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AR226-0981

ANALYTICAL LABORATORY REPORT

FROM THE

26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

ON THE

Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples

Project Identification

3M Medical Department Study: T-6295.7
Covance In-Life Study: #6329-223

Analytical Study: FACT TOX-030
3M Laboratory Request No. U2279

Study Completion Date

At signing

Total Number of Pages

233

Contain NO CBI

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030
Laboratory Request Number-U2279

GLP COMPLIANCE STATEMENT

Study Title: Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples

Study Identification Number: FACT TOX-030, T-6295.7, Covance #6329-223

This study was conducted in compliance with United States Environmental Protection Agency Good Laboratory Practice (GLP) Standards 40 CFR Part 792, with the exceptions in the bulleted list below. All raw data and samples for this study are retained in archives at the 3M Lab and will be retained for a period of at least ten years. The analytical phase completed at the 3M Lab was performed in accordance with 3M ET&SS Standard Operating Procedures.

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.
- On a few occasions, data were not recorded or corrected exactly as required by the GLPs.
- The 3M TOX 030 protocol states in the Regulatory Compliance section that "This study will be conducted in accordance with the United States Environmental Protection Agency Good Laboratory Practices Standards, 40 CFR 792, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor." Analyses were, however, completed on the concentration and homogeneity of the test material mixture, according to non-GLP validated methods, and are included in this report. As per the protocol, solubility and stability determinations were not conducted.

Andrew M. Seacat — 9/19/00

Study Director — Date

John L. Butenhoff — 19 SEPT 2000

Study Sponsor — Date

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GLP Study—Quality Assurance Statement

Study Title: Analytical Laboratory Report from the 26-Week Capsule Toxicity Study with Perfluorooctanesulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in Liver and Serum Samples

Study Identification Number: FACT TOX-030, T-6295.7, Covance #6329-223

The analytical phase of 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 management.

Inspection Dates	Phase	Date Reported to Management	Date Reported to Study Director
December 01/98	Sample receipt	1/17/00	1/17/00
March 19,22,23/99	Analysis	3/25/99	3/25/99
October 14/99	Extraction	10/20/99	10/20/99
May 3,8–12,15–19,22–26,29–31/00, June 1,2,5,7,8/00	Data	6/14/00	6/14/00
June 1,5,7,12–16/00	Draft report	6/16/00	6/16/00
September 14/00	Draft report	9/14/00	9/14/00

QAU Representative

9/22/00
Date

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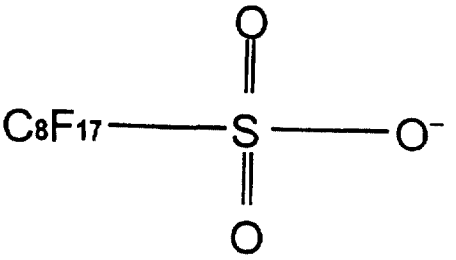
INTRODUCTION AND PURPOSE



Perfluorooctanesulfonate (PFOS)

CAS Number: 2759-39-3

Chemical Formula: $C_8F_{17}SO_3^-$ Molecular Weight: 498.98

The purpose of the analytical phase of this study is to determine the presence and concentration of PFOS ($C_8F_{17}SO_3^-$) in liver and serum specimens collected during the study of Cynomolgus monkeys orally dosed with perfluorooctane sulfonic acid potassium salt (T-6295).

Test System

The test system species and strain selected was the Cynomolgus monkey from Covance Research Products, Inc., identified using a collar tag. At the initiation of treatment, the Cynomolgus monkeys were young adult to adult, and weighed approximately 3–5 kg.

Twenty-two male and 22 female Cynomolgus monkeys were used as the test system in the present study. Four groups of test animals were established according to dosage levels. Group 1 consisted of control Cynomolgus monkeys that did not receive the test substance, but received the equivalent amount of lactose in gelatin capsules as that administered to the Group 4 animals. Groups 2, 3, and 4 were administered daily with 0.03 (low dose), 0.15 (mid dose), and 0.75 (high dose) mg respectively, of T-6295 per kg of body weight/day (mg/kg/day) triturated with lactose in gelatin capsules (see Table 1 for Dosage and Group Characteristics).

Table 1. Dosage and Group Characteristics of Test System in Study T-6295.7

STUDY GROUP	NUMBER OF ANIMALS	TOTAL	DOSAGE LEVEL (mg/kg/day)	DOSAGE RATIO (w:w)a
Total: Test System	22 males 22 females	44*	—	—
Group 1 (Control)	6 males 6 females	12	0	—
Group 2 (Low Dose)	4 males 4 females	8	0.03	1:499
Group 3 (Mid Dose)	6 males 6 females	12	0.15	1:39
Group 4 (High Dose)	6 males 6 females	12	0.75	1:39

a Test substance triturated with lactose
* 48 animals were included in the baseline sera collection, but 44 animals were assigned for treatment

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All treatment groups were dosed for a minimum period of 26 weeks. Sera specimens were collected from all test animals at various time points during the in-life phase of the 26-week study and sent to the 3M Lab for analysis (see Attachment D, Tables D-1a, D-1b).

Four animals each from Groups 1, 3, and 4 were designated as recovery group animals. Treatment was discontinued and the animals were monitored for elimination of compounds for one year post-treatment. The recovery groups were observed after the cessation of treatment until February 25, 2000 (Week 79) for Group 1 and Group 4 recovery animals, and until March 7, 2000 (Week 80) for Group 3 recovery animals.

Specimen Collection and Analysis

In the analytical phase reported here, liver and sera specimens collected from all test animals were sent to the 3M Lab and analyzed for the presence of PFOS (some samples were analyzed to determine the presence of EtFOSE, PFOSA, POAA, PFOSEA, M556, PFOSAA, and the monoester; however, these data were collected for informational purposes only, and are not reported). Specimens other than serum and liver tissues were collected and received from Covance Laboratories (6329-223), but were not part of the current scope of analysis determined by the study director and sponsor. Additional analyses of feces are being completed and will be issued as an amendment to this final report.

Blood specimens were centrifuged within one hour of collection. The serum was then harvested and stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Liver specimens collected from each animal were flash frozen in liquid nitrogen and then stored in a freezer set to maintain specimens at -60 to -80°C until shipped to the 3M Lab. Liver and sera specimens were shipped to the 3M Lab frozen and on dry ice. Liver specimens from Group 3 (3/01/00) and Group 4 (9/22/99) recovery animals were collected via biopsy.

Sera and liver samples were extracted using an ion-pairing reagent and methyl-*tert*-butyl ether (MtBE). Liver samples were homogenized prior to the extraction procedure. Sample extracts were analyzed using high-pressure liquid chromatography-electrospray/tandem mass spectrometry (HPLC-ES/MS/MS) in the multiple response mode. PFOS levels were quantitated by external standard calibration. Analytical details are included in this report.

Specimens Collected from Study Groups 1 through 4 (through 2/25/99):
Serum Specimens—550 specimens: 9–14 specimens/animal
Liver Specimens—30 specimens

Specimens Collected from the Recovery Group from 2/27/99 to 3/07/00:
Serum Specimens—224 specimens: 18–20 specimens/animal
Liver Specimens—12 specimens from Group 3 and Group 4 animals (8 via biopsy)

Specimen Receipt

Specimens were received from Covance Laboratories periodically, during the in-life phase of this study, from August 1998 through March 2000. Specimens received were frozen and on dry ice. Specimens were logged in with the 3M Lab and transferred to freezers for storage at either -55°C ±10–20°C or -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-030 were obtained from commercial sources and are presented in Attachment A (see Table A-1). Samples analyzed at the 3M Lab will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

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Dose Confirmation Analyses

Dose confirmation analyses were performed on lactose dose samples (1:39 and 1:499) collected on 8/11/98 during the in-life phase of the study: the results are presented in Attachment A (see Table A-2, A-3). The dose confirmation data were collected according to a method that was not fully validated.

Dose confirmation was performed by diluting the lactose dose samples (1:39 - 1,000x and 1:499 - 1,000x) with Milli-Q water, then extracted using the ion-pair procedure, diluted 1:50 and 1:5 respectively into the linear range of the instrument. For each sample (top, middle, bottom), a matrix spike was prepared (approximately 5000 µg/g and 400 µg/g) by spiking the dose solution and then diluting and extracting as described above. In all cases, samples were analyzed versus an unextracted curve using HPLC-ES/MS/MS. The instrumental parameters and analytical conditions described in ETS-8-5.1 were used for dose solution analyses. The average dose level measured was confirmed to be 99 ±27% of the target concentration. Matrix spikes were recovered at >60%..

MATERIALS AND METHODS

Chemical Characterization

Table 2 presents information regarding characterization of the test substance used in the in-life phase of this study, and the analytical reference substance used in the analytical phase of this study.

Table 2. Characterization of Test and Analytical Reference Substances in Study FACT TOX-030

	TEST SUBSTANCE	ANALYTICAL REFERENCE SUBSTANCES		
CHEMICAL NAME	**KPFOS** Potassium Perfluorooctanesulfonate	**KPFOS** Potassium Perfluorooctanesulfonate	**THPFOS** 1H,1H,2H,2H-perfluorooctanesulfonic acid	
SOURCE	3M Specialty Chemicals Div.	3M Specialty Chemicals Div.	ICN Biomedics, Inc.	
EXPIRATION DATE	8/31/2001	8/31/2001	1/01/2020	
STORAGE CONDITIONS	Frozen ≤-10°C	Frozen ≤-10°C	Ambient temperature	
CHEMICAL LOT NUMBER	217	171	59909	53406
PHYSICAL DESCRIPTION	FC-95, White crystalline powder	White crystalline powder	Brown powder	Brown waxy solid
PURITY	86.9%	86.4%	N/A	N/A

Reserve samples of the analytical reference substance will be stored at the 3M Lab for a period of 10 years, as will any reserve samples of test substance returned from the in-life phase of the study.

Method Summaries

Following is a brief description of the latest methods used during the analytical phase of this study by the 3M Lab. Detailed descriptions of the methods used in this analytical phase are located in Attachment C. As the present analytical phase of this study progressed, more advanced methods evolved and earlier methods were used with deviations until amendments to the protocol were written. Changes to the methods included the use of methyl-*tert*-butyl ether (MtBE) instead of ethyl acetate, curves plotted by linear regression weighted 1/x instead of unweighted curves, a reduction in the size of the analytical column from 100mm to 50mm, gradient changes, and faster HPLC cycle times. A summary of protocol and method deviations is presented in Attachment B (see Table B-1) of this report.

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Preparatory Methods:

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

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

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

  Sera samples were spiked with THPFOS and extracted using an ion-pairing extraction procedure. An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into MtBE. The MtBE extract was removed and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol and passed through a 0.2 µm nylon filter, using a 3 cm^3 disposable plastic syringe into glass autosampler vials.

Analytical Methods:

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

  The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC/ES/MS/MS. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3$-) analysis, was fragmented to produce ion 99 (FSO_3-). The characteristic 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 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 in water
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 10 minutes

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Start at 10%B
Hold at 10%B for 1.0 minute
Increase to 95%B over 4.5 minutes
Hold at 95%B for 2.0 minutes
Return to 10%B over 0.5 minutes
Hold at 10%B for 2.0 minutes

Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.2
Cone Voltage: 60V
Collision Gas Energy: 40–60eV
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 FACT TOX-030

TARGET ANALYTE	PRIMARY ION (amu)	PRODUCT ION (amu)
PFOS	499.0	99.0
THPFOS	427.0	80.0

Deviations

It should be noted that as the analytical phase of this study progressed, method parameters were evaluated to improve analyses. Earlier methods were used with deviations until amendments to the protocol were written. Deviations from the original protocol and methods are documented in the Attachment B (see Table B-1).

DATA QUALITY OBJECTIVES AND DATA INTEGRITY

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

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.98
- **Limits of Quantitation (LOQ):** The Method Detection Limit (MDL) for PFOS is 12 ppb for serum and 15 ppb for liver. The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Duplicate/Acceptable Precision:** Precision was reproducible to within 30%
- **Spike/Acceptable Recoveries:** 70–130%
- **Confirmatory Methods:** Indeterminate samples may be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol should be written.
- **Demonstration of Specificity:** Specificity to be demonstrated by chromatographic retention time and mass spectral daughter ion characterization.

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DATA SUMMARY, ANALYSES, AND RESULTS

Data quality objectives for the analytical phase of this study outlined in the 3M Lab protocol for FACT TOX-030 (see Attachment B) were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curve was ≥0.985.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis (unweighted – prior to 3/5/99, unweighted or 1/x weighted–3/5/99 to 3/19/99, and 1/x weighted–3/19/99 to end of the study) of two extracted matrix curves bracketing each group of samples, except as noted in the deviation summary. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier was deactivated. Quantitation of each analyte was based on the response of one specific product ion using the multiple response-monitoring mode of the instrument (see Attachment C).
- **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. This value does not exceed the validated LOQ of the method for data that is accepted (see Attachment D, Table D-6).

Table 4. Determination of PFOS LOQ in TOX-030 Analyses

ANALYTE-MATRIX	LOQ
PFOS-Sera	4.39–15.2 ng/mL
PFOS-Liver	26.9–60.1 ng/g

- **Blanks:** All blanks were below the lower limit of quantitation for the compounds of interest. To simplify analyses that were complicated by endogenous levels of fluorochemicals in unexposed monkey sera, rabbit sera was selected as a suitable surrogate matrix.
- **Duplicate/Acceptable Precision:** Precision was determined by analysis of MS/MSD and was reproducible to within 30%.
- **Matrix Spikes:** Matrix spikes and matrix spike duplicates were extracted with each set of samples and analyzed during analytical runs at the 3M Lab. All sera matrix spikes were within ±30% of the theoretical concentration. Matrix spikes prepared in liver were compliant within ±30%, with the exception of one spike that was prepared with Day-393 samples and had a low recovery. The matrix spike was reextracted and the recovery was within ±30% of the theoretical concentration.
- **Spike/Acceptable Recoveries:** Spike recoveries of ±30% of expected values were achieved for all matrix spikes prepared in sera. With one exception (noted earlier), matrix spikes prepared in liver were within ±30%.
- **Use of Surrogates:** The surrogate (THPFOS) was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. After 11/04/99, 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. No problems were observed with these data.

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Assuming spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitative to ±30%. The validity of this assumption has not been verified by other techniques.

Summary of Sample Results

- **Samples from Control Animals:** Low levels of PFOS were often detected in the sera and liver of the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, PFOS levels found in the sera and liver of the test animals increased with dose group. PFOS levels increased as dosage increased; significant differences between male and female PFOS levels were not observed in sera. However, Group 4 males had notably higher PFOS levels in liver samples than Group 4 females. Detailed sample data is presented in Attachments D and E.

STATISTICAL METHODS AND CALCULATIONS

Statistical methods were limited to the calculation of means and standard deviations. See Attachment F for example calculations used to generate the liver and serum sample data in TOX-030.

STATEMENT OF CONCLUSION

Under the conditions of the present analytical phase of this study, PFOS was detected in the sera and liver samples of Groups 2, 3, and 4 animals. The Control Group 1 animals showed minimal amounts of PFOS. PFOS levels increased as dosage increased; significant differences between male and female PFOS levels were not observed in sera. However, Group 4 (high dose) males had notably higher PFOS levels in liver samples than Group 4 females.

Data quality objectives for the analytical phase of this study outlined in the 3M Lab protocol for FACT TOX-030 (see Attachment B) were met with the exceptions noted in this report.

LIST OF ATTACHMENTS

- **Attachment A:** Control Matrix Characterization and Dose Confirmation Analyses
- **Attachment B:** Protocol and Deviation Summary
- **Attachment C:** Extraction and Analytical Methods
- **Attachment D:** Data Summary Tables
- **Attachment E:** Data Spreadsheets
- **Attachment F:** Example Calculations
- **Attachment G:** Interim Certificate of Analyses
- **Attachment H:** Report Signature Page

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ATTACHMENT A
CONTROL MATRIX CHARACTERIZATION AND DOSE CONFIRMATION ANALYSES

Table A-1. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT TOX-030

CONTROL MATRIX	RABBIT SERUM	RABBIT SERUM	MONKEY SERUM	MONKEY SERUM	MONKEY SERUM
Source	Sigma Chemicals	Sigma Chemicals	Lampire Biological	Sierra Biomedical	N/R
Expiration Date	01/01/2010	01/01/2010	N/R	01/01/2010	01/01/2010
Storage Conditions	Frozen -20°C	Frozen -20°C	Frozen -50°C	Frozen -20°C	Frozen -20°C
Chemical Lot #	118H8418	47H4641	111022515	#LY2N0	N/R
Physical Description	Rabbit Serum	Rabbit Serum	Monkey Serum	Monkey Serum	Monkey Serum
CONTROL MATRIX	**RABBIT LIVER**	**RABBIT LIVER**	**RABBIT LIVER**	**RABBIT LIVER**	**MONKEY LIVER**
Source	Corning Hazleton	N/R	Corning Hazleton	Corning Hazleton	Sierra Biomedical
Expiration Date	12/01/1999	12/01/1999	01/01/2010	01/01/2010	01/01/2010
Storage Conditions	Frozen -20°C	Frozen -20°C	Frozen -20°C	Frozen -20°C	Frozen -50°C
Chemical Lot #	F00007	N/R	F00005	F00009	N/R
Physical Description	Rabbit Liver	Rabbit Liver	Rabbit Liver	Rabbit Liver	Monkey Liver

N/R—not recorded

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Table A-2. Lactose Dose Verification (PFOS) for Study 6329-223—8/21/99

	Expected Conc. (ng/mL)	Measured Conc. (ng/mL)	%Rec. for ng/mL	Expected Calc. Conc. (µg/g)	Measured Calc. Conc. (µg/g)	Average	Std Deviation	%Rec. for µg/g
1:39 Dose (25000 ppm PFOS)								
Top	580	479	83%	25000	20647			83%
Middle	500	650	130%	25000	32537			130%
Bottom	500	422	84%	25000	21108	24764	6736 ±27%	84%
							average / std. deviation=	99% ±27%
1:499 Dose (2000 ppm PFOS)								
Top	410	305	74%	2000	1490			74%
Middle	404	287	71%	2000	1425			71%
Bottom	400	272	68%	2000	1361	1425	64.5 ±5%	68%
							average / std. deviation=	71% ±3%

Actual MS Concentration–Actual background concentration, divided by expected, times 100
(Spiked too low which accounts for the wide differences in recovery)

Table A-3. Lactose Dose Verification (PFOS—Matrix Spikes) for Study 6329-223—8/21/99

	Expected Conc. (ng/mL)	Actual Conc. (ng/mL)	%Rec. for ng/mL	Calculated Conc. (µg/g)	Expected Conc. (µg/g)	Actual Calc. Conc. (µg/g)	Average	Std Deviation	%Rec. for µg/g	Average
1:39 Dose (25000 ppm PFOS) MS										
Top	604	507	84%	21858	10.8	12.1			112%	
Middle	524	485	92%	24252	12.5	-82.8			-664*	
Bottom	524	438	83%	21889	12.5	7.82	22666	1374 ±6%	63%	87%
1:499 Dose (2000 ppm PFOS) MS										
Top	606	475	78%	2320	9.77	8.30			85%	
Middle	600	447	75%	2217	9.92	7.93			80%	
Bottom	596	440	74%	2202	10.0	8.41	2246	64.5 ±3%	84%	83%

* This value is an outlier and was not used in any calculations

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ATTACHMENT B
PROTOCOL AND DEVIATION SUMMARY

Table B-1. Deviation Summary for FACT TOX-030

DEVIATION	DATES OF OCCURRENCE	IMPACT ON STUDY
MtBE was used as an extraction solvent instead of ethyl acetate.	2/5/99, 2/9/99, 5/18/99, 6/11/99	No negative impact on the study—MtBE improved the absolute recoveries and shortened extraction time.
Pipette was used instead of Oxford dispenser.	10/14/99	No negative impact on the study.
Curves plotted by linear regression weighted 1/x rather than by linear regression as specified in the protocol.	2/13/99, 3/5/99, 3/12/99, 3/19/99, 3/20/99, 3/21/99, 3/23/99, 3/24/99, 3/25/99, 4/7/99, 4/11/99, 4/12/99, 4/17/99, 5/19/99, 5/22/99, 6/9/99, 6/14/99	No negative impact on the study—1/x weighted curves improved the precision and accuracy of analysis.
A second extracted matrix curve was not used to bracket samples.	3/5/99, 3/9/99, 3/15/99, 3/16/99, 5/19/99, 5/22/99, 10/26/99, 1/21/00, 3/24/00, 4/27/00	No negative impact on the study—The accuracy of calibration checks analyzed every five to ten samples was monitored to ensure continued accuracy of the analysis. The QC provided by the calibration checks is sufficient and the data quality will not be adversely affected.
Recorded extraction method FACT-M-1.0 rather than FACT-M-1.1.	6/11/99	No negative impact on the study—New method was followed, even though old method was recorded.
Followed extraction method ETS-8-6.0 rather than FACT-M-1.1.	10/14/99, 10/25/99, 1/19/00, 3/22/00	No negative impact on the study—New validated method provides improvements in precision and extraction time.
Followed analytical method ETS-8-7.0 rather than FACT-M-2.1.	7/29/99, 10/20/99, 10/22/99, 10/26/99, 10/27/99, 1/28/00, 3/24/00, 3/28/00	No negative impact on the study—New validated method provides improvements in precision, accuracy and analysis time.
Followed analytical method ETS-8-5.0 rather than FACT-M-4.1.	3/05/99, 3/08/99	No negative impact on the study—New validated method provides improvements in precision, accuracy and analysis time.
Samples extracted using 0.5 mL rather than 1.0 mL due to insufficient sample.	10/25/99	No negative impact on the study—Studies indicate that data quality is not jeopardized using 0.5 mL of sera.
Followed extraction method ETS-8-4.0 rather than FACT-M-3.1.	3/02/99, 3/03/99	No negative impact on the study—New validated method provides improvements in precision and extraction time.
Matrix spikes were not spiked with standard (Used as blanks).	11/3/99	Adequate QC was prepared with the sample set; unspiked samples pose no negative impact on the study.
Continuing calibration standards were not spiked with standard due to analyst error.	11/3/99	Mid-level curve standards were substituted as QC for the non-spiked calibration check standards; the unspiked calibration standards pose no negative impact to the study.
Samples extracted using <0.5 mL due to insufficient initial sample volume.	2/5/99, 2/9/99, 3/2/99, 3/3/99, 3/10/99, 3/12/99, 3/15/99, 3/16/99, 4/6/99, 4/8/99, 8/25/99, 11/3/99, 4/21/00	Studies indicate that data accuracy and precision may be affected when sera samples less than 0.5 mL were extracted. Data reported from extraction of samples less than 0.5 mL is noted in the data tables.

3M Environmental Technology and Services
PO Box 33331
St. Paul, MN 55133-3331
612 778 6442

3M

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

PROTOCOL

Author
Lisa Clemen

Date:
January 25, 1999

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

Laboratory Project Identification
FACT-TOX-030

Study Identification

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Andrew Seacat, Ph.D. 3M Toxicology Services Telephone: 612-575-3161 Facsimile: 612-733-1773
Study Director	Kristen Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Covance Laboratories, Inc. 3301 Kinsman Boulevard Madison, Wisconsin 53704
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106
Proposed Study Timetable	
Study Initiation Date	January 25, 1999
Study Completion Date	January 25, 2000

1. STUDY

Twenty-six week capsule toxicity study with potassium perfluorooctane sulfonic acid (T-6295) in cynomolgus monkeys.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in the liver and serum of cynomolgus monkeys. Additional tissues or fluids may be analyzed. The in-life portion of this study was conducted at Covance Laboratories, study #6329-223.

3. REGULATORY COMPLIANCE

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

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit will review the protocol and audit study conduct, data, and final report to determine compliance with Good Laboratory Practice Standards and with 3M Environmental Laboratory Standard Operating Procedures.

5. TEST MATERIAL

5.1 Refer to Covance Laboratory protocol for study #6329-223.

6. CONTROL MATRICES

6.1 *Identification* Monkey liver and serum and/or rabbit liver and serum, traceability numbers will be recorded in the raw data and included in the final report

6.2 *Source* Covance Research and/or Sigma Chemical

6.3 *Physical Description* Monkey liver and serum and/or rabbit liver and serum

6.4 *Purity and Stability* Not applicable

6.5 *Storage Conditions* Frozen at -20 °C ± 10 °C or –55 °C ± 10 °C

6.6 *Reserve Matrix* A portion of the control matrix will be retained in the archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 *Disposition* Matrices will be retained per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

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

7. *Reference Material*

7.1 *Identification* Potassium perfluorooctanesulfonate (PFOS), lot #s 171, 215, or 217 (equivalent lots)

7.2 *Source* 3M Specialty Chemicals

7.3 *Physical Description* White powder

7.4 *Purity and Stability* Responsibility of the Sponsor

7.5 *Storage Conditions* Room temperature

7.6 *Reserve Material* A reserve sample from each batch of PFOS used in this study will be retained as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

7.7 *Disposition* Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.

