00, 05/24/00, 05/25/00, 06/12/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH | | |
| Date of Data Reduction/Analyst: | 05/30/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH | | |

**Sample Data**

**DAY 719 RAT LIVER** Lot 171

| Group Dose | Sample # | Surrogate Verified | Initial Wt. g | Total Mass of Liver g | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Filename (optional) | Concentration of PFOS ug/g or % Rec | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | 05160-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.740 | 1 | 0.643 | D061400077 | < LOQ (0.0107 ug/g) | | |
| | 05160-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400078 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200073 | < LOQ (0.0107 ug/g) | | |
| | 052200-H2OBlk-3 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400088 | < LOQ (0.0107 ug/g) | | NA |
| | 052200-H2OBlk-4 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400089 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| Matrix Blk | RBL05160-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400079 | < LOQ (0.0107 ug/g) | | |
| | RBL05160-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400080 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.570 | 1 | 0.495 | A052200074 | < LOQ (0.0107 ug/g) | | |
| | RBL052200-LiverBlk-3 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400090 | < LOQ (0.0107 ug/g) | | NA |
| | RBL052200-LiverBlk-4 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400091 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| QC | C92503M G1-MS-250ppb-3 | NA | 1.0161 | NA | NA | NA | 357 | 1 | 177 | A073100018 | 60% * | | |
| | C92503M G1-MSD-250ppb-3 | NA | 1.0161 | NA | NA | NA | 411 | 1 | 230 | A073100019 | 78% | 69% | 26% |
| | C92505M G1-MS-250ppb-5 | NA | 0.9927 | NA | NA | NA | 496 | 1 | 377 | A052200020 | 126% | | |
| | C92505M G1-MSD-250ppb-5 | NA | 0.9927 | NA | NA | NA | 478 | 1 | 359 | A052200021 | 120% | 123% | 5% |
| | C92866F-G1-MS-250ppb-4 | NA | 0.9665 | NA | NA | NA | 125 | 10 | 389 | A061200060 | 126% | | |
| | C92866F-G1-MSD-250ppb-4 | NA | 0.9665 | NA | NA | NA | 135 | 10 | 488 | A061200061 | 158% * | 142% | 22% |
| | C92868F G1-MS-250ppb-6 | NA | 1.0055 | NA | NA | NA | 379 | 1 | 253 | D072800055 | 85% | | |
| | C92868F G1-MSD-250ppb-6 | NA | 1.0055 | NA | NA | NA | 433 | 1 | 306 | A052200025 | 102% | 94% | 20% |
| | C92510M G1-MS-250ppb-6 | NA | 0.9973 | NA | NA | NA | 803 | 1 | 217 | D072800056 | 72% | | |
| | C92510M G1-MSD-250ppb-6 | NA | 0.9973 | NA | NA | NA | 966 | 1 | 380 | A052200076 | 126% | 99% | 55% |
| | C92991F G3-MS-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 492 | 100 | 5430 | D061400045 | 1816% * a | | |
| | C92991F G3-MSD-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 517 | 100 | 7923 | D061400046 | 2650% * a | 2233% | 37% |
| | C92821M G6-MS-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 319 | 50 | 6423 | D061400047 | 2134% * a | | |
| | C92821M G6-MSD-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 309 | 50 | 5914 | D061400048 | 1965% * a | 2049% | 8% |
| **Group 3** | C92991F | NA | 1.0024 | NA | 0.9949 | 0.8640 | 504 | 100 | 43639 | A052500015 | 43.6 | | |
| Mid Dose | C93003F | NA | 0.9967 | NA | 0.9949 | 0.8640 | 593 | 100 | 51692 | A052500016 | 51.7 | | |
| 2.0 ppm | C93007F | NA | 1.0010 | NA | 0.9949 | 0.8640 | 1121 | 100 | 97210 | A052500017 | 97.2 | | |
| in Diet | C93011F | NA | 1.0018 | NA | 0.9949 | 0.8640 | 153 | 100 | 13222 | A052500018 | 13.2 | | |
| | C93022F | NA | 1.0008 | NA | 0.9949 | 0.8640 | 104 | 100 | 9018 | A052500019 | 9.02 | | |
| | C93039F | NA | 1.0030 | NA | 0.9949 | 0.8640 | 921 | 100 | 79737 | A052500022 | 79.7 | | |
| | C93040F | NA | 0.9967 | NA | 0.9949 | 0.8640 | 1080 | 100 | 94108 | A052500023 | 94.1 | | |
| | C93047F | NA | 1.0016 | NA | 0.9949 | 0.8640 | 615 | 100 | 53315 | A052500024 | 53.3 | | 57.2 |
| | C93048F | NA | 1.0020 | NA | 0.9949 | 0.8640 | 620 | 100 | 53775 | A052500025 | 53.8 | 55.1 | 31.5 |

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00

* Reanalyzed with the same results. LAC 08/14/00

a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. KJH 10/03/00

NA = Not applicable

Date Entered/Analyst: 06/01/00, 06/21/00, 06/22/00, 08/14/00 CSH/LAC
Date Verified/Analyst: 10/03/00 KJH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 | | |
| Matrix: | Rat Liver | Filename: | See Below |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.4 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 05/16/00-05/18/00, 05/22/00 SAL/KJK | | |
| Date of Analysis/Analyst: | 05/19/00, 05/22/00, 05/24/00, 06/12/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH | | |
| Date of Data Reduction/Analyst: | 05/22/00, 05/23/00, 05/24/00, 05/25/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00, 11/20/00 IAS/MMH/KJH | | |