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

8. *Test System*

Cynomolgus monkeys were used as the test system, and were maintained and dosed as described in Covance protocol #6329-223. Group 1 control animals did not receive the test substance. Groups 2, 3, and 4 received the test substance daily for 26 weeks, at concentrations of 0.02, 0.5, and 2.0 mg/kg/day, respectively. Refer to Covance protocol #6329-223 for tabular presentation of data. Two animals each from Groups 1, 3, and 4 were designated as recovery animals and were allowed at least a 13 week, which may be extended, recovery period after cessation of treatment.

9. *Specimen and Sample Receipt*

The 3M Environmental Laboratory will receive homogeneity samples for dose analysis and specimens of the following body tissues and fluids from the indicated points in the study. All specimens will be packed on dry ice for shipping.

Body tissue/fluid	Collected	Expected # of specimens
Serum – all animals	7 days prior to treatment 7 days post treatment every two weeks during treatment and recovery	616 from main study 24 additional from recovery
Urine and feces – recovery animals	Day zero of recovery 6, 30, and 90 (with a potential 180) days of recovery	24 urine 24 feces
Liver – all animals	After termination of the study	44

Total number of test animals: 32
Total number of control animals: 12

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

10. Preparatory Methods

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

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

10.3 If preparatory methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

11. Analytical Methods

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

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

11.3 If analytical methods other than those listed above are used, an amendment to this protocol will be written. Any deviations from these methods will be documented and included with the study data.

12. Data Quality Objectives

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

12.1 Linearity $r^2 \geq 0.98$

12.2 Limits of detection / quantitation

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 12 ppb

b) Liver: 15 ppb

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

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

12.5 Use of confirmatory methods Indeterminate samples will be re-analyzed using a confirmatory method. If a confirmatory method is used, an amendment to this protocol will be written.

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

13. Sub-Contracted Analysis

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

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than the 3M Environmental Laboratory.

14. Statistical Analysis

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

14.1 Data transformations and analysis Data will be reported as the concentration (weight/weight or weight/vol) of PFOS or metabolite per tissue or fluid.

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

15. REPORT

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

15.1 Name and address of the facility performing the study

15.2 Dates upon which the study was initiated and completed

15.3 A statement of compliance by the Study Director addressing any exceptions to Good Laboratory Practice Standards

15.4 Objectives and procedures as stated in the approved protocol, including any changes in the original protocol

15.5 The test substance identification by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics, if provided by the Sponsor

15.6 Stability and the solubility of the test substances under the conditions of administration, if provided by the Sponsor

15.7 A description of the methods used to conduct the test(s)

15.8 A description of the test system

15.9 A description of any circumstances that may have affected the quality or the integrity of the data

15.10 The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study

15.11 A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the analytical chemistry data, and a statement of the conclusions drawn from the analyses

15.12 Statistical methods used to evaluate the data, if applicable

15.13 The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable

15.14 The location where raw data and the final report are to be stored

15.15 A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management

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

16. Location of Raw Data, Records, and Final Report

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

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

- ***16.1.1*** Approved protocol and amendments
- ***16.1.2*** Study correspondence
- ***16.1.3*** Shipping records
- ***16.1.4*** Raw data
- ***16.1.5*** Approved final report (original signed copy)
- ***16.1.6*** Electronic copies of data

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

- ***16.2.1*** Training records
- ***16.2.2*** Calibration records
- ***16.2.3*** Instrument maintenance logs
- ***16.2.4*** Standard Operating Procedures, Equipment Procedures, and Methods

17. Specimen Retention

Specimens will be maintained in the laboratory specimen archives for a period of time as specified by regulation or as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

18. Protocol Amendments and Deviations

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

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Andrew M. Seacat 1/28/98

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

Kristen Hansen 1/29/98

Kristen Hansen, Ph.D., 3M Environmental Laboratory Study Director — Date

GLP Study

Protocol Amendment

Study Number: FACT- TOX-030 Covance 6329-223

Study Title: 26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys

Study Director: Kris Hansen

Amendment Date: 03/09/99 Amendment Number: 1

This amendment modifies the following portion of the protocol:
Section 10 Preparatory Methods and Section 11 Analytical Methods

These sections list FACT-m-3.1 and FACT-m-4.1 as the serum extraction and analytical methods. The methods have been updated to ETS-8-4.0 and ETS-8-5.0. These updated methods will be used for the remaining serum extractions and analyses.

Approved by:

Kris Hansen 4/5/99
Study Director Date

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS, T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 2

Amendment Date:

April 29, 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-030
LIRN U2279

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

1. ***PROTOCOL READS:*** In amendment 1, section 10 Preparatory Methods and Section 11 Analytical Methods were updated to ETS-8-4.0 and ETS-8-5.0 as the revised serum extraction and analytical methods.

AMEND TO READ: These methods were revised on 4/~~29~~27/99 (RE) UC 7/08/99 to ETS-8-4.1 and ETS-8-5.1 which will be used for all future analyses.

REASON: The methods were revised for clarification and to include linear regression, 1/x weighting for initial curves.

Amendment Approval

Andrew M. Seacat — 7/30/99

Andrew Seacat Ph.D., Sponsor Representative — Date

Kris J. Hansen — 7/7/99

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

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (PFOS, T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 3

Amendment Date:

June 03, 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-030
LIRN U2279

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

1. ***PROTOCOL READS:*** Section 10 Preparatory Methods and Section 11 Analytical Methods list FACT-M-1.0 and FACT-M-2.0 as the liver extraction and analytical methods.

   AMEND TO READ: These methods were revised on 06/03/99 to FACT-M-1.1 and FACT-M-2.1 which will be used for all future analyses.

   REASON: The methods were revised for clarification and to expand on the list of target analytes.

Amendment Approval

Andrew Seacat 7/30/99

Andrew Seacat Ph.D., Sponsor Representative — Date

Kris Hansen 7/7/99

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

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 4

Amendment Date:
April 25, 2000

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

Laboratory Project Identification
FACT Tox-030
ET&SS LRN-U2279

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

1. ***PROTOCOL READS:***
The sponsor for the present study was identified as Andrew Seacat, Ph.D.
AMEND TO READ:
The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D. as of the date of signature approval of this protocol amendment.
REASON:
To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

2. ***PROTOCOL READS:***
On page 2 of the protocol, Kris Hansen is identified as the study director for the analytical phase of the study. Peter Thomford is also identified as a study director, but for the in-life phase of the study (see Covance Laboratories Protocol 6329-223).

AMEND TO READ:
On page 2 of the protocol, Andrew Seacat will be identified as the study director, Kris Hansen will perform the duties of the principal analytical investigator, and Peter Thomford will be identified in the final report as the principal in-life investigator as of the date of signature approval of this protocol amendment.

REASON:
The original study design identified two study directors; one for the in-life phase of the study and one for the analytical phase of the study. The role of study director has been reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements.

3. ***PROTOCOL READS:***
10. Preparatory Methods:
10.1 **FACT-M-1.1**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
AMEND TO READ:
10. Preparatory Methods:
10.1 **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
REASON:
The method was revised to include extractions of other tissue types and the use of methyl-*tert*-butyl ether (MtBE) instead of ethyl acetate in the extraction process.

4. ***PROTOCOL READS:***
11. Analytical Methods:

11.1 **FACT-M-2.1**, "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ:

11. Analytical Methods:

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

REASON:

The method was revised to include curves plotted by linear regression weighted 1/x and a reduced cycle time from 13.5 minutes to 9.0 minutes.

5. ***PROTOCOL READS:***

8. Test System

Refer to the Covance protocol #6329-223 for tabular presentation of data. Two animals each from Groups 1, 3, and 4 were designated as recovery animals and were allowed at least a 13 week, which may be extended, recovery period after cessation of treatment.

AMEND TO READ:

8. Test System

A tabular presentation of the test system data will be included in the final report for this project (FACT Tox-030). Four animals each (two/gender) from Groups 1, 3, and 4 were designated as recovery animals and were observed for a recovery period after cessation of treatment until study cut-off on March 7, 2000 (Week 80). The observation and analysis of the recovery group III animals beyond the cut-off date of this study will be reported in a new long-term recovery study.

REASON:

Additional animals were assigned to the recovery group following approval of the protocol for FACT Tox-030. A new study will report the long-term recovery of the remaining animals from recovery group III (mid-dose group).

6. ***PROTOCOL READS:***

9. Specimen and Sample Receipt: [Sample Receipt Table]

Expected # of Specimens:

Serum–all animals: 616 from main study, 24 additional from recovery
Urine and feces–recovery animals: 24 urine, 24 feces
Liver–all animals: 44

Collected:

Urine and feces–recovery animals: Day 0, 6, 30, 90 (potential 180)

Total number of test animals: 32

AMEND TO READ:

9. **Specimen and Sample Receipt:** [Sample Receipt Table]
   # of Specimens:
   Serum–all animals: 516 from main study, 280 additional specimens from recovery
   Liver–all animals: 42 (eight specimens via biopsy from recovery group)
   Total number of test animals: 32
   Total number of animals: 48 (12 animals in control group)
   Note: 4 animals were not assigned to a study group. Day 0 serum specimens were collected. Specimens of body tissues and fluids other than serum and liver specimens will be collected and received from Covance Laboratories (6329-223, Study T-6295.7).

REASON:

Additional animals were assigned to the recovery group following approval of the protocol for FACT Tox-030. The recovery period has been extended for the recovery group. Specimen collection figures shown are through the end of the study 3/07/00.

Amendment Approval

John L. Butenhoff — 4/28/2000

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

Andrew M. Seacat — 4/27/2000

Andrew Seacat, Ph.D., Incoming Study Director — Date

Kristen J. Hansen — May-2-2000

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

Dale L. Bacon — 5/4/2000

Dale L. Bacon, Laboratory Manager — Date

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 5

Amendment Date:

August 14, 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX-030
Covance #6329-223
LIMS #U2279

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

1. ***PROTOCOL READS: 2. PURPOSE:*** This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in the liver and serum of cynomolgus monkeys. Additional tissues or fluids may be analyzed.

   AMEND TO READ: Add that select feces samples will be analyzed for PFOS at Centre.

   REASON: Feces samples were added after the original protocol was written.

2. ***PROTOCOL READS: STUDY LOCATIONS(PAGE 2):*** Analytical Testing Laboratory: 3M Environmental Laboratory

   AMEND TO READ: 3M Environmental Laboratory ***and*** Centre Analytical Laboratories Inc. (Centre), 3048 Research Drive, State College, PA 16801

   REASON: Analyses of feces are assigned to Centre, in addition to the analyses of other tissues at the 3M Environmental Laboratory.

3. ***PROTOCOL READS: SECTIONS 10. PREPARATORY METHODS, 11. ANALYTICAL METHODS AND 12. DATA QUALITY OBJECTIVES***

   AMEND TO READ: Add to these sections the most current version of "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS," Centre method number 00M-023-003, with an LOQ in feces of 10 ng/g. The method will incorporate an initial homogenization step immediately after adding the extraction solvent, using a micro-homogenizer, to allow for complete dispersion of the specimen in the solvent.

   REASON: To specify the validated method to be used for feces analyses along with its analytical limits. NOTE: LC/MS/MS is an abbreviation for "liquid chromatography/mass spectrometry/mass spectrometry."

4. ***PROTOCOL READS: SECTION 10. PREPARATORY METHODS AND 11. 3M ANALYTICAL METHODS***

   AMEND TO READ: Change to specify that the most current version of the methods listed should be used.

   REASON: To specify that the most appropriate version of the preparatory and analytical methods should be used during the course of the study.

5. ***PROTOCOL READS:*** The amended protocol (Amendment #4) states that Kris J. Hansen, Ph.D. is the Principal Analytical Investigator for the entire study.

   AMEND TO READ: Add Enaksha Wickremesinhe, Ph.D. as the Principal ~~In-life~~ Analytical Investigator at Centre for feces analyses.

   REASON: To specify the PAI at Centre for feces analysis.

6. ***PROTOCOL READS: SECTION 16. LOCATION OF RAW DATA, RECORDS AND FINAL REPORTS***

   AMEND TO READ: Add that Centre will forward all original study-specific raw data to 3M Environmental Laboratories, together with copies of appropriate facility-specific raw data applicable to this study. Centre will maintain a copy of the applicable study-specific raw data, protocol and analytical report in the Centre archives, as well as all original facility records.

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

7. ***PROTOCOL READS: SECTION 17. SPECIMEN RETENTION***

   AMEND TO READ: Add that after the analytical report on feces is signed by the study director, all feces specimens of this study will be returned to 3M Environmental Laboratory. These specimens may then be discarded by written direction of the study director. Specimens of serum and urine may also be discarded by written direction of the study director after the analytical report for the 3M Environmental Laboratory analyses is signed by the study director.

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

Protocol TOX-030
Amendment 5

Amendment Approval

John L. Butenhoff, Ph.D., Sponsor's Representative — Date: 9/14/2000

Andrew Seacat, Ph.D., Study Director — Date: 9/1/00

Enaksha Wickremesinhe, Ph.D., Principal In-life Investigator — Date: 9/15/00

Study Title

26-Week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

PROTOCOL AMENDMENT NO. 6

Amendment Date:

September 11, 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT TOX 030
Covance #6329-223
LIMS #U2279

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

1. ***PROTOCOL READS:***
8. TEST SYSTEM: Groups 2, 3, and 4 received the test substance daily for 26 weeks, at concentrations of 0.02, 0.5, and 2.0 mg/kg/day, respectively.

AMEND TO READ:
Groups 2, 3, and 4 received the test substance daily for 26 weeks, at concentrations of 0.03, 0.15, and 0.75 mg/kg/day.

REASON:
Test substance doses were changed after protocol was written. The Covance protocol reflects the actual doses of test substance that were given.

PROTOCOL READS:
The amended protocol (Amendment Number 4, section 2.) states: On page 2 of the protocol, Andrew Seacat will be identified as the study director, Kris Hansen will perform the duties of the principal analytical investigator, and Peter Thomford will be identified in the final report as the principal in-life investigator as of the date of signature approval of this protocol amendment.
AMEND TO READ:
Peter Thomford will remain as the Covance Study Director for the purpose of issuing the Covance final report for the in-life phase of the study.
REASON:
Study directorship was not relinquished by Covance for the in-life phase of the study, since the animal experimental phase was completed before Amendment Number 4 was issued.

Amendment Approval

John T. Butenhoff 9/14/00

John L. Butenhoff, PH.D., Sponsor Representative — Date

Andrew M. Seacat 9/14/00

Andrew Seacat, Ph.D., Study Director — Date

Study Title

26-week Capsule Toxicity Study with Perfluorooctane Sulfonic Acid Potassium Salt (T-6295) in Cynomolgus Monkeys

Protocol Amendment No. 7

Amendment Date:

September 14, 2000

Performing Laboratory

3M Environmental Technology and Safety Services
3M Environmental Laboratory
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Laboratory Project Identification

ET&SS FACT-TOX-030
Covance #6329-223
LIMS #U2279

Protocol TOX-030
Amendment 7

Amendment Approval

John L. Butenhoff, Ph.D., Sponsor's Representative — Date 9/15/00

Andrew Seacat, Ph.D., Study Director — Date 9/14/00

3M Medical Department Study: T-6295.7
Report No. FACT TOX-030
Laboratory Request Number-U2279

ATTACHMENT C
EXTRACTION AND ANALYTICAL METHODS

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

Blank	Liver Homogenate: Std #	Liver amount = g
Liver Extraction Method		**Date & Initials**
Spike surrogate and Standard mix, Vortex 15 sec.		
Pipette 1 mL of Liver Solution		
Pipette 1 mL of †0.5 M TBA, pH 10. pH =	Std. #	
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer	Std. #	
Dispense 5ml of Methyl-t-Butyl Ether	TN-A-	
Shake 20 min.	Shaker Speed	
Centrifuge 20-25 min.	Centrifuge Speed	
Remove a 4 mL aliquot of organic layer		
Put on Nitrogen Evaporator to dryness	Evaporator Temperature	
Add 1.0 mL of Methanol	TN-A-	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into autosample vial		

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

MDL/LOQ values for rabbit liver

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

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

Refer to LOQ Summary and MDL study in ETS-8-6.0 & 7.0-V-1 for further information
* EtFOSE-OH estimates only for MDL and LOQ. Did not meet criteria for validation.

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

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

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-7.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — 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):

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

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

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:

D. T. Lin — 4/27/99
Laboratory Manager — Date

Victor Ho — 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 Approximate spiking amounts for standards and spikes Using 1.0 mL of matrix		
Working standard (approx. conc.)	µL	Approx. final conc. of analyte in matrix
- -	-	Blank
0.500 ppm	10	0.005 ppm
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm
50.0 ppm	20	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw at room temperature or in a lukewarm waterbath.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube.

12.3 Return unused samples to freezer after extraction amounts have been removed.

12.4 Record the initial volume on the extraction worksheet.

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

12.6 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2**.

12.7 Spike each matrix with the appropriate amount of standard as described in **11.1**, or **Table 1** in that section, for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

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

12.9 Check to ensure the 0.5 M TBA reagent is at pH 10. If not, adjust accordingly.

12.10 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.11 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

12.12 Cap each sample and put on the shaker at a setting of 300 rpm, for 20 minutes.

12.13 Centrifuge for 20 to 25 minutes at a setting of 3500 rpm, or until layers are well separated.

12.14 Label a fresh 15 mL centrifuge tube with the same information as in **12.5**.

12.15 Remove 4.0 mL of the organic layer to this clean 15 mL centrifuge tube.

12.16 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

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

12.18 Vortex mix for 30 seconds.

12.19 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.20 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.21 Cap and store extracts at room temperature or at approximately 4 °C until analysis.

12.22 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

14.3 Refer to section 14 of ETS-8-5.1 for method performance criteria.

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1.**

18.2 FACT-M-3.1, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

Blank	Std #	amount =	mL
Serum Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix	Volume	mL	
Pipette 1 mL of 0.5 M TBA, pH 10. pH =	Std. #		
Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #		
Dispense 5 mL of methyl-t-butyl ether	TN-A-		
Shake 20 min.	Shaker speed:		
Centrifuge 20-25 min.	Centrifuge speed:		
Remove a 4 mL aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Temperature:		
Add methanol	Volume mL	TN-A-	
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2µm filter into a 1.5 mL autosample vial			

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

MDL/LOQ values for rabbit serum

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Fluids

Prep date(s):
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

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

Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager — Date: 4/26/99

Group Leader — Date: 4/26/99

Technical Reviewer — Date: 04/26/99

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 ort the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode, flow rate 10 – 500 µL/min

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

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **ETS-8-4.1, Attachment B,** for a listing of current validated MDL and LOQ values.

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

14.4.1 Matrix spike percent recoveries are must be within ± 30% of the spiked concentration.

14.5 Continuing Calibration Verifications

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

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

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

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

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: ETS-8-5.1 Data summary spreadsheet.

18.0 References

18.1 FACT-M-4.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

19.0 Affected Documents

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

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. Section 11.1 Average of two curves, not standard values, are used for plotting linear regression and added the 1/x weighting of the curve. Section 12.2.2.4 Clarification of solvent ramp. Section 17.1 Changed from attachment B to A.	04/02/99

Laboratory Study

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

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-4.0

Adoption Date: 3/1/99

Revision Date:

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager	3/1/99 Date
Group Leader	3/1/99 Date
Technical Reviewer	3/1/99 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 using an ion pairing reagent and 5.0 mL of 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.

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

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.

10.0 Quality Control

10.1 Method blanks and matrix blanks

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

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

10.3.2 Prepare one 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 fall within the mid-range of the initial calibration curve. Additional spikes may be included that fall in the low-range of the initial

calibration curve. This is necessary if the analyst must quantitate using only the low end of the calibration curve (for example, 5 ppb – 100 ppb, rather than 5 ppb – 1000 ppb).

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract less than 0.50 mL of matrix. Record each sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 ml centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards, two matrix blanks, and two method blanks.

11.1.5 Refer to validation report **ETS-8-4.0 & ETS-8-5.0-V-1,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or continuing check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb - 1000 ppb.

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

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

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw.

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 samples to freezer after extraction amount has been removed.

12.4 Record the 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 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25M sodium carbonate/sodium bicarbonate buffer.

12.10 Using an Oxford Dispenser, add 5 mL methyl-*tert*-butyl ether.

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

12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.

12.13 Remove 4.0 mL of the organic layer to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in **12.5**.

12.14 Put each sample on the analytical nitrogen evaporator until dry, approximately 1 to 2 hours.

12.15 Add 1.0 mL or other appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume to add equals the initial volume of sample used for the extraction.

Note: If the initial volume is less than 0.500 mL the final methanol volume will equal 1.0 mL.

12.16 Vortex mix for 30 seconds.

12.17 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.19 Cap and store extracts at approximately 4 °C until analysis.

12.20 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks.

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction.

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

16.1 Complete the extraction worksheet attached to this method, and tape 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.**

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-5.0, "Analysis of Serum or Other Fluid Extracts for Fluorochemicals using HPLC-Electrospray Mass Spectrometry"

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date

Extraction Worksheet ETS-8-4.0

Study #/matrix	Surrogate Std approx. ppm actual ppm #	FC-Mix approx. 0.5 ppm actual ppm #	FC-Mix approx. 5 ppm actual ppm #	FC-Mix approx. 50 ppm actual ppm #	Date and Initials for Std. or Comments
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[1] Study number where the original worksheet is located.

Blank	Std #	amount =	mL
Serum Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix	Volume	ml	
Pipette 1 ml of 0.5 M TBA, pH 10.	Std. #		
Pipette 2 ml of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #		
Dispense 5 ml of methyl-t-butyl ether	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 filter into a 1.5 ml autosample vial			

MS/MSD/___ Cont. Checks: Spiked _____ uL of a _____ ppm std (_____) for a final concentration of _____ ppm. MS/MSD used sample _____. Cont. Checks used same matrix as for std curve.
Surrogate Standard: Spiked _____ uL of a _____ ppm std (_____) to all samples, standards, and blanks

MDL/LOQ values for rabbit serum

Compound	MDL (ppb)	LOQ (ppb)	Linear Calibration Range (LCR) Approximate concentrations to be used for preparing the Standard Calibration Curve
PFOS	4.76	15.2	5 ppb - 1000 ppb
PFOSA	2.89	9.19	5 ppb - 1000 ppb
PFOSAA	2.94	9.33	5 ppb - 1000 ppb
EtFOSE-OH	13.2	41.9	5 ppb - 1000 ppb
M556	12.6	40.2	5 ppb - 1000 ppb
PFOSEA	11.1	35.4	5 ppb - 1000 ppb

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

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

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

Compound	Matrix	MDL	LOQ	Low std	High std
PFOS	All	4.76	15.2	25.1	1002
PFOSA	All	2.89	9.19	25.0	1000
PFOSAA	All	2.94	9.33	25.0	998
EtFOSE-OH*	All	13.2*	41.9*	50.0	1000
PFOSEA	All	12.6	40.2	25.0	1000
M556	All	11.1	35.4	25.0	1000

* MDL and LOQ are estimates only for EtFOSE-OH.

Compound: PFOS

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 - 1002	1.00 - 1002	25.1 - 1002	5.01 - 250	5.01 - 250	100 - 1002	100 - 1002

Compound: PFOSA

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 - 1000	1.00 - 1000	10.0 - 1000	5.00 - 100	5.00 - 100	25.0 - 1000	25.0 - 1000

Compound: PFOSAA

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 – 998	1.00 - 998	25.0 - 998	4.99 - 250	9.98 - 250	49.9 - 998	99.8 - 998

Compound: EtFOSE-OH

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 - 1000	1.00 - 1000	50.0 - 1000	5.00 - 100	10.0 - 100	50.0 - 1000	100 - 1000

Compound: PFOSEA

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 - 1000	1.00 - 1000	25.0 - 1000	5.00 - 250	5.00 - 250	50 - 1000	50 - 1000

Compound: M556

Serum matrix	Prepared range of standards (ppb) (ng/mL)	Range of average curve (ppb) (ng/mL)	LCR from ave curve (ppb) (ng/mL)	Range of low std curve (ppb) (ng/mL)	LCR from low std curve (ppb) (ng/mL)	Range of high std curve (ppb) (ng/mL)	LCR from high std curve (ppb) (ng/mL)
Rabbit	1.00 - 1000	1.00 - 1000	25.0 - 1000	5.00 - 100	5.00 - 100	100 - 1000	100 - 1000

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	25.1-1002	25.0-1000	25.0-998	50.0-1000	25.0-1000	25.0-1000
Bovine	Estimates only. Use values for rabbit.					
Rat	Estimates only. Use values for rabbit.					
Monkey	Estimates only. Use values for rabbit.					
Human	Estimates only. Use values for rabbit.					

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-5.0

Adoption Date: 3/1/99

Revision Date:

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager — Date: 3/1/99

Group Leader — Date: 3/1/99

Technical Reviewer — Date: 3/1/99

1.0 SCOPE AND APPLICATION

1.1 Scope: This method is for the analysis of extracts from serum 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, using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, m/z= 499. Samples may also be analyzed using an API-ES/MS/MS system to further verify compound identification.

3.0 Definitions

3.1 **Atmospheric Pressure Ionization (API):** The Micromass Quattro II systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II 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 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 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 MassLynx or MassLynx NT USER'S GUIDE).

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

4.1.1 Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.

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

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 Interferences

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

6.0 Equipment

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

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler

7.0 Supplies and Materials

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi (House air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 Reagents and Standards

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water 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-8-4.0**.

9.0 Sample Handling

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C until analysis can be performed.

10.0 Quality Control

10.1 Method Blanks and Matrix Blanks

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

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

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

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

10.3 Continuing Calibration Checks

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

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

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression (r^2)for the calibration curve using MassLynx or other suitable software.

11.2 The r^2 value for the data should be 0.980 or greater. Lower values may be acceptable at the discretion of the analyst and approval of the Project Lead.

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

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

12.0 Procedures

12.1 Acquisition Set up

12.1.1 Click on start button in the Acquisition Control Panel. Set up a sample list. Assign a filename using 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 with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 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.

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode flow rate 10 – 500 µL/min

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

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Record tune parameters in the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button. 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-8-4.0, Attachment B,** for a listing of current validated MDL and LOQ values.

14.2 Method Blanks and Matrix Blanks

14.2.1 Method blanks and matrix blanks will be analyzed with each sample set for possible contamination or carryover. Values are expected to fall below the lowest standard in the calibration curve.

14.3 Matrix Spikes

14.3.1 Matrix spikes are analyzed with each sample set and the percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.4 Continuing Calibration Checks

14.4.1 Continuing calibration checks are analyzed at a minimum of after every 10 samples with each sample set. The percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.5 If any criteria listed in the method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. All actions will be documented in the instrument runlog, the maintenance log, or on the summary sheet with the sample results.

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and store in appropriate study folder.

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment B: ETS-8-5.0 Data reporting spreadsheet.

17.2 The validation report associated with this method is **ETS-8-4.0 & 5.0-V-1**.

18.0 REFERENCES

18.1 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II Systems"

19.0 AFFECTED DOCUMENTS

19.1 ETS-8-4.0, "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

Attachment A

Laboratory Study

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

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M Environmental Laboratory

Method

Extraction of Potassium Perfluorooctanesulfonate or Other 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:

Laboratory Manager — 5/26/98 Date

Group Leader — 5/26/98 Date

Technical Reviewer — 5/27/98 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	-	-	-	
-					
-	-				
-					
-					
-					
-					
-					
-					
[1]	-	-	-	-	
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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 laver	
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.

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 J'Jm — 5/26/98
Laboratory Manager — Date

Kirsten Hoz — 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

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-1.1

Adoption Date: 05/26/98

Revision Date: 06/03/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 6/3/99

Group Leader — Date: 6/1/99

Technical Reviewer — Date: 6/15/99

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 liver or other liver as designated in the validation report.

2.0 Summary of Method

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

3.0 Definitions

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 POAA: perfluorooctanoate (anion of ammonium salt) $C_7F_{15}COO^-$

3.6 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.7 FC-807 monoester $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O\text{-}PO_3H)$

3.8 Surrogate standard 1H,1H,2H,2H perfluorooctane sulfonic acid

4.0 Warnings and Cautions

4.1 **Health and safety warnings:**

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

5.0 Interferences

5.1 There are no known interferences at this time.

6.0 Equipment

6.1 The following equipment is used while carrying out this method. Equivalent equipment is acceptable.

6.1.1 Ultra-Turrax with T25 grinder attachment for grinding/dispersing/emulsifying

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen evaporator, Organomation

6.1.6 Balance, (± 0.100 g)

7.0 Supplies and Materials

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

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

8.0 Reagents and Standards

8.1 ASTM Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system.
8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent
8.3 Tetrabutylammonium hydrogen sulfate (TBA), Kodak or equivalent
8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent
8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent
8.6 Ethyl acetate, Omnisolv, glass distilled or HPLC grade
8.7 Methanol, Omnisolv, glass distilled or HPLC grade
8.8 Liver tissue, frozen from supplier
8.9 Control matrix or blank matrix for standards, QC checks, blanks, etc.
8.10 **Fluorochemical standards**
8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538
8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499
8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 571

8.10.5 POAA (3M Specialty Chemical Division), molecular weight = 431

8.10.6 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.10.7 FC-807 monoester (3M Specialty Chemical Division). FC-807 is a mixture of triester, diester, and monoester fluorochemical components. The monoester molecular weight = 650

8.10.8 Surrogate Standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H,2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.10.9 Other fluorochemicals, as appropriate

8.11 Reagent preparation

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200g NaOH. Pour into a 1000 ml beaker containing 500 ml Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10N NaOH 1:10. Measure 10 ml of 10N NaOH solution into a 100 ml volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 ml Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 grams of TBA into a 1 L volumetric containing 500 ml Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 ml of 10N NaOH and dilute to volume with Milli-Q™ water. While adding the last few ml's of NaOH, add slowly because the pH changes abruptly. Store in a 1 L Nalgene bottle.

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

8.11.4 0.25M Sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

8.12 Standards

8.12.1 Prepare PFOS standards for the standard curve.

8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (e.g. one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.12.3 Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight.

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/ml).

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

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

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

8.13 Surrogate stock standard preparation

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

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

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

8.14 Liver homogenate preparation

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

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

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

8.14.3

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

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

9.0 Sample Handling

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

10.0 Quality Control

10.1 Matrix blanks and method blanks

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 Calibration and Standardization

11.1 Prepare liver homogenate standards

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

11.1.2 If the volumes of sample liver homogenates are limited, extract standards with liver homogenate volumes equal to the sample volumes. Do not extract below 0.50 ml of liver homogenate. Record the sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots of liver homogenate in 15 ml centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 ml, or appropriate aliquots, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table I (at the end of this section) to spike, in duplicate, two standard curves, for a total of eighteen standards and two matrix blanks.

11.1.5 Refer to the validation reports **FACT-M-1.1-V-1** and **FACT-M-2.1-V-1** which list the working ranges and Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section 13.0 to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or QC check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 10 ppb – 1000 ppb.

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

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

Working Standard (Approx. Conc.)	µL	Approx. final conc. of PFOS in liver
-	-	Blank
0.500 ppm	4	0.012 ppm
0.500 ppm	10	0.030 ppm
0.500 ppm	20	0.060 ppm
0.500 ppm	40	0.120 ppm
5.00 ppm	10	0.300 ppm
5.00 ppm	20	0.600 ppm
5.00 ppm	30	0.900 ppm
50.0 ppm	4	1.20 ppm
50.0 ppm	6	1.80 ppm

12.0 PROCEDURES

12.1 Obtain frozen liver samples and homogenize as described in **8.14.3**.

12.2 Vortex mix homogenate for 15 seconds, then transfer 1.0 ml or other appropriate volume to a 15 ml polypropylene centrifuge tube.

12.3 Return liver homogenate samples to freezer after extraction amount has been removed.

12.4 Record the liver homogenate volume on the extraction worksheet. The final methanol volume will equal the initial homogenate volume. For example, if 1 ml of homogenate is transferred for extraction, then the final reconstitution methanol volume equals 1 ml.

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

12.6 Spike blank liver homogenate aliquots with the appropriate amount of standard as described in Section **11.1** or **Table I** in that section for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.7 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in Section **11.2**.

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

12.9 To each sample, add 1 ml 0.5 M TBA and 2 ml of the 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.10 Using a volumetric pipette, add 5 ml ethyl acetate.

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

12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.

12.13 Transfer 4 ml of organic layer, using a 5 ml graduated glass pipette, to a clean 15 ml centrifuge tube. Label this fresh tube with the same information as in **12.5**.

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

12.15 Add 1.0 ml or appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume equals the initial volume of liver homogenate used for the extraction.

12.16 Vortex mix for 30 seconds.

12.17 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 ml glass autovial (or low-volume autovial when necessary).

12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) who performed the extraction.

12.19 Cap and store extracts at approximately 4° C until analysis.

12.20 Complete the extraction worksheet, attached to this document, and tape to page of study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

Final Concentration (µg/g) of PFOS in Liver

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

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to evaluate the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve.

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

17.0 Attachments

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values

17.3 Attachment C, LOQ summary

17.4 Attachment D, Calibration standard concentration worksheet

18.0 References

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

19.0 Affected Documents

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

20.0 REVISIONS

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

Study#____ Matrix____ Box#____ Analyst/Date	Surrogate Std. approx. ppm actual ppm #W____	FC Mix Std approx. 0.5 ppm actual ppm #W____	FC Mix Std approx. 5 ppm actual ppm #W____	FC Mix Std approx. 50 ppm actual ppm #W____	Comments
H_2O Blank					
____ Blank					

Blank Std # amount =

Extraction Method/Revision:

		Date & Initials
Add Surrogate, Vortex 15 sec.		
Pipette Sample	Volume ml	
Pipette 1 ml of 0.5 M TBA, pH 10. PH = ____	Std. # ____	
Pipette 2 ml of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. # ____	
Pipette 5 ml of ethyl acetate	TN-A-____	
Shake 20 min.	Shaker Speed	
Centrifuge 20-25 min.	Centrifuge speed:	
Remove a 4 ml aliquot of organic layer		
Put on Nitrogen Evaporator to dryness	Temperature:	
Add methanol Volume ____ ml	TN-A-____	
Vortex 30 sec.		
Filter using a 3cc B-D syringe with a 0.2μm SRI filter into a 1.5 ml autosample vial		

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

MDL/LOQ values for Rabbit Liver:

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

MDL/LOQ values for Rat Liver:

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

MDL/LOQ values for Monkey Liver:

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

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

Summary Table: Limits of Quantitation

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

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

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

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

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

Compound		PFOSA					
Liver Matrix	Prepared Range of Standards	Range of Average Curve	LCR from Average Curve	Range of Low Std. Curve	LCR from Low Std. Curve	Range of High Std. Curve	LCR from High Std. Curve
	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)
Rabbit	6.04 - 1810	6.04 - 1210	302 - 1210	6.04 - 302	12.1 - 302	121 - 1210	302 - 1210
Bovine	5.95 - 1190	5.95 - 1190	297 - 1190	n/a	n/a	n/a	n/a
Rat	6.17 - 1240	6.17 - 1240	617 - 1240	n/a	n/a	n/a	n/a
Monkey	5.88 - 1180	5.88 - 1180	588 - 1180	n/a	n/a	n/a	n/a

Compound		PFOSAA					
Liver Matrix	Prepared Range of Standards	Range of Average Curve	LCR from Average Curve	Range of Low Std. Curve	LCR from Low Std. Curve	Range of High Std. Curve	LCR from High Std. Curve
	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)
Rabbit	6.33 - 1900	127 - 1900	633 - 1900	n/a	n/a	n/a	n/a
Bovine	5.99 - 1200	120 - 1200	599 - 1200	n/a	n/a	n/a	n/a
Rat	6.21 - 1240	62.1 - 1240	621 - 1240	n/a	n/a	n/a	n/a
Monkey	5.92 - 1180	59.2 - 1180	592 - 1180	n/a	n/a	n/a	n/a

Compound		EtFOSE-OH					
Liver Matrix	Prepared Range of Standards	Range of Average Curve	LCR from Average Curve	Range of Low Std. Curve	LCR from Low Std. Curve	Range of High Std. Curve	LCR from High Std. Curve
	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)
Rabbit	5.96 - 1790	119 -1790	596 - 1790	n/a	n/a	n/a	n/a
Bovine	5.87 - 1170	58.7 - 1170	587 - 1170	n/a	n/a	n/a	n/a
Rat	6.09 - 1220	122 - 1220	609 - 1220	n/a	n/a	n/a	n/a
Monkey	5.80 - 1160	29.4 - 1160	580 - 1160	n/a	n/a	n/a	n/a

Compound		POAA					
Liver Matrix	Prepared Range of Standards	Range of Average Curve	LCR from Average Curve	Range of Low Std. Curve	LCR from Low Std. Curve	Range of High Std. Curve	LCR from High Std. Curve
	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)
Rabbit	6.06 - 1820	30.3 - 1820	303 - 1820	30.3 - 606	303 - 606	303 - 1820	303 - 1820
Bovine	5.93 - 1190	59.3 - 1190	593 - 1190	n/a	n/a	n/a	n/a
Rat	6.15 - 1230	61.5 - 1230	615 - 1230	n/a	n/a	n/a	n/a
Monkey	5.86 - 1170	117 - 1170	117 - 1170	n/a	n/a	n/a	n/a

Compound		PFOSEA					
Liver Matrix	Prepared Range of Standards	Range of Average Curve	LCR from Average Curve	Range of Low Std. Curve	LCR from Low Std. Curve	Range of High Std. Curve	LCR from High Std. Curve
	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)	(ppb)(ng/g)
Rabbit	6.20 - 1860	62.0 - 1240	62.0 - 1240	n/a	n/a	n/a	n/a
Bovine	5.92 - 1180	29.6 - 1180	29.6 - 1180	n/a	n/a	n/a	n/a
Rat	6.14 - 1230	123 - 1230	123 - 1230	n/a	n/a	n/a	n/a
Monkey	5.85 - 1170	58.5 - 1170	117 - 1170	n/a	n/a	n/a	n/a

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

Ion Pair Standard Curves--Tissues

Prep Date(s):	11/2/98	**Study Number:**	Cross Validation
Analyst(s):	RWW-IAS	**Equipment Number:**	
Sample Matrix:	Monkey Liver	**Final Solvent & TN Number:**	MeOH TN-A-2076
Method/Revision	FACT-M-1.0	**Blank Tissue/Identifier:**	Liver
Target Analyte(s):	FC-Mix		

FC Mix Std Approx. 0.500 ppm:	W398-1004
FC Mix Std Approx. 5.00 ppm:	W398-1003
FC Mix Std Approx. 50.0 ppm:	W398-1002
Surrogate Std Approx. 16.5 ppm:	W398-989

Actual Concentrations of Standards in the FC Mix

PFOS Std Conc.	PFOSA Std Conc.	PFOSAA Std Conc.	EtFOSE Std Conc.	POAA Std Conc.	PFOSEA Std Conc.	All Am't Spiked	All Liver conc.
ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	ug/mL	mL	g/ml
0.501	0.497	0.500	0.490	0.495	0.494	0.002	0.169
0.501	0.497	0.500	0.490	0.495	0.494	0.004	0.169
0.501	0.497	0.500	0.490	0.495	0.494	0.010	0.169
0.501	0.497	0.500	0.490	0.495	0.494	0.020	0.169
0.501	0.497	0.500	0.490	0.495	0.494	0.040	0.169
5.01	4.97	5.00	4.90	4.95	4.94	0.010	0.169
5.01	4.97	5.00	4.90	4.95	4.94	0.020	0.169
5.01	4.97	5.00	4.90	4.95	4.94	0.030	0.169
50.1	49.7	50.0	49.0	49.5	49.4	0.004	0.169

Calculated Concentrations of Standards in the Sample Matrix.

PFOS Final Conc.	PFOSA Final Conc.	PFOSAA Final Conc.	EtFOSE Final Conc.	POAA Final Conc.	PFOSEA Final Conc.
ng/g	ng/g	ng/g	ng/g	ng/g	ng/g
5.93	5.88	5.92	5.80	5.86	5.85
11.9	11.8	11.8	11.6	11.7	11.7
29.6	29.4	29.6	29.0	29.3	29.2
59.3	58.8	59.2	58.0	58.6	58.5
119	118	118	116	117	117
296	294	296	290	293	292
593	588	592	580	586	585
889	882	888	870	879	877
1186	1176	1183	1160	1172	1169

Surrogate Std Conc.	All Am't Spiked
ug/mL	mL
16.50	0.005

Surrogate Final Conc.
ng/g
488.2

Validated Ranges -- Approximate Concentrations

Liver	PFOS	PFOSA	PFOSAA	EtFOSE-OH	POAA	PFOSEA
Rabbit	40 - 1000 ppb	20 - 1200 ppb	180 - 1900 ppb	190 - 1800 ppb	80 - 1800 ppb	60 - 1200 ppb
Bovine	60 - 1200 ppb	30 - 1200 ppb	120 - 1200 ppb	120 - 1200 ppb	80 - 1200 ppb	30 - 1200 ppb
Rat	60 - 1200 ppb	70 - 1200 ppb	60 - 1200 ppb	120 - 1200 ppb	60 - 1200 ppb	120 - 1200 ppb
Monkey	60 - 1200 ppb	90 - 1200 ppb	120 - 1200 ppb	60 - 1200 ppb	120 - 1200 ppb	120 - 1200 ppb

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-2.1

Adoption Date: 05/26/98

Revision Date: 06/03/99

Author: Lisa Clemen

Approved By:

D J Bruin — 6/3/99
Laboratory Manager — Date

Kristen Hanson — 6/1/99
Group Leader — Date

Lisa A Clemen — 6/01/99
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 **Atmospheric Pressure Ionization (API):** The Micromass platform systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API platforms are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass platform systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these platform systems. Currently MassLynx has Windows 95 and WindowsNT 3.1 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Platform II or Quattro II MassLynx or MassLynx NT USER'S GUIDE).

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

4.1.1 Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi

7.1.2 HPLC column, specifics to be determined by the analyst.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent.

8.1.2 ASTM, Type I water, Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system.

8.1.3 Ammonium acetate, reagent grade or equivalent.

8.2 Standards

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

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 mL centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples may be stored at room temperature or refrigerated at 4° C until analysis can be performed.

10.0 Quality Control

10.1 Matrix Blanks and Method Blanks

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

10.2 Matrix Spikes

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

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

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

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

10.3.2 See section 13 to calculate percent difference.

11.0 Calibration and Standardization

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

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

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

12.0 Procedures

12.1 Acquisition Set up

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

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

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

12.1.3 Typically the sample list begins with the first set of liver standards and ends with the second set of standards.

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	40%	60%
8.0 min.	90%	10%
11.0 min.	90%	10%
12.0 min.	40%	60%

12.2.2.5 Press the "Start" button.

12.3 Instrument Sep-up

12.3.1 Refer to **ETS-9-24.0** for more details .

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

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

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe. Readjust the tip of the probe if no mist is observed.

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 LC constant flow mode flow rate 10 – 500 uL/min

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

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Print the tune page, with its parameters, and store it in the study binder with a copy taped into the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel. Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.1 Calculate matrix spike percent recoveries using the following equation:

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

13.1.2 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

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

14.1 14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.2 Calibration Curves

14.2.1 The r^2 value for the calibration curve must be 0.980 or better.

14.3 Matrix Spikes

14.3.1 Matrix spike percent recoveries are must be within ± 30% of the spiked concentration.

14.4 Continuing Calibration Verifications

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

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

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

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

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

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

18.0 REFERENCES

18.1 FACT-M-1.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry

18.2 ETS-9-24.0, "Operation and Maintenance of the Micromass Atmospheric Pressure Ionization/Mass Spectrometer Quattro II triple quadrupole Systems"

18.3 The validation report associated with this method is **FACT-M-1.1-V & 2.1-V-1**.

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Laboratory Study

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

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-3.1

Adoption Date: 04/22/98

Revision Date: 10/01/98

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager	Date 10/1/98
Group Leader	Date 9/28/98
Technical Reviewer	Date 9/28/98

1.0 SCOPE AND APPLICATION

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

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

1.3 **Matrices:** Rabbit, rat, bovine, and monkey serum, rat whole blood, and rat milk curd.

2.0 Summary of Method

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

3.0 Definitions

3.1 PFOS: perfluorooctanesulfonate (anion of potassium salt) $C_8F_{17}SO_3^-$

3.2 PFOSA: perfluorooctane sulfonylamide $C_8F_{17}SO_2NH_2$

3.3 PFOSAA: perfluorooctane sulfonylamido (ethyl)acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2CO_2^-$

3.4 EtFOSE-OH: 2(N-ethylperfluorooctane sulfonamido)-ethyl alcohol $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$

3.5 POAA: perfluorooctanoate (anion of ammonium salt) $C_7F_{15}COO^-$

3.6 PFOSEA: perfluorooctane sulfonyl ethylamide $C_8F_{17}SO_2N(CH_2CH_3)H$

3.7 FC-807 monoester $C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2O\text{-}PO_3H)$

3.8 Surrogate standard: 1H-1H-2H-2H perfluorooctane sulfonic acid

4.0 Warnings and Cautions

4.1 **Health and safety warnings**

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

5.0 Interferences

5.1 There are no known interferences at this time.

6.0 Equipment

6.1 The following equipment is used while performing this method. Equivalent equipment is acceptable.

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Eppendorf or disposable pipettes

7.3 Electronic pipettor, Eppendorf or equivalent

7.4 Graduated pipettes

7.5 Nalgene bottles, capable of holding 250 mL and 1 L

7.6 Volumetric flasks, glass, type A

7.7 Volumetric pipets, glass, type A

7.8 I-CHEM vials, glass, 40 mL glass

7.9 Crimp cap autovials

7.10 Centrifuge tubes, polypropylene, 15 mL

7.11 Labels

7.12 Syringes, capable of measuring 5 µL to 50 µL

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

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

8.0 REAGENTS AND STANDARDS

8.1 Type I reagent grade water, Milli-Q™ or equivalent; all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus™ system

8.2 Sodium hydroxide (NaOH), J.T Baker or equivalent

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

8.4 Sodium carbonate (Na_2CO_3), J.T. Baker or equivalent

8.5 Sodium bicarbonate ($NaHCO_3$), J.T. Baker or equivalent

8.6 Ethyl acetate, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 Control matrix or blank matrix for purpose of standards, QC checks, blanks, etc.

8.10 **Fluorochemical standards**

8.10.1 PFOS (3M Specialty Chemical Division), molecular weight = 538

8.10.2 PFOSA (3M Specialty Chemical Division), molecular weight = 499

8.10.3 PFOSAA (3M Specialty Chemical Division), molecular weight = 585

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 571

8.10.5 POAA (3M Specialty Chemical Division), molecular weight = 431

8.10.6 PFOSEA (3M Specialty Chemical Division), molecular weight = 527

8.10.7 FC-807 monoester (3M Specialty Chemical Division). FC-807 is a mixture of triester, diester, and monoester fluorochemical components. The monoester molecular weight = 650

8.10.8 Surrogate standard: 4-H, perfluorooctane sulfonic acid (1-H,1-H, 2-H, 2-H $C_8F_{13}SO_3H$) molecular weight = 428

8.10.9 Other fluorochemicals, as appropriate

8.11 Reagent preparation

8.11.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.11.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.11.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH and dilute to volume with Milli-Q™ water. While adding the last mL of NaOH, add slowly because the pH changes abruptly. Store in a 1 L Nalgene bottle.

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

8.11.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

8.12.2 Prepare other fluorochemical standards, as appropriate. Multicomponent fluorochemical standards are acceptable (for example, one working standard solution containing 1.00 ppm PFOS, 1.02 ppm PFOSA, 0.987 ppm PFOSAA, and 1.10 ppm EtFOSE-OH.)

8.12.3 Weigh approximately 100 mg of PFOS into a 100 ml volumetric flask and record the actual weight.

8.12.4 Bring to volume with methanol for a stock standard of approximately 1000 ppm (µg/ml).

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

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

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

8.13 Surrogate stock standard preparation

8.13.1 Weigh approximately 50-60 mg of surrogate standard 1-H,1-H, 2-H, 2-H, $C_8F_{17}SO_3H$ into a 50 ml volumetric flask and record the actual weight.