**Sample Data**

**WEEK 105 RAT LIVER** Lot 171

| Group Dose | Sample # | Surrogate Verified | Initial Wt. g | Total Mass of Liver g | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Filename (optional) | Concentration of PFOS ug/g or % Rec | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | 05160-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.740 | 1 | 0.643 | D061400077 | < LOQ (0.0107 ug/g) | | |
| | 05160-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400078 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200073 | < LOQ (0.0107 ug/g) | | |
| | 052200-H2OBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400088 | < LOQ (0.0107 ug/g) | | NA |
| | 052200-H2OBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400089 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| Matrix Blk | RBL05160-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400079 | < LOQ (0.0107 ug/g) | | |
| | RBL05160-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400080 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 1.14 | 1 | 0.990 | A052200074 | < LOQ (0.0107 ug/g) | | |
| | RBL052200-LiverBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400090 | < LOQ (0.0107 ug/g) | | NA |
| | RBL052200-LiverBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400091 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| QC | C92503M G1-MS-250ppb-3 | NA | 1.0161 | NA | NA | NA | 357 | 1 | 177 | A073100018 | 60% * | | |
| | C92503M G1-MSD-250ppb-3 | NA | 1.0161 | NA | NA | NA | 411 | 1 | 230 | A073100019 | 78% | 69% | 26% |
| | C92505M G1-MS-250ppb-5 | NA | 0.9927 | NA | NA | NA | 496 | 1 | 377 | A052200020 | 126% | | |
| | C92505M G1-MSD-250ppb-5 | NA | 0.9927 | NA | NA | NA | 478 | 1 | 359 | A052200021 | 120% | 123% | 5% |
| | C92866F-G1-MS-250ppb-4 | NA | 0.9665 | NA | NA | NA | 125 | 10 | 389 | A061200060 | 126% | | |
| | C92866F-G1-MSD-250ppb-4 | NA | 0.9665 | NA | NA | NA | 135 | 10 | 488 | A061200061 | 158% * | 142% | 22% |
| | C92868F G1-MS-250ppb-6 | NA | 1.0055 | NA | NA | NA | 379 | 1 | 253 | D072800055 | 85% | | |
| | C92868F G1-MSD-250ppb-6 | NA | 1.0055 | NA | NA | NA | 433 | 1 | 306 | A052200025 | 102% | 94% | 20% |
| | C92510M G1-MS-250ppb-6 | NA | 0.9973 | NA | NA | NA | 803 | 1 | 217 | D072800056 | 72% | | |
| | C92510M G1-MSD-250ppb-6 | NA | 0.9973 | NA | NA | NA | 965 | 1 | 380 | A052200076 | 126% | 99% | 55% |
| | C92991F G3-MS-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 492 | 100 | 5430 | D061400045 | 1816% * a | | |
| | C92991F G3-MSD-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 517 | 100 | 7923 | D061400046 | 2650% * a | 2233% | 37% |
| | C92821M G6-MS-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 319 | 50 | 6423 | D061400047 | 2134% * a | | |
| | C92821M G6-MSD-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 309 | 50 | 5914 | D061400048 | 1965% * a | 2049% | 8% |
| **Group 1** | C92503M | NA | 1.0161 | NA | 0.9949 | 0.8640 | 177 | 1 | 152 | A052400025 | 0.152 | | |
| Control | C92505M | NA | 0.9927 | NA | 0.9949 | 0.8640 | 122 | 1 | 106 | A052200026 | 0.106 | | |
| 0.0 ppm | C92510M | NA | 0.9973 | NA | 0.9949 | 0.8640 | 587 | 1 | 511 | A052200077 | 0.511 | | |
| in Diet | C92513M | NA | 0.9947 | NA | 0.9949 | 0.8640 | 75.9 | 1 | 66.3 | A052200080 | 0.0663 | | |
| | C92514M | NA | 0.9873 | NA | 0.9949 | 0.8640 | 265 | 1 | 233 | A052400026 | 0.233 | | |
| Males | C92518M | NA | 0.9939 | NA | 0.9949 | 0.8640 | 8.00 | 1 | 6.99 | D061400081 | < LOQ (0.0107 ug/g) | | |
| | C92541M | NA | 1.0035 | NA | 0.9949 | 0.8640 | 32.7 | 1 | 28.3 | A052200081 | 0.0283 | | |
| | C92542M | NA | 0.9861 | NA | 0.9949 | 0.8640 | 38.9 | 1 | 34.2 | A052400027 | 0.0342 | | |
| | C92544M | NA | 0.9913 | NA | 0.9949 | 0.8640 | 33.2 | 1 | 29.1 | A052200082 | 0.0291 | | |
| | C92551M | NA | 1.0077 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400084 | < LOQ (0.0107 ug/g) | | 130 |
| | C92569M | NA | 1.0067 | NA | 0.9949 | 0.8640 | 80.0 | 1 | 69.0 | A052200029 | 0.0690 | 0.114 | 0.148 |
| **Group 1** | C92866F | NA | 0.9665 | NA | 0.9949 | 0.8640 | 101 | 10 | 907 | A061200062 | 0.907 | | |
| Control | C92867F | NA | 0.9915 | NA | 0.9949 | 0.8640 | 419 | 1 | 367 | A052200083 | 0.367 | | |
| 0.0 ppm | C92868F | NA | 1.0055 | NA | 0.9949 | 0.8640 | 125 | 1 | 108 | A052200032 | 0.108 | | |
| in Diet | C92869F | NA | 1.0065 | NA | 0.9949 | 0.8640 | 290 | 1 | 250 | A052200084 | 0.250 | | |
| | C92871F | NA | 1.0005 | NA | 0.9949 | 0.8640 | 25.0 | 1 | 21.7 | D061400085 | < LOQ (0.0107 ug/g) | | |
| Females | C92873F | NA | 1.0150 | NA | 0.9949 | 0.8640 | 297 | 1 | 254 | A052400031 | 0.254 | | |
| | C92874F | NA | 0.9957 | NA | 0.9949 | 0.8640 | 173 | 1 | 151 | A052200034 | 0.151 | | |
| | C92877F | NA | 0.9906 | NA | 0.9949 | 0.8640 | 309 | 1 | 271 | A052200087 | 0.271 | | |
| | C92881F | NA | 0.9994 | NA | 0.9949 | 0.8640 | 104 | 1 | 90.4 | A052400032 | 0.0904 | | |
| | C92882F | NA | 1.0042 | NA | 0.9949 | 0.8640 | 264 | 1 | 228 | A052400033 | 0.228 | | |
| | C92885F | NA | 1.0344 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400092 | < LOQ (0.0107 ug/g) | | |
| | C92890F | NA | 0.9635 | NA | 0.9949 | 0.8640 | 275 | 1 | 248 | A052400035 | 0.248 | | |
| | C92892F | NA | 0.9984 | NA | 0.9949 | 0.8640 | 56.3 | 1 | 49.0 | A052200088 | 0.0490 | | |
| | C92895F | NA | 1.0074 | NA | 0.9949 | 0.8640 | 131 | 1 | 113 | A052200089 | 0.113 | | |
| | C92896F | NA | 1.0100 | NA | 0.9949 | 0.8640 | 135 | 1 | 116 | A052200035 | 0.116 | | |
| | C92897F | NA | 0.9983 | NA | 0.9949 | 0.8640 | 182 | 1 | 158 | A052200036 | 0.158 | | |
| | C92906F | NA | 1.0148 | NA | 0.9949 | 0.8640 | 384 | 1 | 328 | A052200039 | 0.328 | | |
| | C92907F | NA | 0.9758 | NA | 0.9949 | 0.8640 | 38.9 | 1 | 34.6 | A052400038 | 0.0346 | | |
| | C92909F | NA | 1.0083 | NA | 0.9949 | 0.8640 | 131 | 1 | 113 | A052200090 | 0.113 | | |
| | C92911F | NA | 1.0094 | NA | 0.9949 | 0.8640 | 239 | 1 | 206 | A052200091 | 0.206 | | |
| | C92912F | Confirmed Low | 0.9960 | NA | 0.9949 | 0.8640 | 76.5 | 1 | 66.7 | A052200092 | 0.0667 | | |
| | C92913F | NA | 1.0035 | NA | 0.9949 | 0.8640 | 211 | 1 | 183 | A052200040 | 0.183 | | |
| | C92925F | NA | 1.0722 | NA | 0.9949 | 0.8640 | 6.16 | 1 | 4.99 | D061400095 | < LOQ (0.0107 ug/g) | | 99.4 |
| | C92927F | NA | 0.9973 | NA | 0.9949 | 0.8640 | 185 | 1 | 161 | A052200041 | 0.161 | 0.185 | 0.184 |
| **Group 2** | C92577M | NA | 1.0121 | NA | 0.9949 | 0.8640 | 186 | 10 | 1596 | A061200098 | 1.60 | | |
| Low Dose | C92583M | NA | 0.9943 | NA | 0.9949 | 0.8640 | 188 | 10 | 1641 | A051900015 | 1.64 | | |
| 0.5 ppm | C92590M | NA | 1.0076 | NA | 0.9949 | 0.8640 | 90.2 | 50 | 3888 | D061400032 | 3.89 | | |
| in Diet | C92597M | NA | 1.0092 | NA | 0.9949 | 0.8640 | 200 | 100 | 17190 | A061300015 | 17.2 | | |
| | C92598M | NA | 1.0050 | NA | 0.9949 | 0.8640 | 233 | 10 | 2016 | A051900017 | 2.02 | | |
| Males | C92601M | NA | 0.9814 | NA | 0.9949 | 0.8640 | 244 | 100 | 21574 | A061200089 | 21.6 | | |
| | C92602M | NA | 1.0729 | NA | 0.9949 | 0.8640 | 124 | 100 | 10022 | A061200090 | 10.0 | | |
| | C92619M | NA | 1.0018 | NA | 0.9949 | 0.8640 | 32.1 | 50 | 1390 | D061400033 | 1.39 | | |
| | C92623M | NA | 1.0866 | NA | 0.9949 | 0.8640 | 167 | 100 | 13353 | A061200091 | 13.4 | | 93.8 |
| | C92626M | NA | 1.0069 | NA | 0.9949 | 0.8640 | 130 | 50 | 5606 | D061400034 | 5.61 | 7.83 | 7.34 |
| **Group 2** | C92936F | NA | 0.9944 | NA | 0.9949 | 0.8640 | 120 | 100 | 10455 | D061400037 | 10.5 | | |
| Low Dose | C92938F | NA | 1.0011 | NA | 0.9949 | 0.8640 | 20 | 100 | 1719 | D061400038 | 1.72 | | |
| 0.5 ppm | C92946F | NA | 1.0030 | NA | 0.9949 | 0.8640 | 176 | 100 | 15281 | D061400039 | 15.3 | | |
| in Diet | C92953F | NA | 1.0082 | NA | 0.9949 | 0.8640 | 234 | 100 | 20115 | A061200092 | 20.1 | | |
| | C92957F | NA | 1.0272 | NA | 0.9949 | 0.8640 | 172 | 100 | 14523 | A061200093 | 14.5 | | |
| Females | C92960F | NA | 1.0800 | NA | 0.9949 | 0.8640 | 614 | 10 | 4938 | A061200099 | 4.94 | | |
| | C92964F | NA | 0.9955 | NA | 0.9949 | 0.8640 | 230 | 100 | 20050 | A051900018 | 20.1 | | |
| | C92970F | NA | 1.0078 | NA | 0.9949 | 0.8640 | 77.7 | 100 | 6691 | A051900019 | 6.69 | | |
| | C92973F | NA | 1.0016 | NA | 0.9949 | 0.8640 | 58.6 | 100 | 5084 | A051900023 | 5.08 | | |
| | C92974F | NA | 0.9968 | NA | 0.9949 | 0.8640 | 181 | 100 | 15787 | D061400040 | 15.8 | | |
| | C92975F | NA | 0.9967 | NA | 0.9949 | 0.8640 | 155 | 100 | 13476 | A061200096 | 13.5 | | |
| | C92983F | NA | 0.9980 | NA | 0.9949 | 0.8640 | 310 | 100 | 26969 | A051900024 | 27.0 | | |
| | C92984F | NA | 1.0023 | NA | 0.9949 | 0.8640 | 99.0 | 100 | 8576 | D061400041 | 8.58 | | |
| | C92986F | NA | 0.9951 | NA | 0.9949 | 0.8640 | 151 | 100 | 13153 | A061200097 | 13.2 | | 52.7 |
| | C92988F | NA | 1.0009 | NA | 0.9949 | 0.8640 | 198 | 100 | 17189 | A051900025 | 17.2 | 12.9 | 6.81 |