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

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

9.0 Sample Handling

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

10.0 Quality Control

10.1 Matrix blanks and method blanks

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

11.1.1 Transfer 1 mL of serum or 1 mL of blood (blood is diluted 1:1 with Milli-Q™ water) to a 15 mL centrifuge tube. The blood is similar in composition to milk curd and can be used in place of milk curd for standard curves when extracting that matrix.

11.1.2 If most sample volumes are less than 1.0 mL, extract standards with matrix volumes equal to the sample volumes. Do not extract below 0.50 mL of matrix. Record the sample volume on the extraction sheet.

11.1.3 While preparing a total of twenty aliquots in 15 ml centrifuge tubes, mix or shake between aliquots.

11.1.4 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen standards and two matrix blanks.

11.1.5 Refer to validation reports **FACT-M-3.1-V-1** and **FACT-M-4.1-V-1**, which list the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.6 Use Attachment D as an aid in calculating the concentrations of the working standards. See Section **13.0** to calculate actual concentrations of PFOS in calibration standards.

11.2 To each standard, blank, or QC check, add appropriate amount of surrogate working standard for the concentration to fall within the calibration curve range 5 ppb -1000 ppb.

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

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

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

12.0 PROCEDURE

12.1 Obtain frozen samples and allow to thaw.

12.2 Vortex mix for 15 seconds, then transfer 1.0 mL or other appropriate volume to a 15 mL polypropylene centrifuge tube. For blood samples, remove 0.5 mL and dilute to 1.0 mL with Milli-Q™ water. As soon after diluting as possible, pipet diluted blood into TBA-buffer mixture shown in step 12.9 and mix well.

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

12.4 Record the volume on the extraction worksheet. The final methanol volume equals the volume transferred from the sample. For example, if 0.5 mL is removed for a blood sample, the final methanol volume will equal 0.5 mL.

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

12.6 Spike each matrix with the appropriate amount of standard as described in **11.1** or **Table 1** or **2** in that section for the calibration curve standards. Also prepare matrix spikes and continuing calibration standards.

12.7 Spike all samples, including blanks and standards, ready for extraction with surrogate standard as described in **11.2**.

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

12.9 To each sample, add 1 mL 0.5 M TBA and 2 mL of 0.25 M sodium carbonate/sodium bicarbonate buffer.

12.10 Using a volumetric pipette, add 5 mL ethyl acetate.

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

12.12 Centrifuge for 20 to 25 minutes at approximately 3500 rpm, until layers are well separated.

12.13 Transfer 4 mL of organic layer, using a 5 mL graduated glass pipette, to a clean 15 mL centrifuge tube. Label this fresh tube with the same information as in **12.5**.

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

12.15 Add 1.0 mL or other appropriate volume of methanol to each centrifuge tube using a graduated pipette. Methanol volume to add equals the initial volume of sample used for the extraction.

12.16 Vortex mix for 30 seconds.

12.17 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial or low-volume autovial when necessary.

12.18 Label the autovial with the study number, animal number and gender, sample timepoint, matrix, final solvent, extraction date, and analyst(s) performing the extraction.

12.19 Cap and store extracts at approximately 4 °C until analysis.

12.20 Complete the extraction worksheet, attached to this document, and tape in the study notebook or include in study binder, as appropriate.

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

14.1 The method detection limit (MDL) is analyte and matrix specific. Refer to MDL report for specific MDL and limit of quantitation (LOQ) values (see **Attachments B and C**).

14.2 The following quality control samples are extracted with each batch of samples to ensure the quality of the extraction and analysis.

14.2.1 Method blanks and matrix blanks

14.2.2 Matrix spike and matrix spike duplicate samples to determine accuracy and precision of the extraction

14.2.3 Continuing calibration check samples to determine the continued accuracy of the initial calibration curve

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

17.0 Attachments

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values

17.3 Attachment C, LOQ Summary

17.4 Attachment D, Calibration standard concentration worksheet

18.0 References

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

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

[1] Study number where the original worksheet is located.

Blank	Std #	amount =	mL
Serum Extraction Method	:		**Date & Initials**
Vortex 15 sec.			
Pipette Matrix	Volume	ml	
Pipette 1 ml of 0.5 M TBA, pH 10.	Std. #		
Pipette 2 ml of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer	Std. #		
Pipette 5 ml of ethyl acetate	TN-A-		
Shake 20 min.			
Centrifuge 20-25 min.	Centrifuge speed:		
Remove a 4 ml aliquot of organic layer			
Put on Nitrogen Evaporator to dryness	Evaporator #:	Temperature:	
Add methanol	Volume ml	TN-A-	
Vortex 30 sec.			
Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 ml autosample vial			

MS/MSD/___ Cont. Checks: Spiked _____ uL of a _____ ppm std (_____) for a final concentration of _____ ppm. MS/MSD used sample _____. Cont. Checks used same matrix as for std curve.
Surrogate Standard: Spiked _____ uL of a _____ ppm std (_____) to all samples, standards, and blanks

MDL/LOQ values for Rabbit Serum:

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

MDL/LOQ values for Rat Serum:

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

MDL/LOQ values for Bovine Serum:

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

No data is available for MDL or LOQ in Monkey Serum. Use validated Linear Calibration Range instead.
Please see Attachment C (LOQ Summary) and MDL study in FACT-M-3.1 & 4.1-V-1 for specifics.

MDL/LOQ values for Monkey Serum:

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

MDL/LOQ values for Rat Whole Blood:

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

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

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: POAA

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

Compound: PFOSEA

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

Compound: Monoester

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

In general, the chromatography for the monoester was very poor (broad peaks, high baseline). Curves for monoester in rabbit and bovine were unacceptable. Any quantitation performed with the monoester is only an estimate and should not be used for reliable, accurate data reporting.

Ion Pair Standard Curves – Fluids

Prep date(s): **Standard number:**
Analyte(s): **Equipment number:**
Sample matrix: **Final solvent and TN:**
Blank fluid/identifier:

Method/revision:
Target analyte(s):
FC mix std approx. 0.500 ppm: W398-641
FC mix std approx. 5.00 ppm: W398-640
FC mix std approx. 50.0 ppm: W398-639
Surrogate std approx. 17.71 ppm: W398-605

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

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3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-4.1

Adoption Date: 4/22/98

Revision Date: 10-1-98

Author: Lisa Clemen, Glenn Langenburg

Approved By:

Laboratory Manager — Date: 10/1/98

Group Leader — Date: 9/29/98

Technical Reviewer — Date: 9/29/98

1.0 SCOPE AND APPLICATION

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

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

1.3 Matrices: Rabbit, rat, bovine, or monkey serum and rat whole blood or milk curd.

2.0 Summary of Method

2.1 This method describes the analysis of fluorochemical surfactants extracted from serum, whole blood, or milk curd using HPLC-electrospray/mass spectrometry, or similar system as appropriate. The analysis is performed by monitoring a single ion characteristic of a particular fluorochemical, such as the potassium perfluorooctanesulfonate (PFOS) anion, M/Z= 499. Samples may also be analyzed using an API/MS/MS system to further verify compound identification.

3.0 Definitions

3.1 **Atmospheric Pressure Ionization (API):** The Micromass platform systems allow for various methods of ionization by utilizing various sources, probes, and interfaces. These include but are not limited to: Electrospray Ionization (ESI), Atmospheric Pressure chemical Ionization (APcI), Thermospray, etc. The ionization process in these techniques occurs at atmospheric pressure (i.e. not under a vacuum).

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ionization occurs through the production of tiny charged droplets in a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API platforms are equipped with quadrupole mass selective detectors. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or a series (MS/MS) for more specific fragmentation information.

3.4 **Conventional vs. Z-spray probe interface:** The latest models of Micromass platform systems (post 1998) utilize a "Z-spray" conformation. The spray emitted from a probe is orthogonal to the cone aperture. In the conventional conformation it is aimed directly at the cone aperture, after passing through a tortuous pathway in the counter electrode. Though the configuration is different, the methods of operation, cleaning, and maintenance are the same. However, Z-spray components and conventional components are not compatible with one another, but only with similar systems (i.e. Z-spray components are compatible with other Z-spray systems, etc.)

3.5 **Mass Lynx Software:** System software designed for the specific operation of these platform systems. Currently MassLynx has Windows 95 and WindowsNT 3.1 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Platform II or Quattro II MassLynx or MassLynx NT USER'S GUIDE).

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

4.1.1 Use caution with the voltage cables for the probe. The probe employs a voltage of approximately 5000 Volts.

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

4.2 Cautions:

4.2.1 Do not operate solvent pumps above capacity of 400 bar (5800 psi) back pressure. If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

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

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade nitrogen gas regulated to approximately 100 psi

7.1.2 HPLC analytical column, specifics to be determined by the analyst

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water, all water used in this method should be Milli-Q™ water and may be provided by a Milli-Q TOC Plus system

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically one method blank, one matrix blank, and ten matrix standards are prepared during the extraction procedure. See **FACT-M-3.1**.

9.0 SAMPLE HANDLING

9.1 Fresh matrix standards are prepared with each analysis. Extracted standards and samples are stored in capped autovials or capped 15 ml centrifuge tubes until analysis.

9.2 If analysis will be delayed, extracted standards and samples can be refrigerated at approximately 4° C until analysis can be performed.

10.0 Quality Control

10.1 Method Blanks and Matrix Blanks

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

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate per forty samples. With a minimum of 2 spikes per batch.

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

10.2.3 See Section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

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

10.3.2 See Section 13 to calculate percent difference.

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The mean of two standard values, at each standard concentration, will be plotted by linear regression (r^2)for the calibration curve using MassLynx or other suitable software.

11.2 The r^2 value for the data should be 0.980 or greater. Lower values may be acceptable at the discretion of the analyst and documented approval of the Project Lead.

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

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

12.0 Procedures

12.1 Acquisition Set up

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

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 15 minutes

12.2.2.4 Solvent ramp =

Time	MeOH	2.0 mM Ammonium acetate
0.00 min.	45%	55%
7.5 min.	90%	10%
11.0 min.	90%	10%
11.5 min.	45%	55%

Note: In this instrument configuration, the run must be set up on the electrospray software with a "Waiting for inlet start" message before the "Start" button is pressed on the HP Workstation.

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to **FACT-EP-3.0** for more details.

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

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

12.3.4 Set HPLC pump to "On". Set the flow to 10 - 500 uL/min or as appropriate. Observe droplets coming out of the tip of the probe. Allow to equilibrate for approximately 10 minutes.

12.3.5 Turn on the nitrogen. A fine mist should be expelled with no nitrogen leaking around the tip of the probe.

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

12.3.6.3 HPLC constant flow mode flow rate 10 – 500 µL/min

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

12.3.7 Carefully guide the probe into the opening. Insert probe until it will not go any further. Connect the voltage cables to the probe.

12.3.8 Record tune parameters in the instrument log.

12.3.9 Using the cross-flow counter electrode in the ES/MS source is recommended for the analysis of biological matrices.

12.3.10 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, see appropriate MassLynx USER'S GUIDE). Press the start button at top of sample list. Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

Final Volume (mL)

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. Please see **FACT-M-3.1, Attachment A** for a listing of current validated MDL and LOQ values.

14.2 Method Blanks and Matrix Blanks

14.2.1 Method blanks and matrix blanks will be analyzed with each sample set for possible contamination or carryover. Values are expected to fall below the lowest standard in the calibration curve.

14.3 Matrix Spikes

14.3.1 Matrix spikes are analyzed with each sample set and the percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.4 Continuing Calibration Checks

14.4.1 Continuing calibration checks are analyzed at a minimum of after every 10 samples with each sample set. The percent recoveries are expected to fall within ± 30% of the spiked concentration.

14.5 If any criteria listed in the method performance section isn't met, maintenance may be performed on the system and samples reanalyzed or other actions as determined by the analyst. All actions will be documented in the instrument runlog, the maintenance log, or on the summary sheet with the sample results.

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

16.2 Plot calibration curve by linear regression and store in the study folder.

16.3 Print sample list from MassLynx and tape into the instrument runlog.

16.4 Print data integration summary from MassLynx and tape into the instrument runlog.

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and store in appropriate study folder.

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: FACT-M-4.1 Data reporting spreadsheet

17.2 The validation report associated with this method is FACT-M-3.1 & 4.1-V-1.

18.0 REFERENCES

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
1	*Validation of method to include 7 fluorochemicals addition of whole blood matrix, surrogate standard, new API/MS(MS) systems, monkey sera cross validation, MDL study, updates in record keeping and storing policies, etc.*	07/01/98

Attachment A

Laboratory Study

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

Group Dose	Sample#	Concentration ug/mL	Initial Vol. mL	Dilution Factor	Final Conc. ug/mL

Slope: Taken from linear regression equation.
Group/Dose: Taken from the study folder.
Sample#: Taken from the study folder.
Concentration (ug/mL): Taken from the MassLynx integration summary.
Initial Volume (mL): Taken from the study folder.
Dilution Factor: Taken from the study folder.
Final Conc. (ug/mL): Calculated by dividing the initial volume from the concentration

3M Medical Department Study: T-6295.7
Report No. FACT TOX-030
Laboratory Request Number-U2279

ATTACHMENT D
DATA SUMMARY TABLES

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030
Laboratory Request Number-U2279

Table D-1a. Sample Intervals and Types by Individual Animal from Study 6329-223

Animal ID	Serum Samples Collected (Qty—Week)	Liver Samples Collected (Date)	Recovery Subgroup Serum Samples Collected (Qty—Week)
Group I (Control)	**151 Samples**	**8 Samples**	**72 Samples**
105508M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105517M	11—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26	2/25/99	
105519M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105520M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	Returned to colony 3/07/00	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105526M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	Returned to colony 3/07/00	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105527M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105529F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	Returned to colony 3/07/00	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105530F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105531F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105535F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105544F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105549F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	Returned to colony 3/07/00	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
Group II (Low Dose)	**99 Samples**	**8 Samples**	—
[illegible]	1—0	Baseline only–Not assigned	Returned to colony
105514M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/26/99	
105515M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105516M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105521M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
[illegible]	1—0	Baseline only–Not assigned	Returned to colony
105537F	11—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26	2/25/99	
105541F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
[illegible]	1—0	Baseline only–Not assigned	Returned to colony
[illegible]	1—0	Baseline only–Not assigned	Returned to colony
105547F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105550F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	

27 Day 183 (2/23/99) 27i Day 184 (2/25/99) 27ii Day 185 (2/26/99) 27iii Day 187 (2/28/99)
** Two samples 2/20/99 79 Sample on 2/23/00 79i Sample on 2/25/00 79ii Sample on 2/25/00

Note: Samples for Week 26 and Week 27 (Recovery Group) taken on same day (Day 183, 2/23/99)

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030
Laboratory Request Number-U2279

Table D-1b. Sample Intervals and Types by Individual Animal from Study 6329-223

Animal ID	Serum Samples Collected (Qty—Week)	Liver Samples Collected (Date)	Recovery SubGroup Serum Samples Collected (Qty—Week)
Group III (Mid Dose)	**152 Samples**	**12 Samples**	**72 Samples**
105505M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, , 27, 27i, 27ii	3/01/00 Biopsy	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105510M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105518M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105523M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	3/01/00 Biopsy	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105524M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105528M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105532F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105538F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105539F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	3/01/00 Biopsy	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
105545F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105548F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105552F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	3/01/00 Biopsy	18—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79
Group IV (High Dose)	**148 Samples**	**14 Samples**	**80 Samples**
105506M	11—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, **	none	
105507M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105509M	9—0, 1, 2, 4, 6, 8, 12, 16, 20	none	
105511M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	9/22/99 Biopsy, 2/25/00	20—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79, 79i, 79ii
105512M	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105522M	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	9/22/99 Biopsy, 2/25/00	20—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79, 79i, 79ii
105533F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	9/22/99 Biopsy, 2/25/00	20—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79, 79i, 79ii
105534F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105536F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27i	2/25/99	
105540F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	
105542F	14—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27, 27i, 27ii	9/22/99 Biopsy, 2/25/00	20—27iii, 28, 29, 30, 31, 35, 39, 43, 47, 51, 53, 57, 61, 65, 69, 73, 77, 79, 79i, 79ii
105551F	12—0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 26, 27ii	2/26/99	

27 Day 183 (2/23/99) 27i Day 184 (2/25/99) 27ii Day 185 (2/26/99) 27iii Day 187 (2/28/99)
** Two samples 2/20/99 79 Sample on 2/23/00 79i Sample on 2/25/00 79ii Sample on 2/25/00

Note: Samples for Week 26 and Week 27 (Recovery Group) taken on same day (Day 183, 2/23/99)

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Table D-2. Average PFOS Concentrations in Serum by Dosage Group and Week from Study FACT Tox-030 (Baseline– Day 185)

Time Point LOQ (µg/mL)	All PFOS values µg/mL	Group 1M 0.0mg/kg/d	Group 1F 0.0mg/kg/d	Group 2M 0.03mg/kg/d	Group 2F 0.03mg/kg/d	Group 3M 0.15mg/kg/d	Group 3F 0.15mg/kg/d	Group 4M 0.75mg/kg/d	Group 4F 0.75mg/kg/d
Baseline	Average PFOS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
LOQ 0.025	Std Deviation	NA	NA	NA	NA	NA	NA	NA	NA
Week 1	Average PFOS	<LOQ	<LOQ	0.869	0.947	4.60	3.71	21.0	20.4
LOQ 0.025	Std Deviation	NA	NA	0.147	0.110	0.782	0.455	1.57	2.71
Week 2	Average PFOS	<LOQ	<LOQ	1.10	1.10	5.81	5.39	26.9	22.0
LOQ 0.025	Std Deviation	NA	NA	0.0835	0.0963	0.933	0.930	3.54	3.25
Week 4	Average PFOS	<LOQ - 1 Anomaly	<LOQ	3.20	3.40	17.8	16.5	95.3	92.7
LOQ 0.0152	Std Deviation	NA	NA	0.577	0.291	1.68	1.87	70.4	39.6
Week 6	Average PFOS	<LOQ	<LOQ	3.61	3.71	20.4	18.8	94.5	90.1
LOQ 0.0152	Std Deviation	NA	NA	0.430	0.417	1.65	2.15	8.07	7.11
Week 8	Average PFOS	<LOQ - 1 Anomaly	0.0226 -2 Anomalies	4.73	4.76	26.0	24.0	109	107
LOQ 0.0152	Std Deviation	NA	0.00189	0.432	0.577	3.30	3.06	18.3	11.8
Week 12	Average PFOS	0.0383 - 1 Anomaly	0.0263 -2 Anomalies	6.69	6.31	35.2	27.8	122	117
LOQ 0.0152	Std Deviation	0.00811	0.00362	0.578	0.717	5.39	3.98	23.9	11.7
Week 16	Average PFOS	0.0407	0.0432 -2 Anomalies	11.2	10.5	56.2	42.1	189	162
LOQ 0.0152	Std Deviation	0.0110	0.00851	2.44	1.90	5.84	4.04	15.9	19.3
Week 20	Average PFOS	0.0400	0.0504	12.3	19.5	63.7	58.1	144	156
LOQ 0.0152	Std Deviation	0.0120	0.0127	1.40	14.5	6.71	14.7	10.9	21.8
Week 24	Average PFOS	0.0440	0.0426- 1 Anomaly	14.5	13.0	65.9	60.4	215	174
LOQ 0.0152	Std Deviation	0.0101	0.00784	3.06	0.675	6.88	7.24	24.9	20.9
Week 25	Average PFOS	0.0459	0.0506	15.8	13.2	82.6	66.8	173	171
LOQ 0.0152	Std Deviation	0.0143	0.0164	1.41	1.42	25.2	10.8	36.5	22.2
Week 27	Average PFOS	0.0529	0.0416	15.9	11.1	68.1	58.5	194	160
LOQ 0.0248	Std Deviation	0.0145	0.0148	5.54	1.52	5.75	4.67	8.93	23.9
Day 183	Average PFOS	0.117	0.0533	—	—	85.0	81.7	249	230
LOQ 0.0152	Std Deviation	0.0764	0.0315	—	—	14.9	35.1	46.8	40.3
Day 184	Average PFOS	0.0233- 1 Anomaly	0.0352	—	—	69.7	78.0	259	245
LOQ 0.0152	Std Deviation	NA	0.00911	—	—	4.68	12.0	110	29.2
Day 185	Average PFOS	0.0432	0.0546	—	—	77.9	84.3	294	321
LOQ 0.0152	Std Deviation	0.00928	0.0306	—	—	10.7	23.3	22.0	170

<LOQ: Less than the Lower Limit of Quantitation

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Table D-3. Average PFOS Concentrations in Serum by Dosage Group and Week from Study FACT TOX-030 (Recovery Group)

Time Point	All PFOS values	Group 1M	Group 1F	Group 3M	Group 3F	Group 4M	Group 4F
LOQ (µg/mL)	µg/mL	0.0mg/kg/day		0.15mg/kg/day		0.75mg/kg/day	
Day 187 LOQ 0.0152	Average PFOS (µg/mL)	0.0300 - 1 Anomaly	0.0457	79.1	39.5	267	258
	Std Deviation	NA	0.0118	4.67	41.4	42.0	15.2
Week 28 LOQ 0.0152	Average PFOS (µg/mL)	0.0371	0.0430	84.6	86.1	249	236
	Std Deviation	0.0124	0.00821	1.51	3.59	21.7	18.3
Week 29 LOQ 0.0152	Average PFOS (µg/mL)	0.0665	0.0742	75.8	69.9	223	194
	Std Deviation	0.00362	0.000664	2.18	11.4	66.9	19.0
Week 30 LOQ 0.0152	Average PFOS (µg/mL)	0.0313	0.0303	65.2	62.0	143	162
	Std Deviation	0.000165	0.000485	4.60	10.6	38.0	7.87
Week 31 LOQ 0.0152	Average PFOS (µg/mL)	0.0358	0.0368	56.6	79.6	161	185
	Std Deviation	0.000567	0.0121	4.77	43.8	46.1	21.9
Week 35 LOQ 0.00555	Average PFOS (µg/mL)	0.0459	0.0723	84.5	74.4	181	171
	Std Deviation	0.00303	0.00352	12.0	9.53	19.5	10.1
Week 39 LOQ 0.00555	Average PFOS (µg/mL)	0.0391	0.0509	60.1	51.7	146	161
	Std Deviation	0.0106	0.000779	3.16	7.29	16.1	11.1
Week 43 LOQ 0.00555	Average PFOS (µg/mL)	0.0319	0.0368	45.7	58.1	78.8	159
	Std Deviation	0.00440	0.0000923	1.12	0.249	16.8	28.4
Week 47 LOQ 0.00555	Average PFOS (µg/mL)	0.0355	0.0459	48.3	42.6	124	98.3
	Std Deviation	0.00221	0.00323	3.69	6.70	25.9	8.32
Week 51 LOQ 0.00555	Average PFOS (µg/mL)	0.0237	0.0341	37.9	35.1	94.7	91.4
	Std Deviation	0.00333	0.000403	2.62	13.2	38.4	6.07
Week 53 LOQ 0.00555	Average PFOS (µg/mL)	0.0331	0.0397	46.2	36.7	80.8	98.2
	Std Deviation	0.0086	0.00311	3.30	6.24	36.8	0.490
Week 57 LOQ 0.00555	Average PFOS (µg/mL)	0.0327	0.0445	30.2	32.3	78.0	106
	Std Deviation	0.00526	0.00385	2.36	1.34	16.3	3.84
Week 61 LOQ 0.00555	Average PFOS (µg/mL)	0.0351	0.0448	31.6	38.2	100	109
	Std Deviation	0.00449	0.00210	5.98	0.283	50.3	0.697
Week 65 LOQ 0.00555	Average PFOS (µg/mL)	0.0210	0.0360	32.9	37.6	91.5	82.8
	Std Deviation	0.00365	0.000522	0.0269	2.32	55.2	9.68
Week 69 LOQ 0.00555	Average PFOS (µg/mL)	0.0406	0.0400	26.4	34.5	84.0	75.0
	Std Deviation	0.00313	0.00301	2.59	3.46	52.4	5.25
Week 73 LOQ 0.00555	Average PFOS (µg/mL)	0.0350	0.0365	27.3	25.8	54.4	147
	Std Deviation	0.0115	0.00284	4.66	2.91	27.3	131
Week 77 LOQ 0.00555	Average PFOS (µg/mL)	0.0296	0.0305	22.5	23.0	60.0	57.0
	Std Deviation	0.00535	0.00167	0.632	6.37	38.3	19.1
Week 79 LOQ 0.00555	Average PFOS (µg/mL)	0.0215	0.0243	19.1	21.4	41.1	41.4
	Std Deviation	0.00296	0.00355	0.805	2.01	25.9	1.15

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Table D-4a. PFOS Amounts Reported in Serum by Individual Animal (Baseline–Week 26)

PFOS Reported	Week 0 (µg/mL)	Week 1 (µg/mL)	Week 2 (µg/mL)	Week 4 (µg/mL)	Week 6 (µg/mL)	Week 8 (µg/mL)	Week 12 (µg/mL)	Week 16 (µg/mL)	Week 20 (µg/mL)	Week 24 (µg/mL)	Week 26 (µg/mL)
Group I (Control)											
105508M	•<LOQ	<LOQ	<LOQ	<LOQ	•<LOQ	<LOQ	0.0381	0.0344	0.0348	0.0449	0.0496
105517M	•<LOQ	•<LOQ	<LOQ	•<LOQ	•<LOQ	<LOQ	0.0430	•0.0408	0.0419	0.0486	•0.0539
105519M	•<LOQ	<LOQ	•<LOQ	•<LOQ	•<LOQ	<LOQ	0.0438	•0.0423	•0.0336	0.0414	0.0417
105520M	•<LOQ	<LOQ	•<LOQ	•<LOQ	<LOQ	<LOQ	<LOQ	0.0332	0.0300	0.0283	0.0254
105526M	•<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0244	0.0320	0.0369	0.0397	0.0376
105527M	•<LOQ	<LOQ	•<LOQ	•<LOQ	•<LOQ	0.0385	0.0423	0.0615	•0.0631	•0.0613	0.0669
105529F	•<LOQ	<LOQ	•<LOQ	•<LOQ	•<LOQ	0.0214	•<LOQ	0.0438	0.0617	0.0498	0.0613
105530F	•<LOQ	<LOQ	•<LOQ	•<LOQ	•<LOQ	0.0244	0.0243	•0.0515	0.0579	•0.0497	0.0736
105531F	•<LOQ	<LOQ	•<LOQ	<LOQ	<LOQ	0.0206	0.0254	0.0314	•0.0493	0.0364	0.0431
105535F	•<LOQ	<LOQ	<LOQ	•<LOQ	•<LOQ	<LOQ	<LOQ	•<LOQ	0.0263	•<LOQ	0.0266
105544F	<LOQ	•<LOQ	•<LOQ	<LOQ	•<LOQ	<LOQ	0.0238	•<LOQ	•0.0505	•0.0325	•0.0433
105549F	<LOQ	<LOQ	•<LOQ	•<LOQ	•<LOQ	0.0240	0.0318	0.0462	0.0570	0.0446	0.0556
Group II (Low Dose)											
105513M	•<LOQ										
105514M	<LOQ	0.793	•1.02	•2.87	•3.87	4.61	6.20	10.4	12.5	•14.7	16.2
105515M	•<LOQ	•0.767	•1.03	2.75	•3.31	4.17	6.48	•9.47	10.7	•10.9	16.5
105516M	<LOQ	0.832	•1.19	3.13	•3.26	5.05	6.55	10.1	11.9	14.1	13.8
105521M	•<LOQ	1.09	•1.13	•4.03	•4.19	5.10	•7.52	•14.8	14.1	•18.4	•16.9
105525M	•<LOQ										
105537F	•<LOQ	1.10	•1.19	•3.73	•4.28	5.28	6.81	11.1	11.0	13.4	•13.5
105541F	•<LOQ	•0.929	1.16	•3.07	3.66	4.85	5.57	•12.3	11.5	13.7	•14.8
105543F	•<LOQ										
105546F	•<LOQ										
105547F	•<LOQ	0.931	•1.06	3.55	•3.85	4.98	•7.03	10.7	14.3	•12.7	13.0
105550F	•<LOQ	0.832	•0.980	•3.27	•3.27	3.94	5.83	7.85	41.1	12.2	•11.4
Group III (Mid Dose)											
105505M	<LOQ	4.60	•6.07	•17.0	21.0	27.9	40.1	•59.4	62.3	77.4	92.9
105510M	•<LOQ	•4.19	4.82	•17.2	22.5	22.1	39.2	52.9	63.3	65.2	•129
105518M	•<LOQ	5.48	•7.01	•20.2	•20.1	25.1	33.7	60.7	63.1	58.8	•69.5
105523M	•<LOQ	3.54	4.56	17.6	•18.6	24.5	•35.3	52.2	75.2	59.7	•72.1
105524M	•<LOQ	4.25	•6.24	15.4	•21.8	31.6	37.8	63.4	64.0	69.6	•69.5
105528M	•<LOQ	5.52	6.15	•19.2	•18.5	24.6	25.4	48.4	54.2	•64.8	62.4
105532F	•<LOQ	•4.31	•4.61	•16.6	•18.4	27.6	25.8	40.4	69.2	•63.1	63.4
105538F	<LOQ	4.12	6.67	18.7	18.9	24.3	•32.8	37.3	47.4	48.6	59.0
105539F	<LOQ	3.31	•4.65	•14.3	•18.4	26.5	27.8	46.3	•78.2	•69.9	72.2
105545F	•<LOQ	•3.69	5.28	•18.7	22.7	24.5	32.2	43.1	45.7	63.5	85.7
105548F	<LOQ	3.72	•6.42	•15.7	•18.4	22.3	23.2	47.0	65.3	56.1	•64.5
105552F	•<LOQ	3.12	4.73	•14.9	16.1	19.1	24.8	38.7	42.7	•61.7	55.6
Group IV (High Dose)											
105506M	•<LOQ	21.3	•29.9	•236	•91.0	125	118	182	158	198	—
105507M	<LOQ	•22.0	•25.1	•72.6	•82.8	86.1	106	172	•138	188	•182
105509M	<LOQ	•18.8	23.9	•54.8	92.3	103	123	197	152	—	—
105511M	<LOQ	•19.3	22.6	•48.4	93.7	92.3	99.4	179	126	207	142
105512M	•<LOQ	22.8	31.3	•77.4	•105	118	167	216	143	247	148
105522M	•<LOQ	•21.6	28.8	•82.2	•102	131	•121	185	145	•233	221
105533F	•<LOQ	•22.6	24.9	•141	93.0	110	126	164	169	190	203
105534F	•<LOQ	•18.1	•26.0	•145	•83.3	121	114	157	143	•200	187
105536F	•<LOQ	•21.0	•23.5	•64.1	•89.8	102	•98.0	•184	146	•169	•155
105540F	•<LOQ	•18.8	•19.5	•67.2	90.8	105	•112	173	193	151	177
105542F	•<LOQ	24.0	•19.9	•78.4	•102	116	131	168	•151	181	165
105551F	<LOQ	•20.1	18.2	•80.2	82.0	87.3	117	127	132	•150	•142

• indicates a sample with an extraction volume of <0.5 mL
Shaded cells=moribund

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Table D-4b. PFOS Amounts Reported in Serum by Individual Animal (Days 183–Day 185 and Week 27)

PFOS Reported	Day 183 (µg/mL)	Day 184 (µg/mL)	Day 185 (µg/mL)	Week 27 (µg/mL)
Group I (Control)				
105508M	—	@	—	0.0461
105517M	—	—	—	—
105519M	—	@	—	0.0430
105520M	0.171	<LOQ	0.0366	—
105526M	•0.0626	0.0233	0.0498	—
105527M	—	@	—	0.0695
105529F	0.0756	0.0416	0.0763	—
105530F	—	@	—	0.0635
105531F	—	—	@	0.0374
105535F	—	—	@	0.0318
105544F	—	@	—	0.0338
105549F	0.0310	0.0287	0.0330	—
Group II (Low Dose)				
105514M	—	@	—	23.9
105515M	—	—	@	13.0
105516M	—	—	@	11.4
105521M	—	—	@	15.5
105537F	—	—	—	—
105541F	—	—	@	12.6
105547F	—	—	@	9.53
105550F	—	—	@	11.2
Group III (Mid Dose)				
105505M	•74.4	66.4	70.3	—
105510M	—	—	@	68.5
105518M	—	—	@	68.7
105523M	95.5	73.0	85.4	—
105524M	—	—	@	60.6
105528M	—	—	@	74.7
105532F	—	@	—	54.3
105538F	—	@	—	56.5
105539F	•107	86.5	101	—
105545F	—	@	—	58.0
105548F	—	@	—	65.1
105552F	56.9	•69.5	•67.8	—
Group IV (High Dose)				
105506M	—	—	—	196*
105507M	—	@	—	184
105509M	—	—	—	—
105511M	•216	181	309	—
105512M	—	@	—	202
105522M	282	336	•278	—
105533F	258	285	•441	—
105534F	—	—	@	182
105536F	—	@	—	189
105540F	—	—	@	184
105542F	201	224	•201	—
105551F	—	—	@	126

@ Sample collection dates for Week 27 data
* Sample collected on 2/20/99
• Indicates a sample with an extraction volume of <0.5 mL

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Table D-5a. PFOS Amounts Reported in Serum by Recovery Group Animal (Day 187– Week 47)

PFOS Reported	Day 187 (µg/mL)	Week 28 (µg/mL)	Week 29 (µg/mL)	Week 30 (µg/mL)	Week 31 (µg/mL)	Week 35 (µg/mL)	Week 39 (µg/mL)	Week 43 (µg/mL)	Week 47 (µg/mL)
Group I (Control)									
105520M	<LOQ	0.0284	•0.0690	0.0314	0.0362	0.0437	0.0316	0.0287	0.0339
105526M	0.0300	•0.0459	0.0639	0.0312	0.0354	0.0480	0.0466	0.0350	0.0370
105529F	0.0373	•0.0488	•0.0738	0.0299	0.0282	0.0748	0.0503	0.0368	0.0481
105549F	•0.0541	•0.0372	0.0747	0.0306	•0.0453	0.0698	•0.0514	0.0367	0.0436
Group III (Mid Dose)									
105505M	82.4	•85.7	77.3	•68.4	60.0	•93.0	•62.4	46.5	50.9
105523M	75.8	•83.5	•74.2	61.9	53.2	76.1	•57.9	44.9	45.7
105539F	10.2	•88.7	77.9	•69.9	•111	•81.2	•56.8	58.3	47.4
105552F	88.8	•83.6	61.9	54.5	•48.6	•67.7	•46.5	•58.0	37.9
Group IV (High Dose)									
105511M	237	•233	175	116	129	167	•135	66.9	105
105522M	297	264	270	170	194	195	•158	90.7	•142
105533F	269	249	207	156	201	164	•169	179	104
105542F	248	223	180	167	•170	178	•154	139	92

• Indicates a sample with an extraction volume of <0.5 mL

Table D-5b. PFOS Amounts Reported in Serum by Recovery Group Animal (Week 51– Week 79)

PFOS Reported	Week 51 (µg/mL)	Week 53 (µg/mL)	Week 57 (µg/mL)	Week 61 (µg/mL)	Week 65 (µg/mL)	Week 69 (µg/mL)	Week 73 (µg/mL)	Week 77 (µg/mL)	Week 79 (µg/mL)
Group I (Control)									
105520M	0.0213	0.0269	0.0289	0.0319	0.0184	•0.0428	•0.0431	•0.0334	0.0194
105526M	0.0261	0.0392	0.0364	0.0382	0.0236	0.0383	0.0269	•0.0259	0.0236
105529F	0.0344	0.0375	0.0472	0.0433	0.0364	0.0421	0.0386	0.0317	0.0268
105549F	0.0338	0.0419	0.0418	0.046	0.0357	0.0379	0.0345	•0.0294	0.0218
Group III (Mid Dose)									
105505M	39.8	43.9	28.5	27.4	32.9	24.6	24.0	•22.1	19.7
105523M	36.0	48.5	31.8	35.9	32.9	28.3	•30.6	•23.0	18.5
105539F	44.5	41.1	33.3	38.0	39.3	36.9	27.9	•27.5	22.8
105552F	•25.8	32.3	31.4	38.4	36.0	32.0	•23.7	•18.5	19.9
Group IV (High Dose)									
105511M	•67.5	54.8	66.5	64.7	•52.5	47.0	35.1	32.9	22.8
105522M	122	107	89.5	136	•131	•121	•73.8	87.0	•59.4
105533F	87.1	98.6	103	106	•89.7	78.7	54.5	70.5	40.6
105542F	95.7	97.9	109	109	•76.0	71.3	•240	•43.4	42.2

• Indicates a sample with an extraction volume of <0.5 mL

Table D-6. LOQ Values Used in Analyses by Method and Usage Dates

Method	Effective Date	LOQ	Usage Dates
Sera		**ng/mL**	
FACT-M-4.1	10/10/98	4.39	1/25/99 to 2/22/99
ETS-8-5.0	3/01/99	15.2	3/05/99 to 4/17/99
ETS-8-5.1	4/26/99	5.55	5/17/99 through the end of study
Liver		**ng/g**	
FACT-M-2.0	5/26/98	30.0	1/25/99 to 5/22/99
FACT-M-2.1	6/03/99	37.4	6/03/99 to 6/14/99
ETS-8-7.0	7/22/99	26.9	7/29/99 through the end of study

3M Medical Department Study: T-6295.7

Report No. FACT TOX-030
Laboratory Request Number-U2279

Table D-7. PFOS Concentrations in Liver by Dosage Group

Dosage Group	Collection Date	PFOS Calculated Concentration (ng/g)	Amount of PFOS (µg/g)
Group I (Control)			
105508M	2/25/99	2 Anomalies	2 Anomalies
105517M	2/25/99	143 - 1 Anomaly	0.143
105519M	2/25/99	91	0.091
105520M	None	—	—
105526M	None	—	—
105527M	2/25/99	129	0.129
105529F	None	—	—
105530F	2/25/99	128	0.128
105531F	2/26/99	112	0.112
105535F	2/26/99	87	0.087
105544F	2/25/99	97	0.097
105549F	None	—	—
Group II (Low Dose)			
105514M	2/26/99	18237	18.2
105515M	2/26/99	22709	22.7
105516M	2/26/99	11417	11.4
105521M	2/26/99	16734	16.7
105537F	2/25/99	22818	22.8
105541F	2/26/99	24847	24.8
105547F	2/26/99	20102	20.1
105550F	2/26/99	20735	20.7
Group III (Mid Dose)			
105505M	3/01/00 Biopsy	8252	8.25
105510M	2/26/99	42169	42.2
105518M	2/26/99	86173	86
105523M	3/01/00 Biopsy	10203	10.2
105524M	2/26/99	58673	58.7
105528M	2/26/99	48203	48.2
105532F	2/25/99	80421	80.4
105538F	2/25/99	49590	49.6
105539F	3/01/00 Biopsy	24728	24.7
105545F	2/25/99	66532	66.5
105548F	2/25/99	81376	81.4
105552F	3/01/00 Biopsy	17690	17.7
Group IV (High Dose)			
105506M	None	—	—
105507M	2/25/99	412474	412
105509M	None	—	—
105511M	9/22/99 Biopsy	142465	142
	2/25/00	23480	23.5
105512M	2/25/99	378723	379
105522M	9/22/99 Biopsy	137561	138
	2/25/00	70781	70.8
105533F	9/22/99 Biopsy	175283	175
	2/25/00	42668	42.7
105534F	2/26/99	280575	281
105536F	2/25/99	256669	257
105540F	2/26/99	267328	267
105542F	9/22/99 Biopsy	421647	422
	2/25/00	57895	57.9
105551F	2/26/99	287223	287

<LOQ: Less than the Lower Limit of Quantitation (26.9–37.4 ng/g)

3M Medical Department Study: T-6295.7
Report No. FACT TOX-030
Laboratory Request Number-U2279

ATTACHMENT E
DATA SPREADSHEETS

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	FACT-M-3.1 & FACT-M-4.1
Analytical Equipment System Number:	Soup 020199
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:	02/09/99 RWW
Date of Analysis/Analyst:	02/13/99 MEE
Date of Data Reduction/Analyst:	02/16/99 KJH

Sample Data

BASELINE MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS02099-H2O Blk-1	0.00	<LOQ		
	RBS02099-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS02099-Sera Blk-1	0.00	<LOQ		
	RBS02099-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS02099-MS	299	121%		
	MKS02099-MSD	285	115%	118%	5%
Group 1 Control 0.0 mg/kg/day	I05508M+	1.28	<LOQ		
	I05517M+	1.32	<LOQ		
	I05519M+	3.16	<LOQ		
	I05520M+	1.46	<LOQ		
	I05526M+	1.62	<LOQ		
	I05527M+	1.48	<LOQ	<LOQ	NA
	I05529F+	2.28	<LOQ		
	I05530F+	1.13	<LOQ		
	I05531F+	1.73	<LOQ		
	I05535F+	1.46	<LOQ		
	I05544F	1.60	<LOQ		
	I05549F	5.42	<LOQ	<LOQ	NA
Group 2 Low-Dose 0.03 mg/kg/day	I05513M+	2.63	<LOQ		
	I05514M	1.59	<LOQ		
	I05515M+	0.540	<LOQ		
	I05516M	2.65	<LOQ		
	I05521M+	1.72	<LOQ		
	I05525M+	1.90	<LOQ	<LOQ	<LOQ
	I05537F+	1.33	<LOQ		
	I05541F+	2.23	<LOQ		
	I05543F+	2.53	<LOQ		
	I05546F+	2.50	<LOQ		
	I05547F+	1.69	<LOQ		
	I05550F+	1.50	<LOQ	<LOQ	<LOQ
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	3.13	<LOQ		
	I05510M+	1.04	<LOQ		
	I05518M+	1.51	<LOQ		
	I05523M+	0.930	<LOQ		
	I05524M+	1.82	<LOQ		
	I05528M+	2.69	<LOQ	<LOQ	<LOQ
	I05532F+	3.13	<LOQ		
	I05538F	2.25	<LOQ		
	I05539F	2.15	<LOQ		
	I05545F+	3.27	<LOQ		
	I05548F	3.60	<LOQ		
	I05552F+	2.02	<LOQ	<LOQ	<LOQ
Group 4 High-Dose 0.75 mg/kg/day	I05506M+	2.19	<LOQ		
	I05507M	2.79	<LOQ		
	I05509M	2.06	<LOQ		
	I05511M	2.69	<LOQ		
	I05512M+	2.64	<LOQ		
	I05522M+	2.73	<LOQ	<LOQ	<LOQ
	I05533F+	2.02	<LOQ		
	I05534F+	2.54	<LOQ		
	I05536F+	2.85	<LOQ		
	I05540F+	3.45	<LOQ		
	I05542F+	1.94	<LOQ		
	I05551F	3.52	<LOQ	<LOQ	<LOQ

Limit of Quantitation (LOQ): 4.39 ng/mL

Data Entered/By: 02/17/99 LAC
Data Verified/ By: 03/23/99 GML, 06/07/00 PW
Data purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC
+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

NA = Not applicable
PFOS = Perfluorooctanesulfonate

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	FACT-M-3.1 & FACT-M-4.1
Analytical Equipment System Number:	Madeline 041098 & Amelia 062498
Instrument Software/Version:	MassLynx 3.1
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	02/05/99 SAH
Date of Analysis/Analyst:	02/08/9, 02/10/99, 02/12/99, 02/17/99 HOJ/DRB
Date of Data Reduction/Analyst:	02/10/99, 02/16/99, 02/26/99 HOJ/DRB

Sample Data

WEEK 1 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS02059-H2O Blk-1	0.00	<LOQ		
	RBS02059-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS02059-Sera Blk-1	0.00	<LOQ		
	RBS02059-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS02059-MS	305	123%		
	MKS02059-MSD	304	123%	123%	0%
Group 1 Control 0.0 mg/kg/day	I05508M	0.00	<LOQ		
	I05517M+	0.00	<LOQ		
	I05519M	0.00	<LOQ		
	I05520M	0.00	<LOQ		
	I05526M	0.00	<LOQ		
	I05527M	0.00	<LOQ	<LOQ	NA
	I05529F	0.00	<LOQ		
	I05530F	0.00	<LOQ		
	I05531F	0.00	<LOQ		
	I05535F	0.00	<LOQ		
	I05544F+	0.00	<LOQ		
	I05549F	0.00	<LOQ	<LOQ	NA
Group 2 Low-Dose 0.03 mg/kg/day	I05514M	495	0.793		
	I05515M+	383	0.767		
	I05516M	519	0.832		16.9
	I05521M	677	1.09	0.869	0.147
	I05537F	684	1.10		
	I05541F+	464	0.929		
	I05547F	581	0.931		11.6
	I05550F	519	0.832	0.947	0.110
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	287	4.60		
	I05510M+	209	4.19		
	I05518M	342	5.48		
	I05523M	221	3.54		
	I05524M	318	4.25		17.0
	I05528M	413	5.52	4.60	0.782
	I05532F+	161	4.31		
	I05538F	257	4.12		
	I05539F	248	3.31		
	I05545F+	184	3.69		
	I05548F	232	3.72		12.3
	I05552F	195	3.12	3.71	0.455
Group 4 High-Dose 0.75 mg/kg/day	I05506M	665	21.3		
	I05507M+	411	22.0		
	I05509M+	351	18.8		
	I05511M+	482	19.3		
	I05512M	711	22.8		7.49
	I05522M+	539	21.6	21.0	1.57
	I05533F+	564	22.6		
	I05534F+	451	18.1		
	I05536F+	393	21.0		
	I05540F+	419	16.8		
	I05542F	749	24.0		13.3
	I05551F+	377	20.1	20.4	2.71

Limit of Quantitation (LOQ): 4.39 ng/mL

Date Entered/By: 02/10/99, 03/02/99 LAC

Date Verified/ By: 03/23/99 GML.