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00
* Reanalyzed with the same results. LAC 08/14/00
a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. KJH 10/03/00
NA = Not Applicable

Date Entered/Analyst: 5/24/00, 5/30/00, 5/31/00, 06/19/00, 06/20/00, 06/21/00, 06/22/00, 08/14/00, 11/20/00 MMH/CSH/LAC
Date Verified/Analyst: 10/03/00 KJH, 11/21/00 MMH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

**AMDT# 112296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 | | |
| Matrix: | Rat Liver | Filename: | See Below |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498, Davey 070799 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.4 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 5/16/00-5/18/00, 5/22/00 SAL/KJK | | |
| Date of Analysis/Analyst: | 5/19/00, 5/22/00, 5/24/00, 5/26/00, 06/12/00, 06/13/00. 06/14/00, 07/28/00, 07/31/00 IAS/MMH | | |
| Date of Data Reduction/Analyst: | 5/22/00, 5/23/00, 5/24/00, 5/25/00, 5/30/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH | | |

**Sample Data**

**WEEK 105 RAT LIVER** Lot 171

| Group Dose | Sample # | Surrogate Verified | Initial Wt. g | Total Mass of Liver g | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Filename (optional) | Concentration of PFOS ug/g or % Rec | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | 05160-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.740 | 1 | 0.643 | D061400077 | < LOQ (0.0107 ug/g) | | |
| | 05160-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400078 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200073 | < LOQ (0.0107 ug/g) | | |
| | 052200-H2OBlk-3 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400088 | < LOQ (0.0107 ug/g) | | NA |
| | 052200-H2OBlk-4 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400089 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| Matrix Blk | RBL05160-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400079 | < LOQ (0.0107 ug/g) | | |
| | RBL05160-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400080 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.570 | 1 | 0.495 | A052200074 | < LOQ (0.0107 ug/g) | | |
| | RBL052200-LiverBlk-3 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400090 | < LOQ (0.0107 ug/g) | | NA |
| | RBL052200-LiverBlk-4 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400091 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| QC | C92503M G1-MS-250ppb-3 | NA | 1.0161 | NA | NA | NA | 357 | 1 | 177 | A073100018 | 60% * | | |
| | C92503M G1-MSD-250ppb-3 | NA | 1.0161 | NA | NA | NA | 411 | 1 | 230 | A073100019 | 78% | 69% | 26% |
| | C92505M G1-MS-250ppb-5 | NA | 0.9927 | NA | NA | NA | 496 | 1 | 377 | A052200020 | 126% | | |
| | C92505M G1-MSD-250ppb-5 | NA | 0.9927 | NA | NA | NA | 478 | 1 | 359 | A052200021 | 120% | 123% | 5% |
| | C92866F-G1-MS-250ppb-4 | NA | 0.9665 | NA | NA | NA | 125 | 10 | 389 | A061200060 | 126% | | |
| | C92866F-G1-MSD-250ppb-4 | NA | 0.9665 | NA | NA | NA | 135 | 10 | 488 | A061200061 | 158% * | 142% | 22% |
| | C92868F G1-MS-250ppb-6 | NA | 1.0055 | NA | NA | NA | 379 | 1 | 253 | D072800055 | 85% | | |
| | C92868F G1-MSD-250ppb-6 | NA | 1.0055 | NA | NA | NA | 433 | 1 | 306 | A052200025 | 102% | 94% | 20% |
| | C92510M G1-MS-250ppb-6 | NA | 0.9973 | NA | NA | NA | 803 | 1 | 217 | D072800056 | 72% | | |
| | C92510M G1-MSD-250ppb-6 | NA | 0.9973 | NA | NA | NA | 966 | 1 | 380 | A052200076 | 126% | 99% | 55% |
| | C92991F G3-MS-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 492 | 100 | 5430 | D061400045 | 1816% * a | | |
| | C92991F G3-MSD-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 517 | 100 | 7923 | D061400046 | 2650% * a | 2233% | 37% |
| | C92821M G6-MS-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 319 | 50 | 6423 | D061400047 | 2134% * a | | |
| | C92821M G6-MSD-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 309 | 50 | 5914 | D061400048 | 1965% * a | 2049% | 8% |
| Group 3 | C92631M | NA | 1.0036 | NA | 0.9949 | 0.8640 | 480 | 100 | 41522 | A051900026 | 41.5 | | |
| Mid Dose | C92633M | NA | 0.9973 | NA | 0.9949 | 0.8640 | 588 | 100 | 51167 | A052500026 | 51.2 | | |
| 2.0 ppm | C92637M | NA | 0.9931 | NA | 0.9949 | 0.8640 | 43.7 | 100 | 3823 | A051900027 | 3.82 | | |
| in Diet | C92641M | NA | 1.0076 | NA | 0.9949 | 0.8640 | 38.9 | 100 | 3352 | A052500029 | 3.35 | | |
| | C92642M | NA | 0.9928 | NA | 0.9949 | 0.8640 | 273 | 100 | 23890 | A051900031 | 23.9 | | |
| Males | C92643M | NA | 0.9943 | NA | 0.9949 | 0.8640 | 212 | 100 | 18505 | A052600015 | 18.5 | | |
| | C92644M | NA | 1.0045 | NA | 0.9949 | 0.8640 | 50.0 | 100 | 4318 | A051900032 | 4.32 | | |
| | C92649M | NA | 1.0021 | NA | 0.9949 | 0.8640 | 623 | 100 | 54004 | A052500030 | 54.0 | | |
| | C92653M | NA | 0.9991 | NA | 0.9949 | 0.8640 | 131 | 25 | 2855 | A061200100 | 2.85 | | |
| | C92657M | NA | 1.0731 | NA | 0.9949 | 0.8640 | 245 | 100 | 19795 | D052600017 | 19.8 | | |
| | C92659M | NA | 1.011 | NA | 0.9949 | 0.8640 | 715 | 100 | 61389 | A052500031 | 61.4 | | |