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

NA = Not applicable

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 0

Extraction Volume Ratio = Initial Volume/Final Volume

Study	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	FACT-M-3.1 & FACT-M-4.1
Analytical Equipment System Number:	Madeline 041098 & Amelia 062498
Instrument Software/Version:	MassLynx 3.1
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	02/09/99 JCP
Date of Analysis/Analyst:	02/15/99, 02/22/99 HOJ
Date of Data Reduction/Analyst:	02/19/99, 02/25/99 HOJ

Sample Data

WEEK 2 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RSB02099-H2O Blk-1	0.00	<LOQ		
	RBS02099-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS02099-Sera Blk-1	0.00	<LOQ		
	RBS02099-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS02099-MS	293	118%		
	MKS02099-MSD	300	121%	120%	2%
Group 1 Control 0.0 mg/kg/day	I05508M	4.09	<LOQ		
	I05517M	3.07	<LOQ		
	I05519M+	4.53	<LOQ		
	I05520M+	2.08	<LOQ		
	I05526M	2.02	<LOQ		
	I05527M+	1.89	<LOQ	<LOQ	NA
	I05529F+	0.520	<LOQ		
	I05530F+	1.02	<LOQ		
	I05531F+	1.90	<LOQ		
	I05535F	3.12	<LOQ		
	I05544F+	0.380	<LOQ		
	I05549F+	0.810	<LOQ	<LOQ	NA
Group 2 Low-Dose 0.03 mg/kg/day	I05514M+	575	1.02		
	I05515M+	513	1.03		
	I05516M+	492	1.19		7.63
	I05521M+	425	1.13	1.10	0.0835
	I05537F+	446	1.19		
	I05541F	722	1.16		
	I05547F+	527	1.06		8.79
	I05550F+	550	0.980	1.10	0.0963
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M+	227	6.07		
	I05510M	301	4.82		
	I05518M+	350	7.01		
	I05523M	285	4.56		
	I05524M+	195	6.24		16.1
	I05528M	384	6.15	5.81	0.933
	I05532F+	173	4.61		
	I05538F	416	6.67		
	I05539F+	232	4.65		
	I05545F	329	5.28		
	I05548F+	320	6.42		17.2
	I05552F	295	4.73	5.39	0.930
Group 4 High-Dose 0.75 mg/kg/day	I05506M+	747	29.9		
	I05507M+	626	25.1		
	I05509M	746	23.9		
	I05511M	706	22.6		
	I05512M	976	31.3		13.1
	I05522M	899	28.8	26.9	3.54
	I05533F	778	24.9		
	I05534F+	650	26.0		
	I05536F+	588	23.5		
	I05540F+	546	19.5		
	I05542F+	372	19.9		14.8
	I05551F	568	18.2	22.0	3.25

Limit of Quantitation (LOQ): 4.39 ng/mL

Date Entered/By 02/22/99, 03/02/99 LAC
Date Verified/ By 03/23/99 GML
Date purity corrected/verified 09/12/00 mmh, 09//12/00 LAC

NA = Not applicable
PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Madeline 041098, Amelia 062498, & Soup 020199
Instrument Software/Version	MassLynx 3.1 and 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	03/02/99 SAH/RWW
Date of Analysis/Analyst:	03/05/99, 03/08/99, 03/25/99 HOJ/MEE/DRB
Date of Data Reduction/Analyst:	03/08/99, 03/26/99 KJH/DRB

Sample Data

WEEK 4 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03029-H2O Blk-1	0.00	<LOQ		
	RBS03029-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03029-Sera Blk-1	0.00	<LOQ		
	RBS03029-Sera Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	MKS03029-Sera Blk-1	7.21	<LOQ		
	MKS03029-Sera Blk-2	7.17	<LOQ		
	MKS03029-Sera Blk-3	7.26	<LOQ		
	MKS03029-Sera Blk-4	6.32	<LOQ	<LOQ	NA
QC - 250 ppb	MKS03029-MS-1	253	102%		
	MKS03029-MSD-1	250	101%	101%	1%
	MKS03029-MS-2	242	98%		
	MKS03029-MSD-2	235	95%	96%	3%
Group 1 Control 0.0 mg/kg/day	105508M	5.43	<LOQ		
	105517M+	6.76	<LOQ		
	105519M+	7.91	<LOQ		
	105520M+	6.11	<LOQ		
	105526M	6.73	<LOQ		
	105527M+	1.98	<LOQ	<LOQ	NA
	105529F+	6.63	<LOQ		
	105530F+	6.11	<LOQ		
	105531F	9.19	<LOQ		
	105535F+	8.17	<LOQ		
	105544F	9.96	<LOQ		
	105549F+	8.74	<LOQ	<LOQ	NA
Group 2 Low-Dose 0.03 mg/kg/day	105514M+	108	2.87		
	105515M	172	2.75		
	105516M	196	3.13		18.1
	105521M+	101	4.03	3.20	0.577
	105537F+	186	3.73		
	105541F+	153	3.07		
	105547F	221	3.55		8.53
	105550F+	163	3.27	3.40	0.291
Group 3 Mid-Dose 0.15 mg/kg/day	105505M+	63.7	17.0		
	105510M+	64.6	17.2		
	105518M+	101	20.2		
	105523M	110	17.6		
	105524M	96.3	15.4		9.47
	105528M+	95.7	19.2	17.8	1.68
	105532F+	41.4	16.6		
	105538F	117	18.7		
	105539F+	35.8	14.3		
	105545F+	46.7	18.7		
	105548F+	78.3	15.7		11.4
	105552F+	55.9	14.9	16.5	1.87
Group 4 High-Dose 0.75 mg/kg/day	105506M+	118	236		
	105507M+	27.2	72.6		
	105509M+	27.3	54.8		
	105511M+	24.2	48.4		
	105512M+	29.0	77.4		73.8
	105522M+	30.8	82.2	95.3	70.4
	105533F+	35.2	141		
	105534F+	36.3	145		
	105536F+	24.0	64.1		
	105540F+	838	67.2		
	105542F+	29.4	78.4		42.7
	105551F+	15.0	60.2	92.7	39.6

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/08/99, 03/26/99 LAC
Date Verified/ By: 03/23/99 GML,
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

NA = Not applicable
PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4 0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498 & Soup 020199
Instrument Software/Version:	MassLynx 3 1 and 3 2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope	See Attachments
Y-Intercept	See Attachments
Date of Extraction/Analyst:	03/10/99 SAH
Date of Analysis/Analyst:	03/12/99, 03/21/99 MEE/DRB
Date of Data Reduction/Analyst:	03/18/99, 03/24/99 LAC/DRB

Sample Data

WEEK 6 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03109-H2O Blk-1	0 00	<LOQ		
	RBS03109-H2O Blk-2	7 74	<LOQ	<LOQ	NA
Matrix Blk	RBS03109-Sera Blk-1	0 00	<LOQ		
	RBS03109-Sera Blk-2	4.19	<LOQ	<LOQ	NA
Matrix Blk	MKS03109-Sera Blk-1	2.56	<LOQ		
	MKS03109-Sera Blk-2	2.55	<LOQ		
	MKS03109-Sera Blk-3	1.93	<LOQ		
	MKS03109-Sera Blk-4	0.500	<LOQ	<LOQ	NA
QC - 250 ppb	MKS03109-MS-1	264	106%		
	MKS03109-MSD-1	253	101%	103%	4%
	MKS03109-MS-2	243	97%		
	MKS03109-MSD-2	249	100%	98%	2%
Group 1 Control 0.0 mg/kg/day	105508M+	0 00	<LOQ		
	105517M+	0 00	<LOQ		
	105519M+	0 00	<LOQ		
	105520M	1 60	<LOQ		
	105526M	0 610	<LOQ		
	105527M+	9 49	<LOQ	<LOQ	NA
	105529F+	1.56	<LOQ		
	105530F+	3.38	<LOQ		
	105531F	4.24	<LOQ		
	105535F+	0.00	<LOQ		
	105544F+	0.00	<LOQ		
	105549F+	3.54	<LOQ	<LOQ	NA
Group 2 Low-Dose 0.03 mg/kg/day	105514M+	36 6	3.67		
	105515M+	33.1	3.31		
	105516M+	32.5	3.26		11.9
	105521M+	41 8	4.19	3 61	0 430
	105537F+	42 7	4.28		
	105541F	45 7	3.66		
	105547F+	36 4	3.65		11 2
	105550F+	32 7	3.27	3 71	0 417
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	87 4	21.0		
	105510M+	56 1	22.5		
	105518M+	66 9	20.1		
	105523M+	61 9	18.6		
	105524M+	72.7	21.8		8 09
	105528M+	61 6	18.5	20 4	1 65
	105532F+	61.2	18 4		
	105538F	94.5	18 9		
	105539F+	61 4	18 4		
	105545F	94 5	22.7		
	105548F+	61.3	18 4		11 4
	105552F	67 0	16 1	18 8	2 15
Group 4 High-Dose 0.75 mg/kg/day	105506M+	182	91 0		
	105507M+	165	82 8		
	105509M	230	92 3		
	105511M	234	93.7		
	105512M+	211	105		8 54
	105522M+	203	102	94 5	8.07
	105533F	232	93.0		
	105534F+	166	83.3		
	105536F+	179	89 8		
	105540F	227	90.8		
	105542F+	203	102		7 90
	105551F	205	82.0	90.1	7 11

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/19/99, 03/24/99 LAC

Date Verified/ By: 08/17/99 SAH, 05/19/00 PJO-PW

Date purity corrected/verified 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

+ Serum initial volume less than 0 50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Soup 020199
Instrument Software/Version:	MassLynx 3.1 and 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	03/03/99 RWW/JCP
Date of Analysis/Analyst:	03/05/99, 03/09/99, 03/10/99, 03/25/99 DRB
Date of Data Reduction/Analyst:	03/08/99, 03/15/99, 03/24/99, 03/26/99 DRB

Sample Data

WEEK 8 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03039-H2O Blk-1	0.00	<LOQ		
	RBS03039-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03039-Sera Blk-1	0.00	<LOQ		
	RBS03039-Sera Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	MKS03039-Sera Blk-1	3.23	<LOQ		
	MKS03039-Sera Blk-2	2.10	<LOQ	<LOQ	NA
QC - 250 ppb	MKS03039-MS-1	174	69%		
	MKS03039-MSD-1	191	76%	72%	9%
	MKS03039-MS-2	205	82%		
	MKS03039-MSD-2	185	73%	77%	10%
	MKS03039-MS-3	181	72%		
	MKS03039-MSD-3	203	81%	76%	11%
Group 1 Control 0.0 mg/kg/day	I05508M	9.43	<LOQ		
	I05517M	7.68	<LOQ		
	I05519M	12.0	<LOQ		
	I05520M	9.03	<LOQ		
	I05526M	11.0	<LOQ		
	I05527M	28.8	0.0385	<LOQ - 1 Anomaly	NA
	I05529F	16.0	0.0214		
	I05530F	18.3	0.0244		
	I05531F	18.0	0.0206		
	I05535F	14.5	<LOQ		
	I05544F	9.46	<LOQ		8.36
	I05549F	15.0	0.0240	0.0226 - 2 Anomalies	0.00189
Group 2 Low-Dose 0.03 mg/kg/day	I05514M	34.5	4.61		
	I05515M	31.2	4.17		
	I05516M	44.1	5.05		9.14
	I05521M	31.8	5.10	4.73	0.432
	I05537F	46.1	5.28		
	I05541F	36.3	4.85		
	I05547F	37.3	4.98		12.1
	I05550F	34.4	3.94	4.76	0.577
Group 3 Mid-Dose 0.15 mg/kg/day	I05503M	122	27.9		
	I05510M	82.9	22.1		
	I05518M	94.1	25.1		
	I05523M	91.6	24.5		
	I05524M	118	31.6		12.7
	I05528M	107	24.6	26.0	3.30
	I05532F	86.1	27.6		
	I05538F	106	24.3		
	I05539F	82.6	26.5		
	I05545F	122	24.5		
	I05548F	69.4	22.3		12.7
	I05552F	59.5	19.1	24.0	3.06
Group 4 High-Dose 0.75 mg/kg/day	I05506M	122	125		
	I05507M	83.8	86.1		
	I05509M	117	103		
	I05511M	89.8	92.3		
	I05512M	115	118		16.7
	I05522M	127	131	109	18.3
	I05533F	107	110		
	I05534F	98.1	121		
	I05536F	100	102		
	I05540F	102	105		
	I05542F	113	116		11.1
	I05551F	99.2	87.3	107	11.8

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/08/99, 03/24/99, 03/26/99 LAC

Date Verified/ By: 08/18/99 SAH, 05/11/00 PJO, 05/23/00 & 05/24/00 PW

Date punty corrected/verified 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Soup 020199
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	03/03/99 RWW/JCP
Date of Analysis/Analyst:	03/05/99, 03/09/99, 03/10/99, 03/25/99 DRB
Date of Data Reduction/Analyst:	03/08/99, 03/15/99, 03/24/99, 03/26/99 DRB

Sample Data

WEEK 12 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03039-H2O Blk-1	0.00	<LOQ		
	RBS03039-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03039-Sera Blk-1	0.00	<LOQ		
	RBS03039-Sera Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	MKS03039-Sera Blk-1	3.23	<LOQ		
	MKS03039-Sera Blk-2	2.10	<LOQ	<LOQ	NA
QC - 250 ppb	MKS03039-MS-1	174	69%		
	MKS03039-MSD-1	191	76%	72%	9%
	MKS03039-MS-2	205	82%		
	MKS03039-MSD-2	185	73%	77%	10%
	MKS03039-MS-3	181	72%		
	MKS03039-MSD-3	203	81%	76%	11%
Group 1 Control 0.0 mg/kg/day	105508M	30.9	0.0381		
	105517M	26.9	0.0430		
	105519M	32.8	0.0438		
	105520M	12.7	<LOQ		
	105526M	21.3	0.0244		21.1
	105527M	30.1	0.0423	0.0383 - 1 Anomaly	0.00811
	105529F+	11.4	<LOQ		
	105530F	16.4	0.0243		
	105531F	19.0	0.0254		
	105535F	9.43	<LOQ		
	105544F	18.1	0.0238		13.8
	105549F	23.3	0.0316	0.0263 - 2 Anomalies	0.00362
Group 2 Low-Dose 0.03 mg/kg/day	105514M	41.8	6.20		
	105515M	44.5	6.48		
	105516M	47.4	6.55		8.65
	105521M+	45.1	7.52	6.69	0.578
	105537F	57.8	6.81		
	105541F	34.8	5.57		
	105547F+	36.9	7.03		11.4
	105550F	48.8	5.83	6.31	0.717
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	143	40.1		
	105510M	135	39.2		
	105518M	133	33.7		
	105523M+	88.0	35.3		
	105524M	118	37.8		15.3
	105528M	79.2	25.4	35.2	5.39
	105532F	86.9	25.8		
	105538F+	94.2	32.8		
	105539F	101	27.8		
	105545F	109	32.2		
	105548F	79.6	23.2		14.3
	105552F	77.4	24.8	27.8	3.98
Group 4 High-Dose 0.75 mg/kg/day	105506M	103	116		
	105507M	86.4	106		
	105509M	116	123		
	105511M	83.9	99.4		
	105512M	149	167		19.6
	105522M+	88.0	121	122	23.9
	105533F	109	126		
	105534F	100	114		
	105536F+	63.6	98.0		
	105540F+	80.0	112		
	105542F	128	131		10.0
	105551F	114	117	117	11.7

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/08/99, 03/24/99, 03/26/99 LAC
Date Verified/ By: 08/18/99 SAH, 05/24/00 PW
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4 0 and ETS-8-5 0
Analytical Equipment System Number:	Soup 020199
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	3/12/99 SAH/RWW
Date of Analysis/Analyst:	03/15/99, 03/19/99 DRB/HOJ
Date of Data Reduction/Analyst:	03/16/99, 03/24/99 DRB

Sample Data

WEEK 16 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03129-H2O Blk-1	0.00	<LOQ		
	RBS03129-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03129-Sera Blk-1	0.00	<LOQ		
	RBS03129-Sera Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	MKS03129-Sera Blk-1	15.0	<LOQ		
	MKS03129-Sera Blk-2	7.6	<LOQ	<LOQ	NA
QC - 250 ppb	MKS03129-MS-1	189	72%		
	MKS03129-MSD-1	199	76%	74%	5%
	MKS03129-MS-2	199	76%		
	MKS03129-MSD-2	235	90%	83%	16%
Group 1 Control 0.0 mg/kg/day	105508M	21.5	0.0344		
	105517M+	20.4	0.0408		
	105519M+	21.1	0.0423		
	105520M	20.7	0.0332		
	105526M	19.9	0.0320		27.1
	105527M	38.4	0.0615	0.0407	0.0110
	105529F	35.5	0.0438		
	105530F+	25.7	0.0515		
	105531F	21.5	0.0314		
	105535F+	9.57	<LOQ		
	105544F+	11.6	<LOQ		19.7
	105549F	28.9	0.0462	0.0432 -2 Anomalies	0.00851
Group 2 Low-Dose 0.03 mg/kg/day	105514M	77.7	10.4		
	105515M+	47.3	9.47		
	105516M	81.7	10.1		21.9
	105521M+	83.1	14.8	11.2	2.44
	105537F	76.4	11.1		
	105541F+	69.2	12.3		
	105547F	66.8	10.7		18.1
	105550F	53.9	7.85	10.5	1.90
Group 3 Mid-Dose 0.15 mg/kg/day	105505M+	222	59.4		
	105510M	330	52.9		
	105518M	379	60.7		
	105523M	326	52.2		
	105524M	475	63.4		10.4
	105528M	362	48.4	56.2	5.84
	105532F	252	40.4		
	105538F	279	37.3		
	105539F	347	46.3		
	105545F	323	43.1		
	105548F	323	47.0		9.59
	105552F	241	38.7	42.1	4.04
Group 4 High-Dose 0.75 mg/kg/day	105506M	273	182		
	105507M	257	172		
	105509M	296	197		
	105511M	268	179		
	105512M	296	216		8.42
	105522M	254	185	189	15.9
	105533F	245	164		
	105534F	196	157		
	105536F+	184	184		
	105540F	258	173		
	105542F	231	168		11.9
	105551F	191	127	162	19.3

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/08/99, 03/17/99, 03/24/99 LAC

Date Verified/ By: 08/18/99 SAH, 05/16/00, 05/24/00 PW

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Soup 020199
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:	3/15/99 JCP/SAH
Date of Analysis/Analyst:	03/16/99, 03/24/99 DRB
Date of Data Reduction/Analyst:	03/17/99, 03/25/99 DRB

Sample Data

WEEK 20 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03159-H2O Blk-1	0.00	<LOQ		
	RBS03159-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03159-Sera Blk-1	0.00	<LOQ		
	RBS03159-Sera Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	MKS03159-Sera Blk-1	0.00	<LOQ		
	MKS03159-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	RBS03159-MS-1	256	103%		
	RBS03159-MSD-1	226	91%	97%	13%
	RBS03159-MS-2	219	88%		
	RBS03159-MSD-2	223	90%	89%	2%
Group 1 Control 0.0 mg/kg/day	105508M	30.4	0.0348		
	105517M	31.4	0.0419		
	105519M+	19.3	0.0336		
	105520M	23.2	0.0300		
	105526M	30.0	0.0369		29.9
	105527M+	31.5	0.0631	0.0400	0.0120
	105529F	31.6	0.0617		
	105530F	38.3	0.0579		
	105531F+	28.3	0.0493		
	105535F	19.4	0.0263		
	105544F+	29.6	0.0505		25.2
	105549F	39.1	0.0570	0.0504	0.0127
Group 2 Low-Dose 0.03 mg/kg/day	105514M	88.6	12.5		
	105515M	86.7	10.7		
	105516M	101	11.9		11.4
	105521M	98.3	14.1	12.3	1.40
	105537F	70.2	11.0		14.6
	105541F	73.2	11.5	12.3	1.79
	105547F	96.6	14.3		74.4
	105550F	359	41.1	19.5	14.5
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	237	62.3		
	105510M	253	63.3		
	105518M	248	63.1		
	105523M	319	75.2		
	105524M	220	64.0		10.5
	105528M	223	54.2	63.7	6.71
	105532F	255	69.2		
	105538F	201	47.4		
	105539F+	210	78.2		
	105545F	171	45.7		
	105548F	204	65.3		25.4
	105552F	154	42.7	58.1	14.7
Group 4 High-Dose 0.75 mg/kg/day	105506M	381	158		
	105507M+	282	138		
	105509M	315	152		
	105511M	352	126		
	105512M	309	143		7.62
	105522M	302	145	144	10.9
	105533F	393	169		
	105534F	303	143		
	105536F+	274	146		
	105540F	441	193		
	105542F+	302	151		14.0
	105551F	330	132	156	21.8

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/08/99, 03/17/99 LAC
Date Verified/ By: 08/18/99 SAH, 05/17/00 PW
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not analyzed, not applicable

* Calculated without sample 105550F
- Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498 & Soup 020199
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:	3/16/99 RWW/SAH
Date of Analysis/Analyst:	03/17/99, 03/20/99, 03/23/99 DRB/MEE
Date of Data Reduction/Analyst:	03/23/99, 03/24/99, 04/02/99 DRB

Sample Data

WEEK 24 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03169-H2O Blk-1	0.00	<LOQ		
	RBS03169-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03169-Sera Blk-1	0.00	<LOQ		
	RBS03169-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	RBS03169-MS-1	218	88%		
	RBS03169-MSD-1	236	95%	92%	8%
	RBS03169-MS-2	231	93%		
	RBS03169-MSD-2	213	86%	89%	8%
	RBS03169-MS-3	224	90%		
	RBS03169-MSD-3	220	89%	90%	2%
Group 1 Control 0.0 mg/kg/day	I05508M	36.4	0.0449		
	I05517M	30.3	0.0486		
	I05519M	25.8	0.0414		
	I05520M	19.4	0.0283		
	I05526M	24.8	0.0397		17.7
	I05527M+	36.7	0.0613	0.0440	0.0101
	I05519F	40.4	0.0498		
	I05530F+	24.2	0.0497		
	I05531F	25.9	0.0364		
	I05535F+	8.36	<LOQ		
	I05544F+	16.2	0.0325		18.4
	I05549F	33.4	0.0446	0.0426 - 1 Anomaly	0.00784
Group 2 Low-Dose 0.03 mg/kg/day	I05514M+	115	14.7		
	I05515M+	85.4	10.9		
	I05516M	132	14.1		21.0
	I05521M+	141	18.4	14.5	3.06
	I05537F	134	13.4		
	I05541F	133	13.7		
	I05547F+	84.8	12.7		5.18
	I05550F	108	12.2	13.0	0.675
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	251	77.4		
	I05510M	186	65.2		
	I05518M	191	58.8		
	I05523M	164	59.7		
	I05524M	201	69.6		10.4
	I05528M+	155	64.8	65.9	6.88
	I05532F+	145	63.1		
	I05538F	153	48.6		
	I05539F+	142	69.6		
	I05545F	168	63.5		
	I05548F	196	56.1		12.0
	I05552F+	139	61.7	60.4	7.24
Group 4 High-Dose 0.75 mg/kg/day	I05506M	287	198		
	I05507M	234	188		
	I05509M	M	M		
	I05511M	258	207		
	I05512M	309	247		11.6
	I05522M+	233	233	215	24.9
	I05533F	280	190		
	I05534F+	225	200		
	I05536F+	207	169		
	I05540F	218	151		
	I05542F	240	181		12.0
	I05551F+	131	150	174	20.9

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/24/99 LAC

Date Verified/ By: 08/18/99 SAH, 05/24/00 PW

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

M = Moribund

NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498, Soup 020199
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:	3/16/99 RWW/SAH
Date of Analysis/Analyst:	03/17/99,03/21/99 DRB/MEE
Date of Data Reduction/Analyst:	03/23/99, 03/22/99 DRB/MEE

Sample Data

WEEK 26 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS03169-H2O Blk-1	0.00	<LOQ		
	RBS03169-H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS03169-Sera Blk-1	0.00	<LOQ		
	RBS03169-Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	RBS03169-MS-1	210	85%		
	RBS03169-MSD-1	232	94%	89%	10%
	RBS03169-MS-2	229	92%		
	RBS03169-MSD-2	193	78%	85%	17%
	RBS03169-MS-3	211	85%		
	RBS03169-MSD-3	213	86%	86%	1%
Group 1 Control 0.0 mg/kg/day	I05508M	30.9	0.0496		
	I05517M+	31.0	0.0539		
	I05519M	27.1	0.0417		
	I05520M	16.8	0.0254		
	I05526M	26.7	0.0376		31.3
	I05527M	41.7	0.0669	0.0459	0.0143
	I05529F	48.2	0.0613		
	I05530F	59.7	0.0736		
	I05531F	30.1	0.0431		
	I05535F	16.6	0.0266		
	I05544F+	21.6	0.0433		32.5
	I05549F	34.7	0.0556	0.0506	0.0164
Group 2 Low-Dose 0.03 mg/kg/day	I05514M	151	16.2		
	I05515M	143	16.5		
	I05516M	131	13.8		8.91
	I05521M+	112	16.9	15.8	1.41
	I05537F+	101	13.5		
	I05541F+	113	14.8		
	I05547F	140	13.0		10.8
	I05550F+	85.3	11.4	13.2	1.42
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	255	92.9		
	I05510M+	149	129		
	I05518M+	142	69.5		
	I05523M+	176	72.1		
	I05524M+	160	69.5		30.4
	I05528M	156	62.4	82.6	25.2
	I05532F	209	63.4		
	I05538F	150	59.0		
	I05539F	216	72.2		
	I05545F	269	85.7		
	I05548F+	145	64.5		16.3
	I05552F	142	55.6	66.8	10.8
Group 4 High-Dose 0.75 mg/kg/day	I05506M	M	M		
	I05507M+	213	182		
	I05509M	M	M		
	I05511M	248	142		
	I05512M	285	148		21.0
	I05522M	298	221	173	36.5
	I05533F	340	203		
	I05534F	308	187		
	I05536F+	155	155		
	I05540F	278	177		
	I05542F	206	165		13.0
	I05551F+	163	142	171	22.2

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 03/23/99 LAC

Date Verified/ By: 08/19/99 SAH, 05/24/00 PW

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

M = Moribund

PFOS = Perfluorooctanesulfonate

NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4 1 and ETS-8-5 1
Analytical Equipment System Number:	Davey070799
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:	06/01/00 SAL/KJK
Date of Analysis/Analyst:	06/06/00, 06/14/00, 06/16/00 IAS/MMH
Date of Data Reduction/Analyst:	06/07/00, 06/15/00, 06/19/00 IAS/MMH

Sample Data

WEEK 27 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	06010-H2O Blk-5	0.00	<LOQ		
	06010-H2O Blk-6	0.00	<LOQ	<LOQ	NA
Matrix Blk	RBS06010-Sera Blk-5	0.00	<LOQ		
	RBS06010-Sera Blk-6	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	105519M-MS-5	350	121%		
	105519M-MSD-5	353	122%	122%	1%
Group 1 Control 0.0 mg/kg/day	105508M	53.1	0.0461		
	105519M	49.5	0.0430		27.4
	105527M	80.1	0.0695	0.0529	0.0145
	105530F	73.1	0.0635		
	105531F	43.1	0.0374		
	105535F	36.6	0.0318		35.5
	105544F	38.9	0.0338	0.0416	0.0148
Group 2 Low-Dose 0.03 mg/kg/day	105514M	172	23.9		
	105515M	187	13.0		
	105516M	165	11.4		34.8
	105521M	223	15.5	15.9	5.54
	105541F	181	12.6		
	105547F	137	9.53		13.7
	105550F	161.2	11.2	11.1	1.52
Group 3 Mid-Dose 0.15 mg/kg/day	105510M	316	68.5		
	105518M	316	68.7		
	105524M	279	60.6		8.44
	105528M	344	74.7	68.1	5.75
	105532F	250	54.3		
	105538F	260	56.5		
	105545F	267	58.0		7.99
	105548F	300	65.1	58.5	4.67
Group 4 High-Dose 0.75 mg/kg/day	105506M *	450	196		
	105507M	424	184		4.61
	105512M	464	202	194	8.93
	105534F	419	182		
	105536F	389	169		
	105540F	377	164		14.9
	105551F	291	126	160	23.9

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 06/07/00, 06/19/00 LAC
Date Verified/ By: 06/08/00 PJW
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

Extraction Volume Ratio = Initial Volume/Final Volume

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/06/99 RWW
Date of Analysis/Analyst:	04/07/99, 04/12/99, 04/17/99, 05/14/99, 05/17/99 HOJ/DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/09/99, 04/15/99, 04/20/99, 05/17/99, 05/18/99 HOJ/DRB/KJH

Sample Data

DAY183 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04069-H2O Blk-1	0.00	<LOQ		
	RBS04069-H2O Blk-2	14.2	<LOQ	NA	NA
Matrix Blk	RBS04069-Sera Blk-1	0.00	<LOQ		
	RBS04069-Sera Blk-2	12.7	<LOQ	NA	NA
QC - 250 ppb	MKS04069-MS-1	254	102%		
	MKS04069-MSD-1	246	99%	101%	3%
	MKS04069-MS-2	267	108%		
	MKS04069-MSD-2	228	92%	100%	16%
Group 1 Control 0.0 mg/kg/day	105520M	128	0.171		65.5
	105526M+	29.7	0.0626	0.117	0.0764
	105529F	66.0	0.0756		59.2
	105549F	21.3	0.0310	0.0533	0.0315
Group 3 Mid-Dose 0.15 mg/kg/day	105505M+	371	74.4		17.6
	105523M	787	95.5	85.0	14.9
	105539F+	479	107		43.0
	105552F	391	56.9	81.7	35.1
Group 4 High-Dose 0.75 mg/kg/day	105511M+	97.1	216		18.8
	105522M	194	282	249	46.8
	105533F	245	258		17.5
	105542F	176	201	230	40.3

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 04/19/99, 05/10/99, 05/20/99 LAC

Date Verified/ By: 08/19/99 SAH, before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Ameba 062498, Soup 020199, & Madeline 041098
Instrument Software/Version	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/06/99 RWW
Date of Analysis/Analyst:	04/07/99, 04/12/99, 04/17/99, 05/14/99, 05/17/99 HOJ/DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/09/99, 04/15/99, 04/20/99, 05/17/99, 05/18/99 HOJ/DRB/KJH

Sample Data

DAY184 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04069-H2O Blk-1	0.00	<LOQ		
	RBS04069-H2O Blk-2	14.2	<LOQ	NA	NA
Matrix Blk	RBS04069-Sera Blk-1	0.00	<LOQ		
	RBS04069-Sera Blk-2	12.7	<LOQ	NA	NA
QC - 250 ppb	MKS04069-MS-1	254	102%		
	MKS04069-MSD-1	246	99%	101%	3%
	MKS04069-MS-2	267	108%		
	MKS04069-MSD-2	228	92%	100%	16%
Group 1 Control 0.0 mg/kg/day	I05520M	12.3	<LOQ		
	I05526M	23.2	0.0233	0.0233 - 1 Anomaly	NA
	I05529F	43.6	0.0416		25.9
	I05549F	28.0	0.0287	0.0352	0.00911
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	621	66.4		6.72
	I05523M	692	73.0	69.7	4.68
	I05539F	810	86.5		15.4
	I05552F-	390	69.5	78.0	12.0
Group 4 High-Dose 0.75 mg/kg/day	I05511M	192	181		42.5
	I05522M	264	336	259	110
	I05533F	242	265		11.9
	I05542F	210	224	245	29.2

Limit of Quantitation (LOQ) PFOS = 15.2 ng/mL

Date Entered/By: 04/19/99, 05/10/99, 05/20/99 LAC

Date Verified/ By: 08/24/99 SAH, before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/06/99 RWW
Date of Analysis/Analyst:	04/07/99, 04/12/99, 04/17/99, 05/14/99, 05/17/99 HOJ/DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/09/99, 04/15/99, 04/20/99, 05/17/99, 05/18/99 HOJ/DRB/KJH

Sample Data

DAY185 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04069-H2O Blk-1	0.00	<LOQ	NA	NA
	RBS04069-H2O Blk-2	14.18	<LOQ		
Matrix Blk	RBS04069-Sera Blk-1	0.00	<LOQ	NA	NA
	RBS04069-Sera Blk-2	12.74	<LOQ		
QC - 2.50 ppb	MKS04069-MS-1	254	102%	101%	3%
	MKS04069-MSD-1	246	99%		
	MKS04069-MS-2	267	108%	100%	16%
	MKS04069-MSD-2	228	92%		
Group 1 Control 0.0 mg/kg/day	I05520M	32.0	0.0366	0.0432	21.5
	I05526M	37.3	0.0498		0.00928
	I05529F	63.8	0.0763	0.0546	56.1
	I05549F	23.0	0.0330		0.0306
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	553	70.3	77.9	13.7
	I05523M	778	85.4		10.7
	I05539F	867	101	84.3	27.6
	I05552F+	322	67.8		23.3
Group 4 High-Dose 0.75 mg/kg/day	I05511M	216	309	294	7.49
	I05522M+	115	278		22.0
	I05533F+	220	441	321	52.8
	I05542F+	121	201		170

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 04/19/99, 05/10/99 LAC

Date Verified/ By: 08/25/99 SAH, before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/06/99 RWW
Date of Analysis/Analyst:	04/07/99, 04/12/99, 04/17/99, 05/14/99, 05/17/99 HOJ/DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/09/99, 04/15/99, 04/20/99, 05/17/99, 05/18/99 HOJ/DRB/KJH

Sample Data

DAY187 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04069-H2O Blk-1	0.00	<LOQ		
	RBS04069-H2O Blk-2	14.2	<LOQ	NA	NA
Matrix Blk	RBS04069-Sera Blk-1	0.00	<LOQ		
	RBS04069-Sera Blk-2	12.7	<LOQ	NA	NA
QC - 250 ppb	MKS04069-MS-1	254	102%		
	MKS04069-MSD-1	246	99%	101%	3%
	MKS04069-MS-2	267	108%		
	MKS04069-MSD-2	228	92%	100%	16%
Group 1 Control 0.0 mg/kg/day	105520M	13.6	<LOQ		
	105526M	22.1	0.0300	0.0300 - 1 Anomaly	NA
	105529F	39.1	0.0373		25.9
	105549F+	31.7	0.0541	0.0457	0.0118
Group 3 Mid-Dose 0.15 mg/kg/day	105503M	699	82.4		5.91
	105523M	662	75.8	79.1	4.67
	105539F	63.7	10.2		105
	105552F	498	68.8	39.5	41.4
Group 4 High-Dose 0.75 mg/kg/day	105511M	225	237		15.7
	105522M	185	297	267	42.0
	105533F	289	269		5.90
	105542F	253	248	258	15.2

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 04/19/99, 05/10/99, 05/20/99 LAC
Date Verified/ By: 08/25/99 SAH, before 06/08/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Study	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/08/99 RWW
Date of Analysis/Analyst:	04/11/99, 04/17/99, 05/14/99 DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/16/99, 04/20/99, 05/17/99 HOJ/DRB/KJH

Sample Data

WEEK 28 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04089-H2O Blk-1	7.80	<LOQ		
	RBS04089-H2O Blk-2	7.01	<LOQ	<LOQ	NA
Matrix Blk	RBS04089-Sera Blk-1	0.00	<LOQ		
	RBS04089-Sera Blk-2	0.680	<LOQ	<LOQ	NA
QC - 250 ppb	MKS04089-MS-1	260	105%		
	MKS04089-MSD-1	267	108%	106%	3%
	MKS04089-MS-2	249	101%		
	MKS04089-MSD-2	264	106%	103%	6%
Group 1 Control 0.0 mg/kg/day	I05520M	18.4	0.0284		33.5
	I05526M+	26.4	0.0459	0.0371	0.0124
	I05529F+	26.8	0.0488		19.1
	I05549F-	20.0	0.0372	0.0430	0.00821
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M+	481	85.7		1.78
	I05523M+	511	83.5	84.6	1.51
	I05539F-	288	88.7		4.16
	I05552F-	355	83.6	86.1	3.59
Group 4 High-Dose 0.75 mg/kg/day	I05511M+	536	233		8.71
	I05522M	659	264	249	21.7
	I05533F	366	249		7.76
	I05542F	423	223	236	18.3

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By: 04/19/99, 05/10/99 LAC

Date Verified/ By: 08/25/99 SAH, before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number:	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/08/99 RWW
Date of Analysis/Analyst:	04/11/99, 04/17/99, 05/14/99 DRB/MEE/KJH
Date of Data Reduction/Analyst:	04/16/99, 04/20/99, 05/17/99 HOJ/DRB/KJH

Sample Data

WEEK 29 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04089-H2O Blk-1	7.80	<LOQ	<LOQ	NA
	RBS04089-H2O Blk-2	7.01	<LOQ		
Matrix Blk	RBS04089-Sera Blk-1	0.00	<LOQ	<LOQ	NA
	RBS04089-Sera Blk-2	0.680	<LOQ		
QC - 250 ppb	MKS04089-MS-1	260	105%	106%	3%
	MKS04089-MSD-1	267	108%		
	MKS04089-MS-2	249	101%	103%	6%
	MKS04089-MSD-2	264	106%		
Group 1 Control 0.0 mg/kg/day	105520M+	42.2	0.0690	0.0665	5.45
	105526M	44.7	0.0639		0.00362
	105529F+	38.7	0.0738	0.0742	0.894
	105549F	68.1	0.0747		0.000664
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	656	77.3	75.8	2.88
	105523M+	417	74.2		2.18
	105539F	613	77.9	69.9	16.2
	105552F	432	61.9		11.4
Group 4 High-Dose 0.75 mg/kg/day	105511M	560	175	223	30.0
	105522M	384	270		66.9
	105533F	641	207	194	9.83
	105542F	549	180		19.0

Limit of Quantitation (LOQ): PFOS = 15.2 ng/mL

Date Entered/By 04/19/99, 05/10/99 LAC

Date Verified/ By: 08/25/99 SAH, before 06/08/00 TKR

Date punty corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.0 and ETS-8-5.0
Analytical Equipment System Number	Amelia 062498, Soup 020199, & Madeline 041098
Instrument Software/Version:	MassLynx 3.2
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	04/08/99 RWW
Date of Analysis/Analyst:	04/11/99, 04/17/99, 05/14/99 DRB/MEE/KJH
Date of Data Reduction/Analyst	04/16/99, 04/20/99, 05/17/99 HOJ/DRB/KJH

Sample Data

WEEK 31 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS04089-H2O Blk-1	7.80	<LOQ		
	RBS04089-H2O Blk-2	7.01	<LOQ	<LOQ	NA
Matrix Blk	RBS04089-Sera Blk-1	0.00	<LOQ		
	RBS04089-Sera Blk-2	0.680	<LOQ	<LOQ	NA
QC - 250 ppb	MKS04089-MS-1	260	105%		
	MKS04089-MSD-1	267	108%	106%	3%
	MKS04089-MS-2	249	101%		
	MKS04089-MSD-2	264	106%	103%	6%
Group 1 Control 0.0 mg/kg/day	105520M	27.1	0.0362		1.58
	105526M	36.7	0.0354	0.0358	0.000567
	105529F	24.3	0.0282		32.9
	105549F+	25.5	0.0453	0.0368	0.0121
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	591	60.0		8.43
	105523M	398	53.2	56.6	4.77
	105539F+	580	111		55.0
	105552F+	291	48.6	79.6	43.8
Group 4 High-Dose 0.75 mg/kg/day	105511M	398	129		28.6
	105522M	668	194	161	46.1
	105533F	591	201		11.8
	105542F+	398	170	185	21.9

Date Entered/By: 04/19/99 LAC
Date Verified/ By: 08/25/99 SAH, before 06/08/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	MassLynx 3.3
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	08/25/99 RWW
Date of Analysis/Analyst:	09/28/99, 10/05/99, 06/14/00 MEE/MMH
Date of Data Reduction/Analyst:	09/29/99, 10/06/99, 06/15/00 MEE/IAS/MMH

Sample Data

WEEK 35 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	0.00	<LOQ	<LOQ	NA
	H2O Blk-2	0.00	<LOQ		
Matrix Blk	Rabbit Sera Blk-1	0.00	<LOQ	<LOQ	NA
	Rabbit Sera Blk-2	0.00	<LOQ		
QC - 250 ppb	MKS08259-MS-1	227	91%	97%	11%
	MKS05289-MSD-1	254	102%		
	MKS08259-MS-2	256	103%	100%	7%
	MKS05289-MSD-2	239	96%		
Group 1 Control 0.0 mg/kg/day	I05520M	27.3	0.0437	0.0459	6.60
	I05526M	35.9	0.0480		0.00303
	I05529F	74.7	0.0748	0.0723	4.86
	I05549F	61.0	0.0698		0.00352
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M+	186	93.0	84.5	14.1
	I05523M	190	76.1		12.0
	I05539F+	122	81.2	74.4	12.8
	I05552F+	135	67.7		9.53
Group 4 High-Dose 0.75 mg/kg/day	I05511M	417	167	181	10.8
	I05522M	486	195		19.5
	I05533F	408	164	171	5.94
	I05542F	444	178		10.1

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 10/05/99, 10/07/99, 06/19/00 LAC
Date Verified/ By: before 06/08/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not analyzed, not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	MassLynx 3.3
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	08/25/99 RWW
Date of Analysis/Analyst:	09/28/99, 10/05/99, 06/14/00 MEE/MMH
Date of Data Reduction/Analyst:	09/29/99, 10/06/99, 06/15/00 MEE/IAS/MMH

Sample Data

WEEK 39 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	0.00	<LOQ		
	H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	0.00	<LOQ		
	Rabbit Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS08259-MS-1	227	91%		
	MKS05289-MSD-1	254	102%	97%	11%
	MKS08259-MS-2	256	103%		
	MKS05289-MSD-2	239	96%	100%	7%
Group 1	105520M	23.7	0.0316		27.1
Control	105526M	29.1	0.0466	0.0391	0.0106
0.0 mg/kg/day	105529F	31.4	0.0503		1.53
	105549F+	25.7	0.0514	0.0509	0.000779
Group 3	105505M+	311	62.4		5.26
Mid-Dose	105523M+	217	57.9	60.1	3.16
0.15 mg/kg/day	105539F+	142	56.8		14.1
	105552F+	174	46.5	51.7	7.29
Group 4	105511M+	270	135		11.0
High-Dose	105522M+	236	158	146	16.1
0.75 mg/kg/day	105533F+	338	169		6.86
	105542F+	230	154	161	11.1

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 10/05/99, 10/07/99, 06/19/00 LAC

Date Verified/ By: before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not analyzed, not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4 1 and ETS-8-5 1
Analytical Equipment System Number:	Amelia 062498
Instrument Software/Version:	MassLynx 3 3
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	08/25/99 RWW
Date of Analysis/Analyst:	09/28/99, 10/05/99, 06/14/00 MEE/MMH
Date of Data Reduction/Analyst:	09/29/99, 10/06/99, 06/15/00 MEE/IAS/MMH

Sample Data

WEEK 43 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	0.00	<LOQ		
	H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	0.00	<LOQ		
	Rabbit Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS08259-MS-1	227	91%		
	MKS05289-MSD-1	254	102%	97%	11%
	MKS08259-MS-2	256	103%		
	MKS05289-MSD-2	239	96%	100%	7%
Group 1 Control 0.0 mg/kg/day	105520M	21.5	0.0287		13.8
	105526M	21.8	0.0350	0.0319	0.00440
	105529F	32.2	0.0368		0.251
	105549F	22.9	0.0367	0.0368	0.0000923
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	162	46.5		2.44
	105523M	112	44.9	45.7	1.12
	105539F	146	58.3		0.429
	105552F+	116	58.0	58.1	0.249
Group 4 High-Dose 0.75 mg/kg/day	105511M	167	66.9		21.4
	105522M	226	90.7	78.8	16.8
	105533F	447	179		17.8
	105542F	347	139	159	28.4

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 10/05/99, 10/07/99, 06/19/00 LAC

Date Verified/ By: before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not analyzed, not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision.	ETS-8-4 1 and ETS-8-5 1
Analytical Equipment System Number	Amelia 062498
Instrument Software/Version:	MassLynx 3.3
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope.	See Attachments
Y-Intercept.	See Attachments
Date of Extraction/Analyst:	08/25/99 RWW
Date of Analysis/Analyst:	09/28/99, 10/05/99, 06/14/00 MEE/MMH
Date of Data Reduction/Analyst:	09/29/99, 10/06/99, 06/15/00 MEE/IAS/MMH

Sample Data

WEEK 47 MONKEY SERA

Group Dose	Sample #	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	0.00	<LOQ		
	H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	0.00	<LOQ		
	Rabbit Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS08259-MS-1	227	91%		
	MKS05289-MSD-1	254	102%	97%	11%
	MKS08259-MS-2	256	103%		
	MKS05289-MSD-2	239	96%	100%	7%
Group 1 Control 0.0 mg/kg/day	I05520M	25.4	0.0339		6.23
	I05526M	27.7	0.0370	0.0355	0.00221
	I05529F	36.0	0.0481		7.04
	I05549F	32.6	0.0436	0.0459	0.00323
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	152	50.9		7.65
	I05523M	114	45.7	48.3	3.69
	I05539F	118	47.4		15.7
	I05552F	114	37.9	42.6	6.70
Group 4 High-Dose 0.75 mg/kg/day	I05511M	263	105		21.0
	I05522M+	212	142	124	25.9
	I05533F	312	104		8.47
	I05542F	277	92	98.3	8.32

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 10/05/99, 10/07/99, 06/19/00 LAC
Date Verified/ By: before 06/08/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not analyzed, not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Ameba 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:	08/25/99 RWW
Date of Analysis/Analyst:	09/28/99, 10/05/99, 06/14/00 MEE/MMH
Date of Data Reduction/Analyst:	09/29/99, 10/06/99, 06/15/00 MEE/IAS/MMH

Sample Data

WEEK 51 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	0.00	<LOQ		
	H2O Blk-2	0.00	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-1	0.00	<LOQ		
	Rabbit Sera Blk-2	0.00	<LOQ	<LOQ	NA
QC - 250 ppb	MKS08259-MS-1	227	91%		
	MKS05289-MSD-1	254	102%	97%	11%
	MKS08259-MS-2	256	103%		
	MKS05289-MSD-2	239	96%	100%	7%
Group 1 Control 0.0 mg/kg/day	105520M	13.3	0.0213		14.0
	105526M	19.5	0.0261	0.0237	0.00333
	105529F	34.3	0.0344		1.18
	105549F	29.6	0.0338	0.0341	0.000403
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	119	39.8		6.92
	105523M	108	36.0	37.9	2.62
	105539F	111	44.5		37.6
	105552F-	52	25.8	35.1	13.2
Group 4 High-Dose 0.75 mg/kg/day	105511M+	135	67.5		40.6
	105522M	365	122	94.7	38.4
	105533F	261	87.1		6.65
	105542F	287	95.7	91.4	6.07

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 10/05/99, 10/07/99, 06/19/00 LAC

Date Verified/ By: before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not analyzed, not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number	Soup020199
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:	11/03/99 SAL
Date of Analysis/Analyst:	11/04/99, 11/11/99, 11/18/99 LAS
Date of Data Reduction/Analyst:	11/08/99, 11/12/99, 11/22/99 MMH/LAS

Sample Data

WEEK 53 MONKEY SERA

Group Dose	Sample #	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-5	0.830	<LOQ		
	H2O Blk-5	1.07	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-5	2.20	<LOQ		
	Rabbit Sera Blk-5	2.50	<LOQ	<LOQ	NA
**	MKS11039-MS-6	29.5	0.0237		0.192
	MKS11039-MSD-6	29.4	0.0236	0.0236	0.0000453
QC - 250 ppb	MKS11039-MS-5	374	112%		
	MKS11039-MSD-5	380	114%	113%	2%
Group 1 Control 0.0 mg/kg/day	I05520M	33.6	0.0269		26.1
	I05526M	48.9	0.0392	0.0331	0.0086
	I05529F	46.8	0.0375		7.82
	I05549F	52.3	0.0419	0.0397	0.00311
Group 3 Mid-Dose 0.15 mg/kg/day	I05505M	109	43.9		7.15
	I05523M	121	48.5	46.2	3.30
	I05539F	103	41.1		17.0
	I05552F+	81	32.3	36.7	6.24
Group 4 High-Dose 0.75 mg/kg/day	I05511M	137	54.8		45.5
	I05522M+	267	107	80.8	36.8
	I05533F	246	98.6		0.499
	I05542F	244	97.9	98.2	0.490

Limit of Quantitation (LOQ) PFOS = 5.55 ng/mL

Date Entered/By: 11/15/99, 11/24/99 LAC

Date Verified/ By: before 06/08/00 TKR

Date purity corrected/verified. 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

** Although labeled as MS/MSD-6, these were not spiked and will be used as blanks. LAC 11/15/99

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Soup020199
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:	11/03/99 SAL
Date of Analysis/Analyst:	11/04/99, 11/11/99, 11/18/99 IAS
Date of Data Reduction/Analyst:	11/08/99, 11/12/99, 11/22/99 MMH/IAS

Sample Data

WEEK 57 MONKEY SERA

Group Dose	Sample #	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-5	0.830	<LOQ		
	H2O Blk-5	1.07	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-5	2.20	<LOQ		
	Rabbit Sera Blk-5	2.50	<LOQ	<LOQ	NA
**	MKS11039-MS-6	29.5	0.0237		0.192
	MKS11039-MSD-6	29.4	0.0236	0.0236	0.0000453
QC - 250 ppb	MKS11039-MS-5	374	112%		
	MKS11039-MSD-5	380	114%	113%	2%
Group 1	105520M	36.1	0.0289		16.1
Control	105526M	45.4	0.0364	0.0327	0.00526
0.0 mg/kg/day	105529F	58.9	0.0472		8.65
	105549F	52.1	0.0418	0.0445	0.00385
Group 3	105505M	71.1	28.5		7.83
Mid-Dose	105523M	79.4	31.8	30.2	2.36
0.15 mg/kg/day	105539F	83.1	33.3		4.13
	105552F	78.4	31.4	32.3	1.34
Group 4	105511M	166	66.5		20.8
High-Dose	105522M+	223	89.5	78.0	16.3
0.75 mg/kg/day	105533F	258	103		3.63
	105542F+	271	109	106	3.84

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 11/15/99, 11/24/99 LAC
Date Verified/ By: before 06/08/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

** Although labeled as MS/MSD-6, these were not spiked and will be used as blanks. LAC 11/15/99
+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Soup020199
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:	11/03/99 SAL
Date of Analysis/Analyst:	11/04/99, 11/11/99, 11/18/99 IAS
Date of Data Reduction/Analyst:	11/08/99, 11/12/99, 11/22/99 MMH/IAS

Sample Data

WEEK 61 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-5	0.830	<LOQ		
	H2O Blk-5	1.07	<LOQ	<LOQ	NA
Matrix Blk	Rabbit Sera Blk-5	2.20	<LOQ		
	Rabbit Sera Blk-5	2.50	<LOQ	<LOQ	NA
**	MKS11039-MS-6	29.5	0.0237		0.192
	MKS11039-MSD-6	29.4	0.0236	0.0236	0.0000453
QC - 250 ppb	MKS11039-MS-5	374	112%		
	MKS11039-MSD-5	380	114%	113%	2%
Group 1 Control 0.0 mg/kg/day	105520M+	39.8	0.0319		12.8
	105526M	47.7	0.0382	0.0351	0.00449
	105529F	54.0	0.0433		4.68
	105549F	57.7	0.046	0.0448	0.00210
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	68.4	27.4		18.9
	105523M	89.5	35.9	31.6	5.98
	105539F+	94.8	38.0		0.742
	105552F	95.8	38.4	38.2	0.283
Group 4 High-Dose 0.75 mg/kg/day	105511M	162	64.7		50.2
	105522M	339	136	100	50.3
	105533F	271	108		0.640
	105542F	273	109	109	0.697