| | C92660M | NA | 0.9929 | NA | 0.9949 | 0.8640 | 121 | 100 | 10581 | A052500032 | 10.6 | | |
| | C92669M | NA | 1.0099 | NA | 0.9949 | 0.8640 | 202 | 100 | 17354 | A052500033 | 17.4 | | |
| | C92674M | NA | 1.004 | NA | 0.9949 | 0.8640 | 682 | 100 | 59020 | A051900033 | 59.0 | | |
| | C92682M | NA | 0.9935 | NA | 0.9949 | 0.8640 | 437 | 100 | 38218 | A051900034 | 38.2 | | |
| | C92683M | NA | 1.0362 | NA | 0.9949 | 0.8640 | 202 | 100 | 16893 | D052600018 | 16.9 | | 77.5 |
| | C92690M | NA | 0.911 | NA | 0.9949 | 0.8640 | 226 | 100 | 21542 | D052600019 | 21.5 | 26.4 | 20.4 |
| Group 4 | C92691M | NA | 0.9909 | NA | 0.9949 | 0.8640 | 317 | 100 | 27748 | A051900035 | 27.7 | | |
| Mid-High Dose | C92693M | NA | 0.9722 | NA | 0.9949 | 0.8640 | 136 | 250 | 30480 | D052600022 | 30.5 | | |
| 5.0 ppm | C92694M | NA | 1.0031 | NA | 0.9949 | 0.8640 | 240 | 1000 | 207597 | A061300017 | 208 | | |
| in Diet | C92695M | NA | 0.9918 | NA | 0.9949 | 0.8640 | 223 | 1000 | 194911 | A061300018 | 195 | | |
| | C92696M | NA | 1.0070 | NA | 0.9949 | 0.8640 | 139 | 1000 | 120002 | A061300019 | 120 | | |
| Males | C92698M | NA | 1.0065 | NA | 0.9949 | 0.8640 | 87.6 | 250 | 18896 | A052500036 | 18.9 | | |
| | C92699M | NA | 1.0000 | NA | 0.9949 | 0.8640 | 189 | 1000 | 163768 | A061300022 | 164 | | |
| | C92700M | NA | 1.0539 | NA | 0.9949 | 0.8640 | 335 | 250 | 69067 | D052600023 | 69.1 | | |
| | C92702M | NA | 0.9997 | NA | 0.9949 | 0.8640 | 60.1 | 250 | 13052 | A052500037 | 13.1 | | |
| | C92704M | NA | 1.0042 | NA | 0.9949 | 0.8640 | 756 | 100 | 65347 | A051900043 | 65.3 | | |
| | C92705M | NA | 0.9979 | NA | 0.9949 | 0.8640 | 311 | 250 | 67702 | D072800048 | 67.7 | | |
| | C92708M | NA | 1.0022 | NA | 0.9949 | 0.8640 | 595 | 100 | 51567 | A051900047 | 51.6 | | |
| | C92716M | NA | 0.9955 | NA | 0.9949 | 0.8640 | 393 | 250 | 85794 | A052500039 | 85.8 | | |
| | C92717M | NA | 1.0129 | NA | 0.9949 | 0.8640 | 638 | 25 | 13674 | A061200103 | 13.7 | | |
| | C92724M | NA | 1.0017 | NA | 0.9949 | 0.8640 | 531 | 100 | 46056 | A051900048 | 46.1 | | |
| | C92726M | NA | 1.0698 | NA | 0.9949 | 0.8640 | 468 | 250 | 95019 | D052600025 | 95.0 | | |
| | C92730M | NA | 1.0073 | NA | 0.9949 | 0.8640 | 83.7 | 250 | 18049 | A052500040 | 18.0 | | |
| | C92735M | NA | 1.0086 | NA | 0.9949 | 0.8640 | 856 | 100 | 73730 | A051900049 | 73.7 | | |
| | C92736M | NA | 0.9886 | NA | 0.9949 | 0.8640 | 68.8 | 250 | 15111 | D052600026 | 15.1 | | |
| | C92739M | NA | 1.0888 | NA | 0.9949 | 0.8640 | 179 | 250 | 35603 | D052600029 | 35.6 | | |
| | C92740M | NA | 0.9962 | NA | 0.9949 | 0.8640 | 711 | 250 | 154965 | D052600030 | 155 | | |
| | C92742M | NA | 1.0035 | NA | 0.9949 | 0.8640 | 33.1 | 250 | 7159 | A052500043 | 7.16 | | |
| | C92747M | NA | 0.9921 | NA | 0.9949 | 0.8640 | 776 | 250 | 169815 | A052500044 | 170 | | |
| | C92748M | NA | 1.0012 | NA | 0.9949 | 0.8640 | 52.9 | 250 | 11473 | A052500045 | 11.5 | | 89.4 |
| | C92750M | NA | 1.0072 | NA | 0.9949 | 0.8640 | 31.5 | 250 | 6796 | A052500046 | 6.80 | 70.5 | 63.1 |
| Group 4 | C93059F | NA | 0.9952 | NA | 0.9949 | 0.8640 | 841 | 250 | 183396 | A052500047 | 183 | | |
| Mid-High Dose | C93060F | NA | 1.0789 | NA | 0.9949 | 0.8640 | 660 | 250 | 132866 | D052600031 | 133 | | |
| 5.0 ppm | C93062F | NA | 1.0068 | NA | 0.9949 | 0.8640 | 112 | 2500 | 241755 | A061300016 | 242 | | |
| in Diet | C93063F | NA | 0.9976 | NA | 0.9949 | 0.8640 | 611 | 250 | 133009 | A051900051 | 133 | | |
| | C93072F | NA | 0.9991 | NA | 0.9949 | 0.8640 | 771 | 250 | 167486 | A052500050 | 167 | | |
| Females | C93077F | NA | 1.0544 | NA | 0.9949 | 0.8640 | 740 | 250 | 152272 | D052600032 | 152 | | |
| | C93078F | NA | 1.0089 | NA | 0.9949 | 0.8640 | 287 | 250 | 61653 | A051900055 | 61.7 | | |
| | C93079F | NA | 0.9979 | NA | 0.9949 | 0.8640 | 519 | 250 | 112894 | A052500051 | 113 | | |
| | C93080F | NA | 1.0316 | NA | 0.9949 | 0.8640 | 709 | 250 | 149290 | D052600033 | 149 | | |
| | C93081F | NA | 0.9939 | NA | 0.9949 | 0.8640 | 539 | 250 | 117702 | A052500052 | 118 | | |
| | C93090F | NA | 1.0008 | NA | 0.9949 | 0.8640 | 858 | 250 | 186045 | A051900056 | 186 | | |
| | C93098F | NA | 0.9906 | NA | 0.9949 | 0.8640 | 87.6 | 250 | 19199 | A052500053 | 19.2 | | |
| | C93101F | NA | 0.9962 | NA | 0.9949 | 0.8640 | 888 | 250 | 193426 | A052500054 | 193 | | |
| | C93102F | NA | 1.0888 | NA | 0.9949 | 0.8640 | 201 | 250 | 40012 | D052600036 | 40.0 | | 46.7 |
| | C93107F | NA | 1.0096 | NA | 0.9949 | 0.8640 | 371 | 250 | 79802 | A051900057 | 79.8 | 131 | 61.4 |

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00
* Reanalyzed with the same results. LAC 08/14/00
a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. KJH 10/03/00
NA = Not Applicable

| | |
|---|---|
| Date Entered/Analyst: | 5/24/00, 5/30/00, 5/31/00, 6/2/00, 06/20/00, 06/21/00, 06/22/00, 08/14/00 MMH/CSH/LAC |
| Date Verified/Analyst: | 10/03/00 KJH |
| Purity Entered/Verified: | 10/04/00 LAC, 10/04/00 KJH |