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Date Entered/By: 11/15/99, 11/24/99 LAC

Date Verified/ By: before 06/08/00 TKR

Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

NA = Not applicable

** Although labeled as MS/MSD-6, these were not spiked and will be used as blanks LAC 11/15/99

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number	Ruby 100699
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:	04/24/00 SAL/KJK
Date of Analysis/Analyst:	04/25/00, 04/27/00, 04/28/00, 05/03/00 IAS/MMH/ADV
Date of Data Reduction/Analyst	04/27/00, 05/01/00, 05/01/00, 05/04/00 IAS

Sample Data

WEEK 65 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04240-H2O Blk-3	0.300	<LOQ		
	04240-H2O Blk-4	0.390	<LOQ	<LOQ	NA
Matrix Blk	RBS04240-Sera Blk-3	0.930	<LOQ		
	RBS04240-Sera Blk-4	2.75	<LOQ	<LOQ	NA
	MKS04240-Sera Blk-3	8.64	0.00750		16.9
	MKS04240-Sera Blk-4	11.0	0.0095	0.00852	0.00144
QC - 250 ppb	MKS04240-Sera-MS-3	310	122%		
	MKS04240-Sera-MSD-3	236	92%	107%	27%
	MKS04240-Sera-MS-4	306	119%		
	MKS04240-Sera-MSD-4	326	127%	123%	6%
Group 1 Control 0.0 mg/kg/day	105520M	12.7	0.0184		17.4
	105526M	14.1	0.0236	0.0210	0.00365
	105529F	31.5	0.0364		1.45
	105549F	24.7	0.0357	0.0360	0.000522
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	208	32.9		0.0820
	105523M	193	32.9	32.9	0.0269
	105539F	344	39.3		6.18
	105552F	261	36.0	37.6	2.32
Group 4 High-Dose 0.75 mg/kg/day	105511M+	254	52.5		60.3
	105522M+	587	131	91.5	55.2
	105533F+	465	89.7		11.7
	105542F+	324	76.0	82.8	9.68

Limit of Quantitation (LOQ) PFOS = 5.35 ng/mL

Date Entered/By: 05/03/00, 05/09/00 LAC/CSH
Date Verified/ By: 06/02/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	16 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Ruby 100499
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:	04/24/00 SAL/KJK
Date of Analysis/Analyst:	04/25/00, 04/27/00, 04/28/00, 05/03/00 IAS/MVH/ADV
Date of Data Reduction/Analyst:	04/27/00, 05/01/00, 05/01/00, 05/04/00 IAS

Sample Data

WEEK 69 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04240-H20 Blk-3	0.300	<LOQ		
	04240-H2O Blk-4	0.390	<LOQ	<LOQ	NA
Matrix Blk	RBS04240-Sera Blk-3	0.930	<LOQ		
	RBS04240-Sera Blk-4	2.75	<LOQ	<LOQ	NA
	MKS04240-Sera Blk-3	8.6	0.00750		16.9
	MKS04240-Sera Blk-4	11.0	0.00954	0.00852	0.00144
QC - 250 ppb	MKS04240-Sera-MS-3	310	122%		
	MKS04240-Sera-MSD-3	236	92%	107%	27%
	MKS04240-Sera-MS-4	306	119%		
	MKS04240-Sera-MSD-4	326	127%	123%	6%
Group 1 Control 0.0 mg/kg/day	105520M+	22.2	0.0428		7.71
	105526M	26.5	0.0383	0.0406	0.00313
	105529F	32.5	0.0421		7.52
	105549F	24.4	0.0379	0.0400	0.00301
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	368	24.6		9.80
	105523M	378	28.3	26.4	2.59
	105539F	459	36.9		10.0
	105552F	406	32.0	34.5	3.46
Group 4 High-Dose 0.75 mg/kg/day	105511M	341	47.0		62.4
	105522M+	544	121	84.0	52.4
	105533F	499	78.7		7.00
	105542F	525	71.3	75.0	5.23

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Data Entered/By: 05/03/00, 05/09/00 LAC/CSH

Data Verified/ By: 06/02/00 TKR

Data purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative 04/28/00 LAC

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number:	Ruby 100699
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	04/24/00 SAL/KJK
Date of Analysis/Analyst	04/25/00, 04/27/00, 04/28/00, 05/03/00 IAS/MMH/ADV
Date of Data Reduction/Analyst:	04/27/00, 05/01/00, 05/01/00, 05/04/00 IAS

Sample Data

WEEK 73 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04240-H20 Blk-3	0.300	<LOQ	<LOQ	NA
	04240-H2O Blk-4	0.390	<LOQ		
Matrix Blk	RBS04240-Sera Blk-3	0.930	<LOQ	<LOQ	NA
	RBS04240-Sera Blk-4	2.75	<LOQ		
	MKS04240-Sera Blk-3	8.64	0.00750	0.00852	16.9
	MKS04240-Sera Blk-4	11.0	0.00954		0.00144
QC - 250 ppb	MKS04240-Sera-MS-3	310	122%	107%	27%
	MKS04240-Sera-MSD-3	236	92%		
	MKS04240-Sera-MS-4	306	119%	123%	6%
	MKS04240-Sera-MSD-4	326	127%		
Group 1 Control 0.0 mg/kg/day	105520M+	22.8	0.0431	0.0350	32.8
	105526M	17.6	0.0269		0.0115
	105529F	22.6	0.0386	0.0365	7.76
	105549F	24.7	0.0345		0.00284
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	163	24.0	27.3	17.1
	105523M+	158	30.6		4.66
	105539F	160	27.9	25.8	11.3
	105552F+	131	23.7		2.91
Group 4 High-Dose 0.75 mg/kg/day	105511M	405	35.1	54.4	50.2
	105522M+	374	73.8		27.3
	105533F	326	54.5	147	89.1
	105542F+	227	240		131

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL

Data Entered/By: 05/03/00, 05/09/00 LAC/CSH
Data Verified/ By: 06/02/00 TKR
Data purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance)	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number	Ruby 100699
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:	04/24/00 SAL/KJK
Date of Analysis/Analyst:	04/25/00, 04/27/00, 04/28/00, 05/03/00 IAS/MOH/ADV
Date of Data Reduction/Analyst:	04/27/00, 05/01/00, 05/01/00, 05/04/00 IAS

Sample Data

WEEK 77 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04240-H2O Blk-3	0.300	<LOQ	<LOQ	NA
	04240-H2O Blk-4	0.390	<LOQ		
Matrix Blk	RBS04240-Sera Blk-3	0.930	<LOQ	<LOQ	NA
	RBS04240-Sera Blk-4	2.75	<LOQ		
	MKS04240-Sera Blk-3	8.64	0.00750	0.00852	16.9
	MKS04240-Sera Blk-4	11.0	0.0095		0.00144
QC - 250 ppb	MKS04240-Sera-MS-3	310	122%	107%	27%
	MKS04240-Sera-MSD-3	236	92%		
	MKS04240-Sera-MS-4	306	119%	123%	6%
	MKS04240-Sera-MSD-4	326	127%		
Group 1 Control 0.0 mg/kg/day	105520M+	12.7	0.0334	0.0296	18.0
	105526M+	11.9	0.0259		0.00535
	105529F	21.9	0.0317	0.0305	5.46
	105549F+	15.2	0.0294		0.00167
Group 3 Mid-Dose 0.15 mg/kg/day	105505M+	120	22.1	22.5	2.90
	105523M+	101	23.0		0.632
	105539F+	101	27.5	23.0	27.7
	105552F+	70.3	18.5		6.37
Group 4 High-Dose 0.75 mg/kg/day	105511M	424	32.9	60.0	63.8
	105522M	511	87.0		38.3
	105533F	463	70.5	57.0	33.6
	105542F+	460	43.4		19.1

Limit of Quantitation (LOQ): PFOS = 5.55 ug/mL

Data Entered/By: 05/03/00, 05/09/00 LAC/CSH
Data Verified/ By: 06/02/00 TKR
Data purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

+ Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study:	24 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Sera
Method/Revision:	ETS-8-4.1 and ETS-8-5.1
Analytical Equipment System Number	Ruby 100699
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:	04/24/00 SAL/KJK
Date of Analysis/Analyst:	04/25/00, 04/27/00, 04/28/00, 05/03/00 IAS/MOH/ADV
Date of Data Reduction/Analyst:	04/27/00, 05/01/00, 05/01/00, 05/04/00 IAS

Sample Data

WEEK 79 MONKEY SERA

Group Dose	Sample #	PFOS Conc. ug/mL	Concentration of PFOS ug/mL or % Rec	Mean PFOS ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04240-H2O Blk-3	0.380	<LOQ		
	04240-H2O Blk-4	0.390	<LOQ	<LOQ	NA
Matrix Blk	RBS04240-Sera Blk-3	0.930	<LOQ		
	RBS04240-Sera Blk-4	2.75	<LOQ	<LOQ	NA
	MKS04240-Sera Blk-3	8.64	0.00750		16.9
	MKS04240-Sera Blk-4	11.0	0.00954	0.00852	0.00144
QC - 250 ppb	MKS04240-Sera-MS-3	310	122%		
	MKS04240-Sera-MSD-3	236	92%	107%	27%
	MKS04240-Sera-MS-4	306	119%		
	MKS04240-Sera-MSD-4	326	127%	123%	6%
Group 1 Control 0.0 mg/kg/day	105520M	16.5	0.0194		13.8
	105526M	17.9	0.0236	0.0215	0.00296
	105529F	22.3	0.0268		14.6
	105549F	18.6	0.0218	0.0243	0.00355
Group 3 Mid-Dose 0.15 mg/kg/day	105505M	152	19.7		4.22
	105523M	111	18.5	19.1	0.805
	105539F	142	22.8		9.40
	105552F	138	19.9	21.4	2.01
Group 4 High-Dose 0.75 mg/kg/day	105511-1M	293	22.8		63.1
	105522-1M-	328	59.4	41.1	25.9
	105533-1F	262	40.6		2.79
	105542-1F	253	42.2	41.4	1.15

Limit of Quantitation (LOQ) PFOS = 5.55 ng/mL

Date Entered/By: 05/03/00, 05/09/00 LAC/CSH
Date Verified/ By: 06/02/00 TKR
Date purity corrected/verified: 09/12/00 mmh, 09/12/00 LAC

PFOS = Perfluorooctanesulfonate
NA = Not applicable

* Serum initial volume less than 0.50 mL, concentrations should be considered tentative. 04/28/00 LAC

Extraction Volume Ratio = Initial Volume/Final Volume

Study: 26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance): T-6295 (PFOS)
Matrix: Monkey Liver Filename: See Attachments
Method/Revision: FACT-M-1.0 & FACT-M-2.0, 2.1, ETS-8-0 R-Squared Value: See Attachments
Analytical Equipment System Number: Amelia 062498, Madeline 041098, Davey0 Slope: See Attachments
Instrument Software/Version: MassLynx 3.2 & 3.3 Y-Intercept: See Attachments
Date of Extraction/Analyst: 05/18/99, 06/11/99, 05/23/00 SAH/SRP/SEE/SAL
Date of Analysis/Analyst: 05/19/99, 05/22/99, 06/09/99, 06/14/99, 07/29/99, 06/14/99, 05/25/00 KJH/HOJ/MEE/SAH/IAS
Date of Data Reduction/Analyst: 05/20/99, 05/25/99, 06/10/99, 06/22/99, 08/04/99, 10/12/99, 50/26/00 KJH/HOJ/MEE/MMH/IAS

Sample Data

MONKEY LIVER Week 27

Group Dose	Sample #	PFOS Calc. Conc. ug/g	Concentration of PFOS ug/g or % Rec.		Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL05189-H2O Blk-3	0.00	<LOQ			
	RBL05189-H2O Blk-4	0.454	<LOQ		<LOQ	NA
Method Blk	RBL06119-H2O Blk-11	0.00	<LOQ			
	RBL06119-H2O Blk-12	NA	NA		<LOQ	NA
Method Blk	05230-H2O Blk-5	0.00	<LOQ			
	05230-H2O Blk-6	1.64	<LOQ		<LOQ	NA
Matrix Blk	RBL05189-Lvr Blk-3	0.00	<LOQ			
	RBL05189-Lvr Blk-4	3.43	<LOQ		<LOQ	NA
Matrix Blk	RBL06119-Lvr Blk-11	0.00	<LOQ			
	RBL06119-Lvr Blk-12	NA	NA		<LOQ	NA
Matrix Blk	RBL05230-Lvr Blk-5	1.25	<LOQ			
	RBL05230-Lvr Blk-6	0.391	<LOQ		<LOQ	NA
Matrix Blk	MKL05230-Lvr Blk-5	15.2	<LOQ			
	MkL05230-Lvr Blk-6	9.7	<LOQ		<LOQ	NA
QC - 250 ppb 05/18/99	105508M-MS	482	174%	**		
	105508M-MSD	305	110%	**	142%	45%
QC - 250 ppb 06/11/99	105517M-MS	300	101%			
	105517M-MSD	305	103%		102%	2%
QC - 250 ppb 05/23/00	MKL05230-MS-5	332	111%			
	MKL05230-MSD-5	292	98%		104%	13%
Group 1 Control 0.0 mg/kg/day	105508M 6/11/99	564	0.564	++		
	105517M 6/11/99	143	0.143			
	105508M 5/18/99	477	0.477	++		
	105517M 5/18/99	119	0.119	R		
	105519M 5/18/99	91	0.091			22.2
	105527M 5/18/99	129	0.129		0.121	0.0269
	105530F	128	0.128			
	105531F	112	0.112			
	105535F	87	0.087			16.8
	105544F	97	0.097		0.106	0.0178
Group 2 Low-Dose 0.03 mg/kg/day	105514M	18237	18.2			
	105515M	22709	22.7			
	105516M	11417	11.4			27.0
	105521M	16734	16.7		17.3	4.66
	105537F	22818	22.8			
	105541F	24847	24.8			
	105547F	282623	283	*		
ext. 5/23/00	105547F	20102	20.1			9.73
	105550F	20735	20.7		22.1	2.15
Group 3 Mid-Dose 0.15 mg/kg/day	105510M	42169	42.2			
	105518M	86173	86			
	105524M	58673	58.7			33.1
	105528M	48203	48.2		58.8	19.5
	105532F	80421	80.4			
	105538F	49590	49.6			
	105545F	66532	66.5			21.4
	105548F	81376	81.4		69.5	14.9
Group 4 High-Dose 0.75 mg/kg/day	105507M	412474	412			6.03
	105512M	378723	379		396	23.9
	105534F	280575	281			
	105536F	256669	257			
	105540F	267328	267			5.00
	105551F	287223	287		273	13.6

Limit of Quantitation (LOQ): PFOS = 30.0 ng/g

Date Entered/By: 05/25/99, 06/10/99, 06/22/99, 10/12/99 LAC
Date Verified/ By: 06/02/00 PJW, 06/05/00 TKR
Date Purity corrected/Verified/ By: 09/12/00 mmh, 09/12/00 LAC

NA = Not analyzed, not applicable
PFOS = Perfluorooctanesulfonate

++ Sample determined an outlier and was not included in any calculations.
** Sample used for MS/MSD appears to be spiked accounting for low recoveries. Sample 105507 & 18 will be reextracted. Sample I05507M will be used for the MS/MSD and I05518M conc. Verified.
R = Sample reextracted along with MS/MSD. This value was not used in any calculations
* Sample determined an outlier and was not included in any calculations. Was reextracted 05/23/00

FACT-TOX-030
Covance# 6329-223

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Soup 020199, Amelia 062499
Instrument Software/Version:	MassLynx 3.2 & 3.3
Date of Extraction/Analyst:	10/14/99, 10/25/99 SEE/RWW
Date of Analysis/Analyst:	10/15/99, 10/20/99, 10/22/99, 10/26/99, 10/27/99 MMH/IAS
Date of Data Reduction/Analyst:	10/18/99, 10/21/99, 10/25/99, 10/27/99, 10/29/99 IAS/MMH

Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments

Sample Data

MONKEY LIVER Day 393 Biopsy

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk 10/14/99	RBL10149-H2O Blk-11	NA	NA	<LOQ	NA
	RBL10149-H2O Blk-12	3.58	<LOQ		
Method Blk 10/25/99	RBL10259-H2O Blk-1*	8.48	<LOQ	<LOQ	NA
	RBL10259-H2O Blk-2	NA	NA		
Matrix Blank 10/14/99	RBL10149-Lvr Blk-11	NA	NA	<LOQ	NA
	RBL10149-Lvr Blk-12	0.00	<LOQ		
Matrix Blank 10/25/99	RBL10259-Lvr Blk-1	0.00	<LOQ	<LOQ	NA
	RBL10259-Lvr Blk-2	NA	NA		
	MKL10259-Lvr Blk-1	2.05	<LOQ	<LOQ	NA
	MKL10259-Lvr Blk-2	NA	NA		
QC-250 ppm 10/25/99 25 ppm & 500 ppb	I05542F-MS-1	198121	79%	68%	30%
	I05542F-MSD-1	145974	58%		
	MKL10259-MS-2	521	87%	85%	5%
	MKL10259-MSD-2	498	83%		
Group 4 High-Dose 0.75 mg/kg/day	I05511M	142465	142	140	2.48
	I05522M	137561	138		3.47
	I05533F	175283	175	298	58.4
	I05542F	421647	422		174

Limit of Quantitation (LOQ): PFOS = 26.9 ng/g

Date Entered/By: 10/22/99, 10/25/99, 10/29/99 LAC, 11/05/99 GML

Date Verified/ By: 06/05/00 TKR

Date Purity correctedVerified/ By:09/12/00 mmh, 09/12/00 LAC

NA= Not analyzed, not applicable

PFOS = Perfluorooctanesulfonate

MS/MSD samples extracted on 10/14/99 were not within recovery criteria, and samples were reextracted on 10/25/99 at a higher concentration. The earlier MS/MSD samples were not spiked at a high enough level in relation to the endogenous PFOS levels. Reextracted MS/MSDs were within criteria. LAC 12/14/99

To calculate the extracted density: (initial liver weight/(initial liver weight +Milli Q Water added)

To calculate the PFOS Calc. Conc.: (PFOS conc. ng/g X density of curve liver homogenate g/mL)/extracted density g/mL) * PFOS correction factor * dilution factor

FACT-TOX-030

Study:	26 Week Capsule Toxicity Study with PFOS in Cynomolgus Monkeys
Product Number(Test Substance):	T-6295 (PFOS)
Matrix:	Monkey Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Davey 070799
Instrument Software/Version:	MassLynx 3.2 & 3.3
Date of Extraction/Analyst:	3/22/00 SAL
Date of Analysis/Analyst:	03/24/00, 03/27/00, 03/28/00, 5/5/00 MMH, IAS
Date of Data Reduction/Analyst:	03/27/00, 03/28/00, 03/29/00, 5/8/00 MMH, IAS

Sample Data

Monkey Liver Wks 79 & 80

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL03220-H2O-Blk-5	0.00	<LOQ (30.6 ng/g)		NA
	RBL03220-H2O-Blk-6	0.00	<LOQ (30.6 ng/g)	<LOQ	NA
Matrix Blk	RBL03220-liverBlk-5	0.00	<LOQ (30.6 ng/g)		NA
	RBL03220-liverBlk-6	0.00	<LOQ (30.6 ng/g)	<LOQ	NA
	MKL03220-liverBlk-5	0.00	<LOQ (30.6 ng/g)		NA
	MKL03220-liverBlk-6	0.00	<LOQ (30.6 ng/g)	<LOQ	NA
QC 250 ppb	MKL03220-MS-250ppb-5-1	216	72%		
	MKL03220-MSD-250ppb-5-2	186	62%	67%	15%
	MKL03220-MS-250ppb-6-1	266	89%		
	MKL03220-MSD-250ppb-6-2	232	77%	83%	14%
	105505-MS-50ppm-5-1	54408	95%		
	105505-MSD-50ppm-5-2	47351	81%	88%	17%
Group 4 High-Dose 0.75 mg/kg/day	105511/M/Wk79	23480	23.5		71.0
	105522/M/Wk79	70781	70.8	47.1	33.4
	105533/F/Wk79	42668	42.7		21.4
	105542/F/Wk79	57895	57.9	50.3	10.8
Group 3 Mid-Dose 0.15 mg/kg/day	105505/M/Wk80	8252	8.25		14.9
	105523/M/Wk80	10203	10.2	9.23	1.38
	105539/F/Wk80	24728	24.7		23.5
	105552/F/Wk80	17690	17.7	21.2	4.98

Limit of Quantitation Limit (LOQ): PFOS = 26.9 ng/g

PFOS = Perfluorooctanesulfonate
NA = Not applicable

Date Entered/Analyst:	04/05/00, 04/06/00, 5/10/00 MMH, CSH
Date Verified/Analyst:	06/05/00 TKR
Date Purity correctedVerified/ By:	09/12/00 mmh, 09/12/00 LAC

ZZ 231

3M Medical Department Study: T-6295.7
Report No. FACT TOX-030
Laboratory Request Number-U2279

ATTACHMENT F
EXAMPLE CALCULATIONS

Formula Used for Sera Analyses in Study FACT TOX-030

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

Calculation Used for Group 3, Week 1, Animal ID 105505M

$$287\ ng/mL \times 10 \times 0.9275 \times \frac{1\ mL}{0.5\ mL} \times \frac{1.0\ \mu g}{1000\ ng} = 5.32\ \mu g/mL \times 0.864 = \textbf{4.60 µg/mL}$$

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%)

Formula Used for Liver Analyses in Study FACT TOX-030

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

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

Calculation Used for Group 3, Week 27, Animal ID 105510M

$$524\ ng/g \times \frac{1\ g/\ 5\ mL}{0.9963\ g/\ 5mL} \times 0.9275 \times 100 \times \frac{1.0\ \mu g}{1000\ ng} = 48.8\ \mu g/g \times 0.864 = \textbf{42.2 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (1 g sample/ 5 mL H_2O)
SC—PFOS salt correction constant (0.9275)
DF— Dilution factor
PC—PFOS purity correction factor (86.4%)

3M Medical Department Study: T-6295.7
Report No. FACT TOX-030
Laboratory Request Number-U2279

ATTACHMENT G
INTERIM CERTIFICATE OF ANALYSES

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 NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.005 wt./wt.% 2. 0.001 wt./wt.% 3. 1.439 wt./wt.% 4. 6.849 wt./wt.% 5. <0.001 wt./wt.% 6. 0.005 wt./wt.% 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) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.59 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.76 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. 0.10 wt./wt.% 4. 0.28 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.48 wt./wt.% 2. 0.244 wt./wt.% 3. 1.74 wt./wt.% 4. 8.84 wt./wt.% 5. 54.1 wt./wt.%

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).

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: _____ _____
David S. Bell — Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ _____
John Flaherty — Date
Laboratory Manager, Centre Analytical Laboratories

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 2. Magnesium 3. Sodium 4. Potassium[2] 5. Nickel 6. Iron 7. Manganese		1. 0.017 wt./wt.% 2. 0.007 wt./wt.% 3. 1.355 wt./wt.% 4. 6.552 wt./wt.% 5. 0.003 wt./wt.% 6. 0.004 wt./wt.% 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 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate[4]		1. <0.015 wt./wt.% 2. 0.27 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. 8.82 wt./wt.%
Organic Acids [5] (IC) 1. TFA 2. PFPA 3. HFBA 4. NFPA		1. <0.1 wt./wt.% 2. <0.1 wt./wt.% 3. <0.1 wt./wt.% 4. <0.25 wt./wt.%
Elemental Analysis[6]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	1. Theoretical Value = 17.8% 2. Theoretical Value = 0% 3. Theoretical Value = 0% 4. Theoretical Value = 5.95% 5. Theoretical Value = 60%	1. 12.08 wt./wt.% 2. 0.794 wt./wt.% 3. 1.61 wt./wt.% 4. 10.1 wt./wt.% 5. 50.4 wt./wt.%

Exact Copy of Original
LAC 09/12/00
Initial Date

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).

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: _____ _____
David S. Bell Date
Scientist, Centre Analytical Laboratories

Reviewed By: _____ _____
John Flaherty Date
Laboratory Manager, Centre Analytical Laboratories