**AMDT# 112296.1**
**Covnace# 6329-183**

| | |
|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats |
| Product Number(Test Substance): | T-6295 |
| Matrix: | Rat Liver — Filename: See Below |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 — R-Squared Value: See Attachments |
| Analytical Equipment System Number: | Amelia 062498, Davey 070799 — Slope: See Attachments |
| Instrument Software/Version: | Masslynx 3.4 — Y-Intercept: See Attachments |
| Date of Extraction/Analyst: | 5/16/00-5/18/00, 5/22/00 SAL/KJK |
| Date of Analysis/Analyst: | 5/19/00, 5/22/00, 5/24/00, 5/26/00, 06/12/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH |
| Date of Data Reduction/Analyst: | 5/22/00, 5/23/00, 5/24/00, 5/25/00, 5/30/00, 06/13/00, 06/14/00, 06/15/00, 06/16/00, 07/31/00, 08/01/00 IAS/MMH |

**Sample Data**

**WEEK 105 RAT LIVER** Lot 171

| Group Dose | Sample # | Surrogate Verified | Initial Wt. g | Total Mass of Liver g | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ug/g | Filename (optional) | Concentration of PFOS ug/g or % Rec | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | 05160-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.740 | 1 | 0.643 | D061400077 | < LOQ (0.0107 ug/g) | | |
| | 05160-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400078 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200073 | < LOQ (0.0107 ug/g) | | |
| | 052200-H2OBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400088 | < LOQ (0.0107 ug/g) | | NA |
| | 052200-H2OBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400089 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| Matrix Blk | RBL05160-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400079 | < LOQ (0.0107 ug/g) | | |
| | RBL05160-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400080 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-5 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-6 | NA | 1.00 | NA | 0.9949 | 0.8640 | 0.570 | 1 | 0.495 | A052200074 | < LOQ (0.0107 ug/g) | | |
| | RBL052200-LiverBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400090 | < LOQ (0.0107 ug/g) | | NA |
| | RBL052200-LiverBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400091 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| QC | C92503M G1-MS-250ppb-3 | NA | 1.0161 | NA | NA | NA | 357 | 1 | 177 | A073100018 | 60% * | | |
| | C92503M G1-MSD-250ppb-3 | NA | 1.0161 | NA | NA | NA | 411 | 1 | 230 | A073100019 | 78% | 69% | 26% |
| | C92505M G1-MS-250ppb-5 | NA | 0.9927 | NA | NA | NA | 496 | 1 | 377 | A052200020 | 126% | | |
| | C92505M G1-MSD-250ppb-5 | NA | 0.9927 | NA | NA | NA | 478 | 1 | 359 | A052200021 | 120% | 123% | 5% |
| | C92868F G1-MS-250ppb-4 | NA | 0.9665 | NA | NA | NA | 125 | 10 | 389 | A061200060 | 126% | | |
| | C92868F G1-MSD-250ppb-4 | NA | 0.9665 | NA | NA | NA | 135 | 10 | 488 | A061200061 | 158% * | 142% | 22% |
| | C92868F G1-MS-250ppb-6 | NA | 1.0055 | NA | NA | NA | 379 | 1 | 253 | D072800055 | 85% | | |
| | C92868F G1-MSD-250ppb-6 | NA | 1.0055 | NA | NA | NA | 433 | 1 | 306 | A052200025 | 102% | 94% | 20% |
| | C92510M G1-MS-250ppb-6 | NA | 0.9973 | NA | NA | NA | 803 | 1 | 217 | D072800056 | 72% | | |
| | C92510M G1-MSD-250ppb-6 | NA | 0.9973 | NA | NA | NA | 966 | 1 | 380 | A052200076 | 126% | 99% | 55% |
| | C92991F G3-MS-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 492 | 100 | 5430 | D061400045 | 1816% * a | | |
| | C92991F G3-MSD-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 517 | 100 | 7923 | D061400046 | 2650% * a | 2233% | 37% |
| | C92821M G6-MS-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 319 | 50 | 6423 | D061400047 | 2134% * a | | |
| | C92821M G6-MSD-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 309 | 50 | 5914 | D061400048 | 1965% * a | 2049% | 8% |
| Group 5 | C92753M | NA | 1.0015 | NA | 0.9949 | 0.8640 | 261 | 500 | 113104 | D052600037 | 113 | | |
| High Dose | C92757M | NA | 1.0100 | NA | 0.9949 | 0.8640 | 765 | 500 | 329066 | A051900058 | 329 | | |
| 20 ppm | C92761M | NA | 1.0065 | NA | 0.9949 | 0.8640 | 186 | 500 | 80173 | A052500057 | 80.2 | | |
| in Diet | C92762M | NA | 0.9941 | NA | 0.9949 | 0.8640 | 400 | 500 | 174603 | A051900059 | 175 | | |
| | C92774M | NA | 0.9768 | NA | 0.9949 | 0.8640 | 88.6 | 500 | 39390 | D052600038 | 39.4 | | |
| Males | C92775M | NA | 1.0108 | NA | 0.9949 | 0.8640 | 81.4 | 500 | 34980 | A052500058 | 35.0 | | |
| | C92776M | NA | 0.9953 | NA | 0.9949 | 0.8640 | 734 | 500 | 320170 | A052500059 | 320 | | |
| | C92780M | NA | 0.9985 | NA | 0.9949 | 0.8640 | 375 | 500 | 163214 | D052600039 | 163 | | |
| | C92782M | NA | 0.9923 | NA | 0.9949 | 0.8640 | 121 | 500 | 53101 | A052500060 | 53.1 | | |
| | C92786M | NA | 1.0004 | NA | 0.9949 | 0.8640 | 227 | 500 | 98636 | A051900063 | 98.6 | | |
| | C92788M | NA | 1.0336 | NA | 0.9949 | 0.8640 | 915 | 500 | 384592 | D052600040 | 385 | | |
| | C92791M | NA | 1.0100 | NA | 0.9949 | 0.8640 | 160 | 500 | 68954 | A051900064 | 69.0 | | |
| | C92792M | NA | 1.0046 | NA | 0.9949 | 0.8640 | 127 | 500 | 54772 | A052500061 | 54.8 | | |
| | C92800M | NA | 1.0003 | NA | 0.9949 | 0.8640 | 366 | 500 | 158784 | A051900065 | 159 | | |
| | C92802M | NA | 0.9946 | NA | 0.9949 | 0.8640 | 93.6 | 5000 | 408544 | A061300023 | 409 | | |
| | C92803M | NA | 1.0428 | NA | 0.9949 | 0.8640 | 987 | 500 | 411159 | D052600043 | 411 | | |
| | C92804M | NA | 1.0029 | NA | 0.9949 | 0.8640 | 762 | 500 | 330049 | A051900067 | 330 | | |
| | C92808M | NA | 0.9908 | NA | 0.9949 | 0.8640 | 271 | 500 | 118664 | A051900071 | 119 | | |
| | C92809M | NA | 1.0161 | NA | 0.9949 | 0.8640 | 1091 | 25 | 23316 | A061200106 | 23.3 | | |
| | C92814M | NA | 1.0275 | NA | 0.9949 | 0.8640 | 847 | 500 | 357873 | D052600045 | 358 | | |
| | C92815M | NA | 0.9995 | NA | 0.9949 | 0.8640 | 166 | 500 | 71998 | A052500064 | 72.0 | | 74.9 |
| | C92818M | NA | 1.0013 | NA | 0.9949 | 0.8640 | 812 | 500 | 351938 | A052500065 | 352 | 189 | 141 |
| Group 5 | C93113F | NA | 0.9987 | NA | 0.9949 | 0.8640 | 85.8 | 5000 | 373041 | A061300024 | 373 | | |
| High Dose | C93115F | NA | 1.0021 | NA | 0.9949 | 0.8640 | 872 | 500 | 377647 | A052500066 | 378 | | |
| 20 ppm | C93124F | NA | 1.0015 | NA | 0.9949 | 0.8640 | 401 | 500 | 173786 | A052500067 | 174 | | |
| in Diet | C93125F | NA | 1.0051 | NA | 0.9949 | 0.8640 | 103 | 5000 | 444108 | A061300025 | 444 | | |
| | C93133F | NA | 0.9705 | NA | 0.9949 | 0.8640 | 900 | 500 | 402766 | D052600046 | 403 | | |
| Females | C93135F | NA | 1.0082 | NA | 0.9949 | 0.8640 | 135 | 5000 | 581680 | A061300026 | 582 | | |
| | C93137F | NA | 0.9941 | NA | 0.9949 | 0.8640 | 643 | 500 | 280805 | A052500068 | 281 | | |
| | C93139F | NA | 0.9735 | NA | 0.9949 | 0.8640 | 999 | 500 | 445629 | D052600047 | 446 | | |
| | C93140F | NA | 0.9965 | NA | 0.9949 | 0.8640 | 21.1 | 500 | 9198 | A052500071 | 9.20 | | |
| | C93146F | NA | 1.0091 | NA | 0.9949 | 0.8640 | 304 | 500 | 130983 | A052500072 | 131 | | |
| | C93149F | NA | 0.9962 | NA | 0.9949 | 0.8640 | 132 | 5000 | 575132 | A061300029 | 575 | | |
| | C93151F | NA | 1.0219 | NA | 0.9949 | 0.8640 | 687 | 500 | 291713 | D052600050 | 292 | | |
| | C93153F | NA | 1.0057 | NA | 0.9949 | 0.8640 | 541 | 500 | 233544 | A052500073 | 234 | | |
| | C93157F | NA | 0.9904 | NA | 0.9949 | 0.8640 | 175 | 500 | 76847 | D052600051 | 76.8 | | |
| | C93158F | NA | 1.0034 | NA | 0.9949 | 0.8640 | 143 | 5000 | 617048 | A061300030 | 617 | | |
| | C93161F | NA | 0.9938 | NA | 0.9949 | 0.8640 | 486 | 500 | 212218 | A051900080 | 212 | | |
| | C93162F | NA | 1.0044 | NA | 0.9949 | 0.8640 | 161 | 5000 | 694812 | A061300031 | 695 | | |
| | C93164F | NA | 1.0078 | NA | 0.9949 | 0.8640 | 118 | 5000 | 509959 | A061300032 | 510 | | |
| | C93165F | NA | 1.0317 | NA | 0.9949 | 0.8640 | 976 | 500 | 410860 | D052600052 | 411 | | |
| | C93166F | NA | 0.9959 | NA | 0.9949 | 0.8640 | 903 | 500 | 393880 | A052500074 | 394 | | |
| | C93168F | NA | 0.9930 | NA | 0.9949 | 0.8640 | 1056 | 500 | 461592 | A052500075 | 462 | | |
| | C93174F | NA | 0.9921 | NA | 0.9949 | 0.8640 | 113 | 5000 | 493038 | A061300033 | 493 | | |
| | C93177F | NA | 1.0740 | NA | 0.9949 | 0.8640 | 154 | 5000 | 622698 | A061200105 | 623 | | |
| | C93178F | NA | 0.9730 | NA | 0.9949 | 0.8640 | 886 | 500 | 395515 | D052600054 | 396 | | 46.1 |
| | C93179F | NA | 1.0472 | NA | 0.9949 | 0.8640 | 792 | 500 | 328294 | D052600057 | 328 | 381 | 176 |

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00
* Reanalyzed with the same results. LAC 08/14/00
a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. Kjh 10/03/00
NA = Not Applicable

Date Entered/Analyst: 05/24/00, 05/30/00, 05/31/00, 06/02/00, 06/20/00, 06/21/00, 06/22/00, 08/14/00 MMH/CSH/LAC
Date Verified/Analyst: 10/03/00 KJH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

**AMDT# 12296.1**
**Covance# 6329-183**

| | | | |
|---|---|---|---|
| 3M Study Title: | 104 Week Dietary Chronic Toxicity and Carcinogenicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Covance Study Title: | 104 Week Dietary Chronic Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS T-6295) in Rats | | |
| Product Number(Test Substance): | T-6295 | | |
| Matrix: | Rat Liver | Filename: | See list to right |
| Method/Revision: | ETS-8-6.0 and ETS-8-7.0 | R-Squared Value: | See Attachments |
| Analytical Equipment System Number: | Amelia 062498, Davey 070799 | Slope: | See Attachments |
| Instrument Software/Version: | Masslynx 3.4 | Y-Intercept: | See Attachments |
| Date of Extraction/Analyst: | 5/16/00 5/18/00 SAL/KJK | | |
| Date of Analysis/Analyst: | 5/25/00, 06/13/00, 06/14/00, 07/28/00, 07/31/00 IAS/MMH | | |
| Date of Data Reduction/Analyst: | 5/30/00, 06/14/00, 06/15/00, 07/31/00, 08/01/00 IAS/MMH | | |

**Sample Data**

**WEEK 106 RAT LIVER**

Lot 171

| Group Dose | Sample # | Surrogate Verified | Initial Wt. g | Total Mass of Liver g | Original Purity Correction Factor | PFOS Purity Correction Factor | PFOS Conc. ng/g | PFOS Dilution Factor | PFOS Calc. Conc. ng/g | Filename (optional) | Concentration of PFOS ug/g or % Rec | Mean PFOS ug/g | RSD Std. Dev. MS/MSD RPD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Method Blk | 05160-H2OBlk-5 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.740 | 1 | 0.643 | D061400077 | < LOQ (0.0107 ug/g) | | |
| | 05160-H2OBlk-6 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400078 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-5 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | 05180-H2OBlk-6 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200073 | < LOQ (0.0107 ug/g) | | |
| | 052200-H2OBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400088 | < LOQ (0.0107 ug/g) | | NA |
| | 052200-H2OBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400089 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| Matrix Blk | RBL05160-LiverBlk-5 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400079 | < LOQ (0.0107 ug/g) | | |
| | RBL05160-LiverBlk-6 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400080 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-5 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | A052200060 | < LOQ (0.0107 ug/g) | | |
| | RBL05180-LiverBlk-6 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.570 | 1 | 0.495 | A052200074 | < LOQ (0.0107 ug/g) | | |
| | RBL052200-LiverBlk-3 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400090 | < LOQ (0.0107 ug/g) | | NA |
| | RBL052200-LiverBlk-4 | NA | 1.0000 | NA | 0.9949 | 0.8640 | 0.00 | 1 | 0.00 | D061400091 | < LOQ (0.0107 ug/g) | < LOQ (0.0107 ug/g) | NA |
| QC | C92503M G1-MS-250ppb-3 | NA | 1.0161 | NA | NA | NA | 357 | 1 | 177 | A073100018 | 60% * | | |
| | C92503M G1-MSD-250ppb-3 | NA | 1.0161 | NA | NA | NA | 411 | 1 | 230 | A073100019 | 78% | 69% | 26% |
| | C92505M G1-MS-250ppb-5 | NA | 0.9927 | NA | NA | NA | 496 | 1 | 377 | A052200020 | 126% | | |
| | C92505M G1-MSD-250ppb-5 | NA | 0.9927 | NA | NA | NA | 478 | 1 | 359 | A052200021 | 120% | 123% | 5% |
| | C92866F-G1-MS-250ppb-4 | NA | 0.9665 | NA | NA | NA | 125 | 10 | 389 | A061200060 | 126% | | |
| | C92866F-G1-MSD-250ppb-4 | NA | 0.9665 | NA | NA | NA | 135 | 10 | 488 | A061200061 | 158% * | 142% | 22% |
| | C92868F G1-MS-250ppb-6 | NA | 1.0055 | NA | NA | NA | 379 | 1 | 253 | D072800055 | 85% | | |
| | C92868F G1-MSD-250ppb-6 | NA | 1.0055 | NA | NA | NA | 433 | 1 | 306 | A052200025 | 102% | 94% | 20% |
| | C92510M G1-MS-250ppb-6 | NA | 0.9973 | NA | NA | NA | 803 | 1 | 217 | D072800056 | 72% | | |
| | C92510M G1-MSD-250ppb-6 | NA | 0.9973 | NA | NA | NA | 966 | 1 | 380 | A052200076 | 126% | 99% | 55% |
| | C92991F G3-MS-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 492 | 100 | 5430 | D061400045 | 1816% * a | | |
| | C92991F G3-MSD-250 ppb-5 | NA | 1.0024 | NA | NA | NA | 517 | 100 | 7923 | D061400046 | 2650% * a | 2233% | 37% |
| | C92821M G6-MS-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 319 | 50 | 6423 | D061400047 | 2134% * a | | |
| | C92821M G6-MSD-250 ppb-5 | NA | 0.9900 | NA | NA | NA | 309 | 50 | 5914 | D061400048 | 1965% * a | 2049% | 8% |
| Group 6 | C92821M | NA | 0.9900 | NA | 0.9949 | 0.8640 | 110 | 100 | 9680 | D061400020 | 9.68 | | |
| | C92823M | NA | 1.0378 | NA | 0.9949 | 0.8640 | 22.9 | 100 | 1915 | D061400023 | 1.91 | | |
| | C92827M | NA | 0.9974 | NA | 0.9949 | 0.8640 | 165 | 1 | 144 | D061300032 | 0.144 | | |
| | C92829M | NA | 1.0069 | NA | 0.9949 | 0.8640 | 136 | 1 | 117 | D061300033 | 0.117 | | |
| | C92833M | NA | 1.0462 | NA | 0.9949 | 0.8640 | 460 | 1 | 382 | D061300034 | 0.382 | | |
| | C92834M | NA | 1.0129 | NA | 0.9949 | 0.8640 | 236 | 1 | 203 | D061300037 | 0.203 | | |
| | C92848M | NA | 0.9561 | NA | 0.9949 | 0.8640 | 197 | 100 | 17890 | D061300016 | 17.9 | | |
| | C92851M | NA | 1.0102 | NA | 0.9949 | 0.8640 | 423 | 1 | 363 | D061300038 | 0.363 | | |
| | C92853M | NA | 1.0350 | NA | 0.9949 | 0.8640 | 514 | 1 | 431 | D061300039 | 0.431 | | 192 |
| | C92856M | NA | 1.0274 | NA | 0.9949 | 0.8640 | 39.3 | 1 | 33.2 | D061400016 | 0.0332 | 3.12 | 5.97 |
| Group 6 | C93181F | NA | 1.0108 | NA | 0.9949 | 0.8640 | 50.2 | 100 | 4316 | D061400017 | 4.32 | | |
| | C93182F | NA | 1.0429 | NA | 0.9949 | 0.8640 | 25.3 | 100 | 2103 | D061400018 | 2.10 | | |
| | C93183F | NA | 0.9968 | NA | 0.9949 | 0.8640 | 104 | 100 | 9079 | D061300019 | 9.08 | | |
| | C93184F | NA | 0.9740 | NA | 0.9949 | 0.8640 | 18.9 | 100 | 1688 | D061400019 | 1.69 | | |
| | C93185F | NA | 1.0186 | NA | 0.9949 | 0.8640 | 186 | 100 | 15896 | D061300023 | 15.9 | | |
| | C93190F | NA | 1.0540 | NA | 0.9949 | 0.8640 | 27.7 | 1000 | 22831 | A052500085 | 22.8 | | |
| | C93192F | NA | 1.0134 | NA | 0.9949 | 0.8640 | 175 | 100 | 15018 | D061300024 | 15.0 | | |
| | C93197F | NA | 1.0425 | NA | 0.9949 | 0.8640 | 104 | 100 | 8650 | D061300025 | 8.65 | | |
| | C93198F | NA | 1.0660 | NA | 0.9949 | 0.8640 | 75.9 | 100 | 6184 | D061300026 | 6.18 | | |
| | C93201F | NA | 1.0054 | NA | 0.9949 | 0.8640 | 222 | 100 | 19198 | D061300027 | 19.2 | | |
| | C93202F | NA | 1.0631 | NA | 0.9949 | 0.8640 | 209 | 100 | 17050 | D061400024 | 17.0 | | |
| | C93205F | NA | 1.0013 | NA | 0.9949 | 0.8640 | 152 | 1 | 132 | D061300044 | 0.132 | | |
| | C93209F | NA | 0.9948 | NA | 0.9949 | 0.8640 | 170 | 100 | 14854 | D061400025 | 14.9 | | |
| | C93211F | NA | 1.0091 | NA | 0.9949 | 0.8640 | 49.5 | 1000 | 42582 | A052500109 | 42.6 | | |
| | C93216F | NA | 1.0096 | NA | 0.9949 | 0.8640 | 171 | 100 | 14687 | D061400027 | 14.7 | | |
| | C93219F | NA | 1.0009 | NA | 0.9949 | 0.8640 | 57.2 | 100 | 4962 | D061400030 | 4.96 | | 80.6 |
| | C93220F | NA | 0.9938 | NA | 0.9949 | 0.8640 | 22.3 | 1000 | 19469 | A052500114 | 19.5 | 12.9 | 10.4 |

Original PFOS LOQ (12.3 ng/g) updated to reflect purity information on 10/04/00. LAC 10/04/00
* Reanalyzed with the same results. LAC 08/14/00
a Endogenous level of PFOS too high for MS/MSD analysis (diluted out of accuracy range); do not include results in the body of the final report. Kjh 10/03/00
NA = Not Applicable

Date Entered/Analyst: 06/01/00, 06/21/00, 06/22/00, 08/14/00 CSH/LAC
Date Verified/Analyst: 10/03/00 KJH
Purity Entered/Verified: 10/04/00 LAC, 10/04/00 KJH

# Appendix F: Example Calculations

### Formula Used for Sera Analyses in Study FACT TOX-002

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

### Calculation Used for Group 3, Day 719, Animal ID C92991F

$$348\ \text{ng/mL} \times 50 \times 0.9275 \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} \times \frac{0.864}{0.9949} = \textbf{14.0 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
SC—PFOS salt correction constant (0.9275)
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted
PC—PFOS purity correction factor (86.4% for lot 171)
OPC—Original PFOS purity correction factor (99.49%)

### Formula Used for Liver Analyses in Study FACT TOX-002

$$\text{AR (ng/g)} \times \frac{\partial\ \text{curve}}{\partial\ \text{specimen}}^{(1)} \times \text{SC} \times \text{DF} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} \times \text{PC} = \textbf{Reported Concentration (µg/g)}$$

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

### Calculation Used for Group 3, Week 105, Animal ID C92631M

$$480\ \text{ng/g} \times \frac{1\ \text{g/}\ 5\ \text{mL}}{1.0036\ \text{g/}\ 5\text{mL}} \times 100 \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} \times \frac{0.864}{0.9949} = \textbf{41.5 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ specimen—Density of the liver specimen (g specimen/ 5 mL $H_2O$)
DF— Dilution factor
PC—PFOS purity correction factor (86.4% for lot 171)
OPC—Original PFOS purity correction factor (99.49%)

Standard deviations were calculated using the equation:

$$SD = \sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n(n-1)}}$$

# Appendix G: Interim Certificates of Analysis

Centre Analytical Laboratories, Inc.
3048 Research Drive State College, PA 16801
Phone: (814) 231-8032 Fax: (814) 231-1253 or (814) 231-1580

# *INTERIM CERTIFICATE OF ANALYSIS*

***Revision 1(9/7/00)***

**Centre Analytical Laboratories COA Reference #: 023-018B**
**3M Product: PFOS, Lot 171**
**Reference #: SD-009**
**Purity: 86.4%**

| Test Name | Specifications | Result |
|---|---|---|
| **Purity[1]** | | **86.4%** |
| Appearance | White Crystalline Powder | Conforms |
| Identification NMR | | Positive |
| Metals (ICP/MS) | | |
| 1. Calcium | | 1. 0.017 wt./wt.% |
| 2. Magnesium | | 2. 0.007 wt./wt.% |
| 3. Sodium | | 3. 1.355 wt./wt.% |
| 4. Potassium[2] | | 4. 6.552 wt./wt.% |
| 5. Nickel | | 5. 0.003 wt./wt.% |
| 6. Iron | | 6. 0.004 wt./wt.% |
| 7. Manganese | | 7. <0.001 wt./wt.% |
| Total % Impurity (NMR) | | 1.00 wt./wt.% |
| Total % Impurity (LC/MS) | | 10.60 wt./wt.% |
| Total % Impurity (GC/MS) | | None Detected |
| Related Compounds – POAA | | 0.30 wt./wt.% |
| Residual Solvents (TGA) | | None Detected |
| Purity by DSC | | Not Applicable[3] |
| Inorganic Anions (IC) | | |
| 1. Chloride | | 1. <0.015 wt./wt.% |
| 2. Fluoride | | 2. 0.27 wt./wt.% |
| 3. Bromide | | 3. <0.040 wt./wt.% |
| 4. Nitrate | | 4. <0.009 wt./wt.% |
| 5. Nitrite | | 5. <0.006 wt./wt.% |
| 6. Phosphate | | 6. <0.007 wt./wt.% |
| 7. Sulfate[4] | | 7. 8.82 wt./wt.% |
| Organic Acids [5] (IC) | | |
| 1. TFA | | 1. <0.1 wt./wt.% |
| 2. PFPA | | 2. <0.1 wt./wt.% |
| 3. HFBA | | 3. <0.1 wt./wt.% |
| 4. NFPA | | 4. <0.25 wt./wt.% |
| Elemental Analysis[6]: | | |
| 1. Carbon | 1. Theoretical Value = 17.8% | 1. 12.08 wt./wt.% |
| 2. Hydrogen | 2. Theoretical Value = 0% | 2. 0.794 wt./wt.% |
| 3. Nitrogen | 3. Theoretical Value = 0% | 3. 1.61 wt./wt.% |
| 4. Sulfur | 4. Theoretical Value = 5.95% | 4. 10.1 wt./wt.% |
| 5. Fluorine | 5. Theoretical Value = 60% | 5. 50.4 wt./wt.% |

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# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-018B**

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.39% +LC/MS impurities, 10.60%+Inorganic Fluoride, 0.27%+NMR impurities, 1.00%+ POAA, 0.30%)
Total impurity from all tests = 13.56%
Purity = 100% - 13.56% = 86.4%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to $SO_4$ and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the $SO_4$ is not considered an impurity.

| | |
|---|---|
| [5]TFA | Trifluoroacetic acid |
| HFBA | Heptafluorobutyric acid |
| NFPA | Nonofluoropentanoic acid |
| PFPA | Pentafluoropropanoic acid |

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-018B**

LC/MS Purity Profile:

| Impurity | wt./wt. % |
|---|---|
| C4 | 1.03 |
| C5 | 1.56 |
| C6 | 6.38 |
| C7 | 1.63 |
| **Total** | **10.60** |

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ 9/10/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 9/11/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

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# *INTERIM CERTIFICATE OF ANALYSIS*

***Revision 1(9/7/00)***

**Centre Analytical Laboratories COA Reference #: 023-018A**
**3M Product: PFOS, Lot 217**
**Reference #: SD-018**
**Purity: 86.9%**

| Test Name | Specifications | Result |
|---|---|---|
| **Purity**[1] | | **86.9%** |
| Appearance | White Crystalline Powder | Conforms |
| Identification<br>NMR | | Positive |
| Metals (ICP/MS)<br>1. Calcium<br>2. Magnesium<br>3. Sodium<br>4. Potassium[2]<br>5. Nickel<br>6. Iron<br>7. Manganese | | <br>1. 0.005 wt./wt.%<br>2. 0.001 wt./wt.%<br>3. 1.439 wt./wt.%<br>4. 6.849 wt./wt.%<br>5. <0.001 wt./wt.%<br>6. 0.005 wt./wt.%<br>7. <0.001 wt./wt.% |
| Total % Impurity (NMR) | | 1.93 wt./wt.% |
| Total % Impurity (LC/MS) | | 8.41 wt./wt.% |
| Total % Impurity (GC/MS) | | None Detected |
| Related Compounds – POAA | | 0.33 wt./wt.% |
| Residual Solvents (TGA) | | None Detected |
| Purity by DSC | | Not Applicable[3] |
| Inorganic Anions (IC)<br>1. Chloride<br>2. Fluoride<br>3. Bromide<br>4. Nitrate<br>5. Nitrite<br>6. Phosphate<br>7. Sulfate[4] | | <br>1. <0.015 wt./wt.%<br>2. 0.59 wt./wt.%<br>3. <0.040 wt./wt.%<br>4. <0.009 wt./wt.%<br>5. <0.006 wt./wt.%<br>6. <0.007 wt./wt.%<br>7. 8.76 wt./wt.% |
| Organic Acids [5] (IC)<br>1. TFA<br>2. PFPA<br>3. HFBA<br>4. NFPA | | <br>1. <0.1 wt./wt.%<br>2. <0.1 wt./wt.%<br>3. 0.10 wt./wt.%<br>4. 0.28 wt./wt.% |
| Elemental Analysis[6]:<br>1. Carbon<br>2. Hydrogen<br>3. Nitrogen<br>4. Sulfur<br>5. Fluorine | <br>1. Theoretical Value = 17.8%<br>2. Theoretical Value = 0%<br>3. Theoretical Value = 0%<br>4. Theoretical Value = 5.95%<br>5. Theoretical Value = 60% | <br>1. 12.48 wt./wt.%<br>2. 0.244 wt./wt.%<br>3. 1.74 wt./wt.%<br>4. 8.84 wt./wt.%<br>5. 54.1 wt./wt.% |

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# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-018A**

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

[1]Purity = 100% - (sum of metal impurities, 1.45% +LC/MS impurities, 8.41%+Inorganic Fluoride, 0.59%+NMR impurities, 1.93%+organic acid impurities, 0.38%+POAA, 0.33%)

Total impurity from all tests = 13.09%
Purity = 100% - 13.09% = 86.9%

[2]Potassium is expected in this salt form and is therefore not considered an impurity.

[3]Purity by DSC is generally not applicable to materials of low purity. No endotherm was observed for this sample.

[4]Sulfur in the sample appears to be converted to $SO_4$ and hence detected using the inorganic anion method conditions. The anion result agrees well with the sulfur determination in the elemental analysis, lending confidence to this interpretation. Based on the results, the $SO_4$ is not considered an impurity.

| | |
|---|---|
| [5]TFA | Trifluoroacetic acid |
| HFBA | Heptafluorobutyric acid |
| NFPA | Nonofluoropentanoic acid |
| PFPA | Pentafluoropropanoic acid |

[6]Theoretical value calculations based on the empirical formula, $C_8F_{17}SO_3^-K^+$ (MW=538)

This work was conducted under EPA Good Laboratory Practice Standards (40 CFR 160).

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# *INTERIM CERTIFICATE OF ANALYSIS*

**Centre Analytical Laboratories COA Reference #: 023-018A**

LC/MS Purity Profile:

| Impurity | wt./wt. % |
|---|---|
| C4 | 1.22 |
| C5 | 1.33 |
| C6 | 4.72 |
| C7 | 1.14 |
| **Total** | **8.41** |

Note: The C4 and C6 values were calculated using the C4 and C6 standard calibration curves, respectively. The C5 value was calculated using the average response factors from the C4 and C6 standard curves. Likewise, the C7 value was calculated using the average response factors from the C6 and C8 standard curves.

Prepared By: _____ 9/10/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ 9/11/00
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories

## Appendix H: Report Signature Page

John L. Butenhoff, Ph.D., *Study Director* — Date: 21 FEB 01

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative* — Date: 21 Feb 2001

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date: 02/14/01

William K. Reagen, Ph.D., *Laboratory Manager* — Date: 02/15/01